OBSTETRICS

Down syndrome maternal serum marker screening after 18 weeks of gestation: a countrywide study

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OBJECTIVE: The objective of the study was to evaluate the efficacy of maternal serum markers in detecting Down syndrome after 18 weeks of gestation in women who book late for maternity care in a large national retrospective study.

STUDY DESIGN: During the period 2007-2012, 27,648 women, regardless of maternal age (17.4% were 35 years old and over), were included in a late Down syndrome screening program (18^{+0} to 35^{+6} weeks) using the maternal serum markers alpha-fetoprotein and human chorionic gonadotrophin-beta. Samples were assayed in a single laboratory. A dataset of median markers previously established in our laboratory was used for risk calculation. The control group consisted of 27,648 women (14^{+0} to 17^{+6} weeks) randomly selected from the routine database.

RESULTS: When the later screening group was compared with the standard second-trimester control group, the median multiples of medians (1.01 vs 0.98 for alpha-fetoprotein, 1.03 vs 0.98 for human chorionic gonadotrophin-beta), median risks (1 of 2414 vs 1 of 2720), false-positive rates (11.1% vs 11.6%), and trisomy 21 detection rates (83.3% vs 85.7%) did not differ significantly.

CONCLUSION: Late Down syndrome maternal serum screening is feasible with a good sensitivity/specificity compromise throughout gestation and is of clinical value in late-booking women.

Key words: alpha-fetoprotein, human chorionic gonadotrophin, late-booking women, trisomy 21

Cite this article as: Dreux S, Nguyen C, Czerkiewicz I, et al. Down syndrome maternal serum marker screening after 18 weeks of gestation: a countrywide study. Am J Obstet Gynecol 2013;208:397.e1-5.

Down syndrome (DS) screening based on maternal serum markers was initially described at 14^{+0} to 18 weeks of gestation.[1] The efficacy of this screening has been largely demonstrated,[2-7] and in recent years this screening has been focused on the first trimester.[8-10] In France, Down syndrome screening is organized on a national scale and is offered to every pregnant woman.

Maternal serum screening concerns 85% of the 820,000 women pregnant in any given year. The specific regulation for this screening stipulates the sampling period: from 14^{+0} to 17^{+6} weeks for second-trimester screening and, since 2009, from 11^{+0} to 13^{+6} weeks for first-trimester screening. However, pregnant women who have access to prenatal care only later in pregnancy may still wish to undergo this screening.

In France, about 6.6% of pregnant women have their first prenatal visit during the second trimester and 1.2% during the third trimester and therefore do not undergo prenatal screening for DS. Most of these are in socially deprived situations.[11,12]

Programs have been implemented locally to promote early access to prenatal care, but despite these efforts, the number of late-booking women remains high.[13] Using a dataset previously established in our laboratory[14] and adapted to new software, we studied the efficacy of DS screening in a large series of 27,648 late-booking women (after 18 weeks of gestation) to provide solid results for the information given to such women.

Materials and Methods

This retrospective study was conducted in our laboratory using the database of women included in second-trimester DS maternal serum marker screening during the period 2007-2012. Twin pregnancies were excluded. Two groups of women were defined: (1) the late screening group (LS group) of the 27,648 women included in a late screening program (18^{+0} to 35^{+6} weeks of gestation) and (2) a control group of 27,648 women (14^{+0} to 17^{+6} weeks) randomly selected from the routine database. No matching was done to allow comparison of maternal age and other confounding factors in the 2 groups. In the vast majority of controls, pregnancy dating was based on first-trimester ultrasound crown-rump length measurement, but when pregnancy was discovered later, dating was based on the last menstrual period or biparietal diameter measurement.[15]

Parameters taken into account in the risk calculation were recorded in the da-

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The ABA Study Group is listed in the Appendix.

Received Oct. 5, 2012; revised Jan. 8, 2013; accepted Jan. 16, 2013.

The authors report no conflict of interest.

Reprints not available from the authors.

0002-9378/$36.00

http://dx.doi.org/10.1016/j.ajog.2013.01.039

TABLE 1
Demographical description of the database

Demographic	Control group (14^{+0} to 17^{+6} wks) (n = 27,648)	LS group (18^{+0} to 35^{+6} wks) (n = 27,648)
Median gestational age, wks		
14 to 17^{+6} (n = 27,648)	15^{+4}	
18 to 21^{+6} (n = 18,753)		19^{+4}
22 to 25^{+6} (n = 6576)		23^{+2}
26 to 35^{+6} (n = 2319)		28^{+2}
Maternal age, y (median and ranges)	30 (14–51)	28 (13–52)[a]
≥38 years, %	8.9	7.3[b]
Smokers, %	12.4	17.1[a]
Maternal weight, kg (median and ranges)	66 (30–180)	72 (32–163)[a]
AFP MoM (median)	0.98	1.01
hCGβ MoM (median)	0.98	1.03
Risk (1/×) (median)	2720	2414
False-positive rate, %	11.6	11.1
Age <38 years	8.6	8.4
Age ≥38 years	43.4	43.7
Trisomy 21		
Screened-positive, n	36	30
Screened-negative, n	6	6
Detection rate, %	85.7	83.3
Frequency (1/×)	658	762
AFP ≥2.5 MoM, %	1.33	2.0
Neural tube defect	7	10
Ventral wall defect	1	5
Congenital nephrotic syndrome	1	1
PPV (1/×)	41	35

AFP, alpha-fetoprotein; *hCGβ*, human chorionic gonadotrophin-beta; *LS*, late screening; *MoM*, multiples of the median; *PPV*, positive predictive value.

[a] $P < .0001$; [b] $P = .002$.

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tabase: maternal age, maternal weight, and smoking status. Markers were human chorionic gonadotrophin-beta (hCGβ) and alpha-fetoprotein (AFP) (Dualkit; AutoDelfia, PerkinElmer, Turku, Finland). Results were expressed in multiple of median (MoM) corrected for maternal weight and smoking status.

We adapted our published reference values for AFP and hCGβ between 18^{+0} and 35^{+6} weeks (Multicalc Wallac software)[14,16] for LifeCycle software (PerkinElmer, Turku, Finland). The reference medians were checked every trimester. DS risk calculation (LifeCycle) was based on a combination of maternal age and maternal serum markers, with a decision cutoff at 1:250. Pregnancy outcomes were recorded, especially fetal karyotyping for at-risk women or karyotyping at birth if DS was clinically suspected.

In accordance with French law, informed consent for biochemical testing was obtained from each woman prior to blood sampling as part of routine antenatal care. If amniocentesis was performed, a second written consent was needed for fetal karyotyping.

The Mann-Whitney test was used for MoM comparisons and Student *t* test for quantitative variables. The χ^2 test was used for comparison of percentages. $P < .05$ was considered as significant.

RESULTS

Table 1 presents a description of the database. Median gestational age at sampling was 20.4 weeks in the LS group and 15.4 weeks in the control group, a 5 week difference. Median maternal age was 28 years (range, 13–52 years) in the LS group, significantly younger than in the control group ($P < .0001$). The percentage of women smoking during pregnancy was significantly higher in the LS group (17.1% vs 12.4% in the control group, $P < .0001$). The maternal weight was higher in the LS group corresponding to the normal increase in the weight of pregnant women over the 5 week difference.

Table 1 also presents the results of the maternal serum marker screening. Median MoMs, median risks, false-positive rates, and trisomy 21 detection rates did not differ between the 2 groups. Detection rates were 80.7% (21 of 26) in an 18 to 21^{+6} week subgroup (n = 18,741) and 90% (9 of 10) in a 22-35 week subgroup (n = 8907), and false-positive rates were 9.8% and 11.3%, respectively in the subgroups.

When the AFP greater than 2.5 MoM is considered, neural tube defects, ventral wall defects, and nephrotic syndrome can be detected at the same level between the 2 groups.

Trisomy 21-affected pregnancies are presented in Table 2. No significant difference was found between the LS group and the control group.

COMMENT

In France, Down syndrome maternal serum marker screening has been routinely proposed free of charge to all pregnant women younger than 38 years of age since 1997 and to women of any maternal age since 2010.[7] For a good compromise of practicability, standardization, and moderate cost ($50 [US] for the test with risk calculation), the strat-

TABLE 2
Comparison of trisomy 21–affected pregnancies, depending on maternal serum markers screening (cutoff risk 1/250)

Variable	Control group (14^{+0} to 17^{+6} weeks)		LS group (18^{+0} to 35^{+6} weeks)	
Trisomy 21	Screened positive	Screened negative	Screened positive	Screened negative
n	36	6	30	6
Maternal age, y	39 (27-46)	33 (23-42)	38 (31-43)	33 (28-40)
GA at sampling, wks	15.1 (14-17.4)	15.3 (14.3-17.4)	20.2 (18.1-30.3)	19.4 (18-23.6)
AFP MoM	0.64 (0.38-1.78)	0.73 (0.62-1.50)	0.73 (0.34-2.45)	0.97 (0.79-1.31)
hCGβ MoM	2.71 (1.1-9.67)	1.1 (0.77-1.35)	2.93 (1.21-8.43)	1.07 (0.61-2.18)

AFP, alpha-fetoprotein; *GA*, gestational age; *hCGβ*, human chorionic gonadotrophin-beta; *LS*, late screening; *MoM*, multiples of the median.

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egy based on only 2 serum markers has been elected.

In addition, since 2010, first-trimester Down syndrome screening has been routinely proposed instead of second-trimester screening. During these 2 years, the proportion of first-trimester screening has progressively increased from 40% to 60%. Despite the opportunity to have second-trimester screening, 2.8% of the women involved in the 2010 French national perinatal survey declared that they did not have DS maternal serum screening because their first prenatal visit was after 18 weeks of gestation.[12] This rate could be higher in deprived areas. Late-booking women wanting to be screened for DS could thus benefit from a test validated in a large series after 18^{+0} weeks or even during the third trimester.

In this study, based on this large series of 27,648 cases (including 36 with DS) in which screening was performed after 18 weeks of gestation, the observed 83.3% detection rate and 11.1% false-positive rate were similar to the results observed in the standard population (85.7% and 11.6%, respectively). These results are similar to those we observed previously in the routine countrywide DS screening program[7] and in the retrospective selected group of women screened later in pregnancy.[14]

The good detection rate we report must be interpreted in view of the high false-positive rate we observed in our population, which comprised 17.4% of women 35 years old and older. When these older women are excluded, our false-positive rate becomes 6.6% and the detection rate 60%, percentages very similar to the 5% and 60% generally observed. It may be objected that our high detection rate is due to poor knowledge of the total number of DS-affected infants at birth. However, in France, maternal serum DS screening is strictly regulated, with only 84 laboratories authorized to perform the screening tests, and only 73 cytogenetic laboratories authorized for fetal karyotyping. In addition, the results of karyotyping for at-risk patients and outcome (trisomy 21 or not) of pregnancies for the not-at-risk patients must be collected because an annual report is mandatory to keep the authorization. Two different sources of data (maternity units and cytogenetic laboratories) allowed us to check the total number of DS cases.

It may be argued that late-booking women do not benefit from early gestational age determination by a first-trimester ultrasound examination and therefore that gestational age is not firmly established. However, whereas determination of gestational age is of major importance for marker screening between 11 and 18 weeks because of the steep slopes of changes in markers over this period of pregnancy, after 18 weeks, AFP and hCGβ levels follow a gentle slope before reaching a plateau. The impact of a precise determination of gestational age at sampling is therefore less in late DS risk calculation.

Altogether, women concerned by late screening are younger, often smoke (17.1%), and are probably less careful about their health. Because socioeconomic factors are very difficult to retrieve and to score, we did not analyze them and this study was focused on overall observation of Down syndrome screening. As reported by others, a late first prenatal visit and late screening are associated with a poor socioeconomic status.[17]

The trisomy 21 frequency observed in the LS group (1 of 762) was paradoxically not significantly different from that observed in the control group (1 of 658), whereas the LS group comprised younger women with therefore a lower age-related risk of trisomy 21. In addition, at late gestational age, fetuses with an abnormal karyotype are less numerous because of in utero fetal death and spontaneous miscarriage. This frequency can perhaps be explained by a small bias in the recruitment of the women of the LS group.

These late-booking women did not in most cases undergo any previous screening, and the discovery at ultrasound examination of an isolated minor sonographic sign suggestive of aneuploidy such as pyelectasis, hyperechoic bowel, short femur, echogenic intracardiac focus, or plexus choroid cysts[18,19] would have led to the inclusion of these women in the late maternal serum screening program, thus increasing the number of detected trisomy 21 cases.

In the LS group, the efficacy of DS screening can be related to the hCGβ values. Maternal age is probably not involved because the median maternal age in the LS group was lower (28 years) than in the control group (30 years). AFP was

not involved in efficacy because the median MoM was higher in the LS group than in the control group. It has been known for years that during the second trimester, the most efficient biological marker is hCG or its free fraction β, with a mean median MoM of 1.93 and 2.24, respectively.[20] The value we observed here, 2.93 MoM, confirms the major role of hCGβ in maternal serum marker screening efficiency.

The pathophysiological relation between fetal DS and high levels of hCGβ could explain the high detection rate of this screening. Human chorionic gonadotrophin is a highly glycosylated (30%) hormone produced by the trophoblast. In primary culture of human cytotrophoblasts isolated from control placentas (n = 44) and trisomy 21 placentas (n = 71), abnormal fusion occurs in more than 90% of the cells. This defect is associated with a dramatic decrease in the synthesis and secretion of hCG.[21] In addition. this hCG is abnormally glycosylated and is weakly bioactive.[22] This abnormally low level of placenta synthesis contrasts with the abnormally high level observed in maternal serum.

The increased maternal hCG levels observed in trisomy 21-affected pregnancies might be related to different abnormalities such as a decreased number (or an abnormality) of hCG receptors in affected placentas or a decrease in binding and/or internalization of this weakly bioactive hCG.[21-23] These anomalies will lead to abnormal placental hCG clearance in the maternal compartment.[22]

In trisomy 21-affected pregnancies, hCG progressively accumulates in the maternal serum from an early stage of gestation, leading to a 1.93 hCGβ median MoM during the first trimester,[24] a 2.24 MoM during the second trimester,[16] and the 2.93 MoM observed in the present study during the second part of the second trimester and the third trimester.

In addition, maternal serum AFP can be used in the late screening group for neural tube defects, ventral wall defects, and nephrotic syndrome screening with a good positive predictive value (1 of 35) as observed in the control group (1 of 41).

Recently, noninvasive prenatal detection of fetal aneuploidies has been achieved by exploitation of the presence of cell-free deoxyribonucleic acid in maternal plasma, based on massive parallel shotgun sequencing.[25-27] With this approach, trisomy 21 has been detected successfully noninvasively. Clinical studies have primarily included women identified by prior screening to be at high risk for aneuploidies, so the at-risk late-booking patients could benefit from this new noninvasive strategy.

In conclusion, late maternal serum screening is feasible with a good sensitivity/specificity compromise throughout gestation and may be of clinical value in late-booking women. ■

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APPENDIX
ABA study group

This is an association of the French laboratories authorized by the Ministry of Health to carry out biochemical Down syndrome screening: Albi (C. Gassier, MB Bleunven); Amiens Centre Hospitalier Universitaire (F. Boitte, M. Brazier); Amiens Vallée des Vignes (L. Maille, S. Jutard, A. Jean); Angers Centre Hospitalier Universitaire (V. Moal, H. Puissant); Annecy (P. Lorenter, M. Jouval); Argenteuil Centre Hospitalier (B. Sitruk-Khalfon); Arras Centre Hospitalier (A. Gruson, S. Verchain); Avignon (V. Gras, T. Roudon); Bayonne (D. Savarit, P. Blouin), Belfort-Montbéliard Centre Hospitalier (M. Laplace); Béziers Labosud Ocbiologie (J. Y. Réal, J. M. Réal, P. Dumas); Blanc-Mesnil (P. Clément, C. Frainais, M. L. Maurin); Bordeaux-Pessac Centre Hospitalier Universitaire (A. Georges, J. Brossaud); Bordeaux Bioffice (I. Fischer); Brest Centre Hospitalier Universitaire (M. P. Moineau, H. Kerspern); Caen Centre Hospitalier Universitaire (M. H. Read, A. Hamel, V. Aze, D. Guenet, M. L. 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