Software for Prenatal Down Syndrome Risk Calculation: A Comparative Study of Six Software Packages, 
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Prenatal diagnosis of Down syndrome is based on fetal karyotyping, but amniocentesis cannot be performed in all patients because of the risk of fetal loss and the cost. It, therefore, is usually applied only to high-risk (and generally older) patients. Noninvasive assays of maternal serum markers have allowed the extension of screening to mothers of all ages. α-Fetoprotein (AFP), human chorionic gonadotropin (hCG) or free β-hCG, and unconjugated estriol have been prospectively evaluated during the second trimester in large populations (1–11). Wald et al. (12) proposed an individual risk calculation for Down syndrome, combining maternal age, maternal serum markers, and gestational age, in which amniocentesis was proposed when the risk was above a cutoff leading to a 60% Down syndrome detection rate and a 5% amniocentesis rate. Software is necessary for risk calculation and should be routinely validated.

To evaluate the influence of software design on risk calculation, we compared six software packages (Prenatal Interpretive Software [Maciel Inc., distributed in France by Abbott], Prisca [DPC France], DIANASoft [BioChem ImmunoSystèmes France], T21 [Chiron], PrenatScreen [CIS bio international], and MultiCalc [Wallac]) in two populations: 529 control patients (ages 18–37 years) selected randomly from 90,000 patients screened in our laboratory, in accordance with the national maternal age structure of pregnant patients in France (Institut National des Statistiques et des Etudes Economiques, 1993), and all 125 Down syndrome-affected pregnancies (patient ages, 20–37 years). AFP (SFRI) and total hCG (13) were expressed in multiples of the median (MoM) for five software packages and in IU/L corresponding to the manufacturer’s MoM for the Abbott package. Gestational age was determined in 99% of cases from ultrasonographic crown-rump length. The cutoff defining the at-risk group was 1 in 250 for all software packages and was calculated at term and not at sampling. The efficacy of the screening tests was evaluated by determining the number of true positives [detection rate for Down syndrome in the Down syndrome-affected population (sensitivity)], and false-positive rate [number of patients above the cutoff in the control population (specificity)].

Risk calculation for Down syndrome combines at least two factors in all cases: the risk of Down syndrome related to maternal age and the risk indicated by maternal serum markers. The maternal age-related risk at term used in each of the six software packages is given in Fig. 1. Although all six software packages yielded a similar risk, substantial variation was apparent, particularly for patients 37 years (1 in 215 to 1 in 273) and 38 years (1 in 167 to 1 in 214) of age.

The percentages of control and Down syndrome-affected pregnancies included in the at-risk group are shown in Table 1. The percentage of cases in which amniocentesis was performed was 2.4–6.8%, and the Down syndrome detection rate was 54.4–66.4%. Software packages that yielded the lowest amniocentesis rate also gave the lowest detection rate, and differences in sensitivity and specificity between the two least sensitive software packages and the four others were significant (P < 0.01). For all software packages, the proportion of patients at risk depended on maternal age. The main differences were observed for patients < 30, for whom the detection rate was 20–40% and the amniocentesis rate was 1.2–3.1%.

To define how these variations influence individual risk estimates, we determined the number of patients below or above the cutoff as a function of the software. In the control population, all six software packages classified 491 (93%) of the 529 patients in the not-at-risk group and 5 (0.9%) in the at-risk group. In the fetal Down syndrome population, 63 of the 125 cases (50.4%) were detected by all six software packages, and 38 cases (30.4%) were detected by none (data not shown). Discordances were therefore observed in 6% of cases in the control population and in a striking 20% of Down syndrome cases.

The cause of these differences can be analyzed by comparing the likelihood ratio, i.e., the ratio of prior risk (age-specific) to final risk. The percentage of patients with a likelihood ratio greater than an arbitrary value of 1.5 varied from 10% to 20% in the control population and from 62% to 73% in the Down syndrome population (data not shown). These large variations demonstrated that factors other than maternal age are responsible for these differences.

Down syndrome risk factor is derived from two statistical functions (14): the age-related risk and a risk derived from the biochemical indices. In all mathematical models tested here, the risk of Down syndrome related to maternal age is taken into account, according to published values (15–18). However, we observed that for a patient 37 years of age, the risk ranged from 1 in 215 to 1 in 273, thereby changing the decision concerning amniocentesis if the cutoff is 1 in 250. These differences could be minimized by considering maternal age in 1-month and not 1-year intervals (19).

The likelihood ratio method (14) is used in all six software packages tested. However, the detection rate and false-positive rate were markedly different. The relative weight given to hCG and AFP and to maternal age can explain these variations. We tested the weight of each biochemical marker by varying hCG from 1 MoM to 2.5 MoM and AFP from 1 MoM to 0.5 MoM when maternal age, gestational age, weight, and ethnic background were fixed. When hCG was within the reference interval (1 MoM), four software packages (MultiCalc, PrenatScreen, Prenatal Interpretive Software, and T21) gave the same weight to AFP and two software packages (DIANASoft
and Prisca) gave a lower weight to AFP. However, a low value for AFP (0.5 MoM) did not assign the patient to the risk group, the risk varying from 1 in 559 to 1 in 800. When hCG was at 2.5 MoM, and AFP was within the reference interval (1 MoM), this high value of hCG was not sufficient for all six software packages to place the patient above the cutoff, the risk varying from 1 in 260 to 1 in 375. To place the patient at a risk of 1 in 250, the necessary AFP value was 0.7 MoM for PrenatScreen, 0.78 MoM for MultiCalc, and 0.95 MoM for the four others. Therefore, the relative weight given to markers depends on the software and markedly affects the risk estimation.

The weight of maternal age in risk calculation was analyzed for a patient with AFP at 0.80 MoM and hCG at 2.2 MoM and a maternal age of 20–38 years. For all software packages, the risk progressively increased with maternal age, but large variations were observed. For example, at 20 years, the risk varied from 1 in 710 (PrenatScreen) to 1 in 378 (DIANASoft and Prisca). Depending on the software, a patient with these marker values would be in the at-risk population at 30 or 34 years.

Another important factor is the choice of marker distribution parameters used in the statistical model. Parameter sets used in software are calculated from relatively few Down syndrome pregnancies and usually are based on women from a single center. Cuckle (20) published variances and covariances obtained by metaanalysis of all studies published to 1995. If all software packages were to use these parameters, differences will be diminished.

Biochemical markers are gestational age-dependent, and the results are expressed in MoM. This supposes that median values are well defined in large populations and that gestational age is given with a high degree of precision (21).

In addition to these main factors, other parameters can be taken into account by the software: choice and number of markers, whether values are corrected for maternal weight, smoking, ethnic background, or diabetes. Risk may be calculated at term or during the second trimester. The fetal death rate in Down syndrome between 15 weeks and term has been estimated as 18–23% (16, 22), but this rate is not always taken into account (Chiron T21). Risk estimation also depends on the extent to which ultrasound is used to estimate gestational age.

Laboratory-related differences must be added to the discrepancies attributable to software. This can lead to great variation in individual risk: Cavalli (23) observed a risk of 1 in 502 and 1 in 80 for the same patient with a Down syndrome-affected pregnancy. The notion of equality between patients, therefore, is clearly flawed.

In conclusion, this study demonstrates that with the same maternal serum markers, variations are observed between software packages, with a mean detection rate of 54.4–66.4% and a false-positive rate of 2.4–6.8%. In practice, in a population of 100 000 patients, including 143 cases of Down syndrome, the least sensitive software will detect 78 cases of Down syndrome through 2400 amniocenteses, whereas the most sensitive will detect 95 cases through 6800 amniocenteses. These differences will have an impact on public health policy (24) and should be minimized. This may be achieved in different ways: use of the same maternal age-related risk, definition for each country of the risk at term or at sampling, use of daily medians, and use of the parameter sets defined by Cuckle.

Table 1. Number of control patients (529 cases) and Down syndrome cases included in the at-risk group (cutoff, 1 in 250).

<table>
<thead>
<tr>
<th></th>
<th>Abbott Prenatal Soft</th>
<th>DPC Prisca</th>
<th>BioChem DIANASoft</th>
<th>Chiron T21</th>
<th>CIS bio PrenatScreen</th>
<th>Wallac MultiCalc</th>
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<td><strong>Control cases</strong></td>
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<tr>
<td>Patients &lt;30 years (n = 321)</td>
<td>10 (3.1%)</td>
<td>7 (2.2%)</td>
<td>7 (2.2%)</td>
<td>6 (1.8%)</td>
<td>6 (1.87%)</td>
<td>4 (1.2%)</td>
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<td>Patients 30–34 years (n = 163)</td>
<td>13 (7.9%)</td>
<td>7 (4.3%)</td>
<td>7 (4.3%)</td>
<td>5 (3.0%)</td>
<td>4 (2.45%)</td>
<td>2 (1.2%)</td>
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<td>Patients 35–37 years (n = 45)</td>
<td>13 (28.8%)</td>
<td>9 (20%)</td>
<td>9 (20%)</td>
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<td>9 (20%)</td>
<td>7 (15.5%)</td>
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<tr>
<td>Total (%) (n = 529)</td>
<td>36 (6.8%)</td>
<td>23 (4.3%)</td>
<td>23 (4.3%)</td>
<td>20 (3.8%)</td>
<td>19 (3.6%)</td>
<td>13 (2.4%)</td>
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<td><strong>Down syndrome cases</strong></td>
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<tr>
<td>Patients &lt;30 years (n = 25)</td>
<td>10 (40%)</td>
<td>9 (36%)</td>
<td>9 (36%)</td>
<td>8 (32%)</td>
<td>7 (28%)</td>
<td>5 (20%)</td>
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<td>Patients 30–34 years (n = 45)</td>
<td>29 (64.4%)</td>
<td>29 (64.4%)</td>
<td>29 (64.4%)</td>
<td>29 (64.4%)</td>
<td>27 (60%)</td>
<td>26 (57.7%)</td>
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<td>Patients 35–37 years (n = 55)</td>
<td>44 (80%)</td>
<td>39 (70.9%)</td>
<td>39 (70.9%)</td>
<td>40 (72.7%)</td>
<td>37 (67.2%)</td>
<td>37 (67.3%)</td>
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<tr>
<td>Total (%) (n = 125)</td>
<td>83 (66.4%)</td>
<td>77 (61.6%)</td>
<td>77 (61.6%)</td>
<td>77 (61.6%)</td>
<td>71 (56.8%)</td>
<td>68 (54.4%)</td>
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</table>
References

Day-to-Day, Postprandial, and Orthostatic Variation of Total Plasma Homocysteine, Poul Thirup1 and Suzanne Ekelund2 (1 Department of Clinical Biochemistry, Hvidovre Hospital, DK-2650 Hvidovre, Denmark, and 2 Department of Clinical Biochemistry, Aalborg Hospital, DK-9100 Aalborg, Denmark; * author for correspondence: fax 45 3675 0977, e-mail hcy@forum.dk)

Increased concentrations of the amino acid homocysteine—hyperhomocysteinemia—are correlated with atherosclerotic and thrombotic diseases (1, 2). High concentrations can be lowered by diet and dietary supplements with vitamins B12, folate, and pyridoxine. Homocysteine is produced in the metabolism of the essential amino acid methionine and is converted by cystathionine β-synthase to cystathionine and by methionine synthase back to methionine. These enzymatic reactions are dependent on sufficient concentrations of the vitamins B12, folate, and pyridoxine.

Knowledge of biological, postprandial, and orthostatic variations are important in judging significant changes in results and error sources in blood sampling conditions (3), and several studies on the biological variation of plasma homocysteine have been published (4–6).

Fasting blood samples traditionally have been recommended for plasma homocysteine measurement because postprandial changes produce a modest decrease in the first hours and an increase after 8 h (7, 8).

Orthostatic changes can also be important in the monitoring of homocysteine in patients with atherosclerotic and thrombotic diseases. Because most homocysteine is bound to albumin, the decrease with supine posture is expected to be 5–10%.

In this study we examined the day-to-day, postprandial, and orthostatic variations of plasma total homocysteine.

Blood samples were obtained from 19 healthy hospital employees (11 women and 8 men) ages 19–60 years (median age, 44 years). None of these individuals was or became pregnant or had medical diseases. The intake of oral contraceptives, intermittent asthma and allergy medicines, nonsteroidal antiinflammatory drugs, acetylsalicylic acid, and multivitamins was allowed during the study. Multivitamins were taken on a regular basis by four persons. Blood hemoglobin, erythrocyte folate, and serum cobalamin concentrations in all subjects were within the reference intervals.

Participants provided fasting blood samples after arrival for work (0800 to 1000) and nonfasting samples after lunch (1215 to 1500). Samples were collected daily for 5 days from subjects in an upright position. In addition, samples for plasma homocysteine, serum albumin, and