2. Hoffman RS, Smilkstein MJ, Howland MA, Goldfrank LR. Osmol gaps revisited: normal values and limitations. J Toxicol Clin Toxicol 1993;31:81–93.
3. Glaser DS. Utility of the serum osmol gap in the diagnosis of methanol or ethylene glycol ingestion. Ann Emerg Med 1996;27:343–6.
4. Krahn J, Khajuria A. Osmolality gaps: diagnostic accuracy and long-term variability. Clin Chem 2006;52:737–9.
5. Rasouli M, Kalantari KR. Comparison of methods for calculating serum osmolality: multivariate linear regression analysis. Clin Chem Lab Med 2005;43:635–40.

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First- and Second-Trimester ADAM12s in Down Syndrome Screening

To the Editor:

ADAM12s[1] (a disintegrin and metalloprotease 12s), a placenta-derived glycoprotein involved in tissue growth and differentiation, has been shown to be an early screening marker for trisomy 21 before the 10th week of gestation *(1)* and a potential second-trimester marker. A recent report suggested that measurements of a serum marker in both the first and second trimesters (repeated measures) could provide better performance than single measurements in either trimester *(2)*.

We sought to demonstrate the potential value of such repeated measures of ADAM12s for the screening of Down syndrome.

A cohort of 7194 women underwent integrated biochemical screening before amniocentesis at the Department of Obstetrics and Gynecology, University of Messina, between December 1, 2006, and March 31, 2009. After collection, the maternal serum samples were stored at −80 °C. We selected paired first- and second-trimester samples from 19 Down syndrome pregnancies (cases) from frozen storage for ADAM12s measurement. Of the Down syndrome cases, 14 were identified from amniocentesis results, and 5 were identified at birth. Two of the latter were excluded owing to maternal smoking and the unknown potential effects of smoking on maternal ADAM12s values in serum. ADAM12s also was measured in frozen samples from 562 unaffected pregnancies matched for gestational age (GA).

GAs ranged between 7 and 10 completed weeks in the first trimester and between 14 and 17 completed weeks in the second.

All the women in the study were Caucasian. Although the patients in the Down syndrome cases had a higher mean maternal age than the unaffected pregnancies (35.3 years vs 30.4 years), previous studies have shown that ADAM12s concentration is unrelated to maternal age *(3)*.

Median maternal weights (cases, 62.6 kg; unaffected pregnancies, 63.4 kg) and GAs in the first trimester (cases, 64 days; unaffected, 65 days) and the second trimester (cases, 112 days; unaffected, 111 days) were comparable.

ADAM12s was measured in 25 μL of serum with a time-resolved fluorescence immunoassay DELFIA assay kit (PerkinElmer Life and Analytical Sciences). This method is a solid-phase assay in which 2 monoclonal antibodies (6E6 and 8F8) are directed against 2 separate antigenic determinants on the ADAM12 molecule. Concentrations were expressed as multiples of the median (MoM) for unaffected pregnancies of the same GA by means of 2 regression equations derived from the first- or second-trimester control groups. QC samples derived from pooled serum stored at −80 °C had ADAM12s concentrations of 86.5, 415.8, and 917.5 ng/L and were measured in duplicate at the beginning and at the end of each run. The mean CVs were 4.9%, 2.9%, and 3.5%, respectively.

Analyse-it (Analyse-it Software) and SPSS (version 14; SPSS) software were used for statistical analysis. In particular, the detection rate (DR) at a 5% false-positive rate (FPR) and the likelihood ratio of a positive were computed with the Analyse-it program. The same results were also calculated with the integrated model described by Royston and Thompson *(4)* with the model parameters (mean and SD) for the first and the second trimesters and taking into account the correlation coefficients between trimesters for ADAM12s in Down syndrome and unaffected pregnancies.

The weighted log-linear regression equations for the first and second trimesters in the unaffected group were, respectively:

$$\text{ADAM12s}_{\text{first}} = 10^{0.0269 \cdot \text{GA} + 0.613}$$

and

$$\text{ADAM12s}_{\text{second}} = 10^{0.0108 \cdot \text{GA} + 1.72},$$

where GA is expressed in days. The ADAM12s MoM values were re-

[1] Nonstandard abbreviations: ADAM12s, a disintegrin and metalloprotease 12s; GA, gestational age; MoM, multiples of the median; DR, detection rate; FPR, false-positive rate.

Table 1. Maternal serum ADAM12s values in 17 singleton pregnancies with Down syndrome and in 562 unaffected pregnancies.[a]

Pregnancy	Marker	Mean (SD)	DR (95% CI), %
Unaffected	A12_1T	0.0135 (0.1919)	
	A12_2T	−0.0041 (0.1473)	
	A12 ratio	−0.0176 (0.1843)	
Down syndrome	A12_1T	−0.1562 (0.2517)	41.2 (18.4–67.1)
	A12_2T	0.1269 (0.2154)	35.3 (14.2–61.7)
	A12 ratio	0.2831 (0.1451)	52.9 (27.8–77.0)

[a] ADAM12s results are expressed as log MoM values in the first trimester (A12_1T) and the second trimester (A12_2T) and as the log ratio of MoM ADAM12s in the second trimester to MoM ADAM12s in the first trimester (A12 ratio). DR values are given at a 5% FPR.

lated to maternal weight and were corrected with a log-linear equation:

$$\mathrm{MoM}_{\mathrm{corrected}} = \frac{\mathrm{MoM}_{\mathrm{measured}}}{10^{0.48 - 0.00741 \cdot W}},$$

where W is body weight in kilograms. The log MoM values are displayed in Table 1. The geometric mean for ADAM12s MoM in the first-trimester cases was 0.654 at a median GA of 9.1 days, significantly lower than in the unaffected group (MoM, 1.003; $P = 0.014$). The geometric mean for the second-trimester ADAM12s MoM for the cases was 1.361, significantly higher than in the unaffected group (MoM, 1.000; $P = 0.024$). The ratio of the second-trimester MoM to the first-trimester MoM for the cases was 1.883, significantly higher than for the unaffected group (MoM, 0.985; $P < 0.0001$).

The between-trimester ADAM-12s correlation coefficient was 0.430 for the unaffected pregnancies and 0.591 for the cases.

At an FPR of 5.0%, the DR was 42.1% in the first trimester and 35.3% in the second trimester; the DR for the ratio of the second trimester value to the first trimester value was 52.9%. In the same cases, a biochemical integrated test (pregnancy-associated plasma protein A in the first trimester; α-fetoprotein, unconjugated estriol, and human chorionic gonadotropin in the second trimester) gave a DR of 70.6% at a 5.0% FPR. These DRs were lower than the expected rate of 90% reported in the literature *(5)*, likely owing to the small number of cases considered and the number of positive pregnancies with spontaneous miscarriage before second-trimester sampling.

Adding the ADAM12s ratio to the serum integrated test increased the DR by about 6%. This performance was obtained with samples at very early GAs (7–10 weeks), whereas at 11–13 weeks, the more commonly tested first-trimester period, the use of ADAM12s was questionable *(6)*.

The present data confirm that the ADAM12s concentration is relatively low in Down syndrome cases before 10 weeks of gestation and increases appreciably in the second trimester. This pattern is similar to that for pregnancy-associated plasma protein A in Down syndrome, and it constitutes the basis for the use of ADAM12s across trimesters in prenatal screening.

Despite the small number of cases, our data suggest that use of the ratio of second-trimester to first-trimester MoM values for ADAM12s could provide better performance than single MoM measurements in either trimester alone. Furthermore, ADAM12s may be useful in combination with other markers in the current integrated biochemical test. Further studies are needed to confirm these first limited results.

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References

1. Spencer K, Vereecken A, Cowans NJ. Maternal serum ADAM12s as a potential marker of trisomy 21 prior to 10 weeks of gestation. Prenat Diagn 2008;28:209–11.
2. Wright DE, Bradbury I. Repeated measures screening for Down's syndrome. BJOG 2005;112:80–3.
3. Laigaard J, Sørensen T, Fröhlich C, Pedersen BN, Christiansen M, Schiøtt K, et al. ADAM12: a novel first-trimester maternal serum marker for Down syndrome. Prenat Diagn 2003;23:1086–91.
4. Royston P, Thompson SG. Model-based screening by risk with application to Down's syndrome. Stat Med 1992;11:257–68.
5. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). J Med Screen 2003;10:56–104.
6. Poon LC, Chelemen T, Minekawa R, Frisova V, Nicolaides KH. Maternal serum ADAM12 (a disintegrin and metalloprotease) in chromosomally abnormal pregnancy at 11–13 weeks. Am J Obstet Gynecol 2009;200:508.e1–6.

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The Effect of Sample Hemolysis on Cardiac Troponin I and T Assays

To the Editor:

Cardiac troponin I and T (cTnI and cTnT)[1] assays are used for the diagnosis of acute myocardial infarction (AMI) *(1)*. Recent improvements in these assays have lowered the imprecision and detection limit so that the assays meet guidelines for using the 99th percentile cutoff concentration. These new cardiac troponin assays have the potential to improve clinical practice by earlier diagnosis, improved risk stratification, and improved monitoring of patients. They also, however, increase the number of true- and false-positive results for patients suspected of AMI *(2)*. Hemolysis is the most common preanalytical interference encountered in the routine laboratory. I tested the effect of sample hemolysis on 1 contemporary and 1 high-sensitivity (hs) cardiac troponin assay.

I prepared hemolysate from erythrocytes that had been washed 3 times with 0.9% saline. After the final centrifugation, the cells were diluted with an equal volume of distilled water, thoroughly mixed, and lysed by freezing. I measured cTnI using the Ortho Clinical Diagnostics TnI ES assay (contemporary) on the Vitros® 5600 Integrated System and cTnT using the Roche TnT hs assay on the Elecsys E170 immunoassay system, which was part of a Modular integrated system. To measure the effect of hemolysate on cTnI and hs cTnT, thawed hemolysate was added to lithium heparin plasma samples that contained concentrations of cardiac troponin selected to be around the 99th percentile cutoffs for the respective assays, namely 34 ng/L for cTnI (24, 36, 49 ng/L) and 13 ng/L for cTnT (6, 12, 23 ng/L). Indices were measured as recommended by the manufacturers. The Vitros 5600 measures the indices using the residual sample left in the sample tip. The Modular measures indices on the chemistry module by taking an aliquot of the patient specimen and diluting it in 0.9% NaCl. For both instruments, algorithms convert the absorbance measured at wavelength pairs into qualitative values that correlate with estimated concentrations of the sample interferent.

According to the recommendations of the National Academy of Clinical Biochemistry, a 20% change in cardiac troponin value is suggestive of an acute myocardial infarction that is either evolving (cardiac troponin increasing) or resolving (cardiac troponin decreasing) *(3)*. For both assays, a hemolysis index of around 150 caused a >20% change in cTn (Fig. 1), which equates to a hemoglobin concentration of 1.9 g/L. It has been suggested that at baseline concentrations of cTn, δ changes of >20% are needed for improved clinical specificity and, thus, laboratories must consider carefully what constitutes a clinically significant change in cardiac troponin *(4)*.

One important aspect of these experiments is that they were carried out at cardiac troponin concentrations close to the 99th percentile for each assay. When the same experiments were done at higher cardiac troponin concentrations, a clinically significant effect (for example, ±20%) was not observed. This is understandable because a change of 10 ng/L at a concentration of 10 ng/L represents a 100% change, whereas at 100 ng/L it represents a 10% change, demonstrating the need to do these types of experiments at critical concentrations for any analyte. Many reagent package inserts contain limited information on interferences and often only on what concentration of interfering material interferes with the assay, with no information on what concentrations of analyte were tested. If the analyte concentration was relatively high, the effect of the interfering material may not be observed. The hs cTnT brochure states that samples are unaffected by Hb <0.1 g/dL, and samples showing visible signs of hemolysis may be interfered. There is no indication of what concentrations of cTnT were tested. In the cTnI brochure, there is a table indicating the effect of increasing sample Hb, but this was tested at 0.006 μg/L, which is half the stated limit of detection for the assay.

Hemolysis has been reported to be as high as 8.8% for samples collected in an emergency department *(5)*. In my own hospital, the number of cTnT requests from the emergency department rejected because of hemolysis interference

[1] Nonstandard abbreviations: cTnI and cTnT, cardiac troponin I and T; AMI, acute myocardial infarction; hs, high sensitivity.