particles, such as red blood cell fragments. Thus, the LBC count on the ADVIA 120 was derived as the sum of all platelet-sized particles measured in the PLT channel (calculated LBC). In contrast, the CBC channels on the Sysmex XE-2100 and the Coulter Gen-S use impedance technology. The Coulter method (conventional impedance) counts particles by detecting changes in electrical resistance when a particle in a conductive liquid goes through a small aperture (17). The size of the electrical pulse generated is proportional to the particle volume. Platelets are identified based on their volume (2–20 fL). The Sysmex technology is different from the Coulter method in that it simultaneously detects conventional (direct current) and radiofrequency impedance (18). The latter is thought to reflect intracellular changes. The Cell-dyn 3500 combines optical scatter and impedance to increase the accuracy of particle counting (19).

The hematology analyzers evaluated in this study produced accurate and precise platelet counts in reference populations (20) and thrombocytopenic patients (21) despite measuring different physical properties. However, our data indicate that the concordance among instruments for enumerating lamellar bodies is poor. For example, eight ADVIA 120 and five Sysmex XE-2100 LBCs indicated intermediate FLM when the Coulter Gen-S LBC indicated maturity ($\geq$50 000/μL), and seven Cell-dyn 3500 LBCs indicated mature fetal lungs when the Coulter Gen-S LBC indicated intermediate FLM (15 000–50 000/μL). Clearly, applying the Coulter LBC cutoff values for FLM to other brands of hematology analyzers could change the positive or negative predictive value of the measurement (depending on instrumental bias), either of which may have adverse clinical consequences.

In summary, it is clear that different hematology analyzers count lamellar bodies differently. It will be necessary to establish analyzer-specific LBC clinical decision limits that are confirmed by outcome-based studies.

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S100B Protein Concentrations in Amniotic Fluid Are Higher in Monoamniotic than in Diamniotic Twins and Singleton Pregnancies,

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S100B is an acidic calcium-binding protein of the EF-hand family present in the central nervous system, where it is located mainly in glial cells (1). It has been suggested that the protein is involved in various cellular functions, but precisely which is still a matter of debate. The protein has been found to act at physiologic concentrations as a cytokine with a neurotrophic role in experimental models, in cell cultures, and in biological fluids such as cord blood, peripheral blood, and urine (1–4). This hypothesis has been corroborated by measurements of S100B protein

in amniotic fluid in the second trimester of pregnancy (5). The present work, following from an earlier study, investigates amniotic fluid S100B concentrations in twins.

We performed a case-control study (between January 1998 and June 2002) of 49 women with physiologic twin pregnancies (27 monoamniotic and 22 diamniotic) who underwent amniocentesis to exclude chromosomal abnormalities between the 15th and 18th weeks of gestation (mean, 16.5 weeks). The control group consisted of 490 singleton pregnancies matched for gestational age and weight at sampling and normal neonatal outcome (5 control fetuses for each twin fetus). Appropriate fetal growth was defined by the presence of ultrasonographic signs (when biparietal diameter and abdominal circumference were between the 10th and 90th percentiles) according to the normograms of Campbell and Thoms (6) and by postnatal confirmation of a birth weight between the 10th and 90th percentiles according to our population standards after correction for maternal height, weight, and parity and the sex of the newborns. Exclusion criteria included intrauterine growth retardation; gestational hypertension; diabetes and infections; fetal malformations; chromosomal abnormalities; maternal exposure to alcohol, cocaine, or smoke; perinatal asphyxia; and dystocia.

The local ethics committee approved the study protocol, and the parents of the fetuses examined gave signed and informed consent.

At the indicated times (15th–18th weeks of gestation), we collected 500 μL of amniotic fluid from the amniotic cavity and immediately centrifuged it at 900g for 10 min; the supernatants were then stored at $-70\ °C$ until measurement. The S100B protein concentration was measured in all samples with use of a commercially available immunoluminometric assay (Lia-mat Sangtec 100; AB Sangtec Medical, Bromma, Sweden). According to the manufacturer's instructions, this assay distinguishes between the A1 and B subunits of the S100 protein and measures the β-subunit as detected by the three monoclonal antibodies SMST 12, SMSK 25, and SMSK 28. The β-subunit of the S100 protein is known to be predominant (80–96%) in the human brain (7, 8). Each measurement was performed in duplicate according to the manufacturer's recommendations, and the averages were reported. As indicated by the manufacturer, the limit of detection of the assay ($B_0 + 3\ SD$) was 0.02 μg/L, and the within- and between-assay imprecision (CV) was $\leq$5.5% and $\leq$10%, respectively, for concentrations of 0.28–4.17 μg/L.

Data are expressed as mean (SD). The amniotic fluid S100B concentrations and neonatal characteristics were analyzed by Kruskal–Wallis one-way ANOVA and Mann–Whitney two-sided U-test when the data did not follow a gaussian distribution. Comparisons between proportions were performed with use of the Fisher exact test. The correlation between the concentrations of S100B in amniotic fluid and weeks of gestation was analyzed by linear regression analysis. A P value <0.05 was considered significant.

At birth all of the newborns showed normal clinical conditions, and no overt neurologic injuries were observed on discharge from the hospital. As expected, mean (SD) gestational age [35 (2) vs 38 (1) weeks] and weight at birth [2127 (231) vs 2936 (342) g] differed (P <0.05 for both) between twins (both monoamniotic and diamniotic) and control groups. There were no differences in Apgar scores at 1 and 5 min, and the incidences of caesarian section, respiratory distress syndrome, and neurologic abnormalities were superimposable in the two groups studied (P >0.05 for all). There were no significant differences in the groups of twins regarding mode of delivery, gestational age and weight at birth, or Apgar scores evaluated at the 1st and 5th min when the data were corrected for monoamniotic (group A) and diamniotic (group B) twins.

At sampling, all fetuses monitored appeared to meet ultrasound scanning parameters for appropriate growth. We observed no differences between controls and twins for gestational age [controls, 16.3 (1) weeks; twins, 16.4 (1) weeks; P >0.05], fetal weight [controls, 179 (22) g; twins, 184 (28) g; P >0.05], head circumference [controls, 131 (12) mm; twins, 133 (9) mm; P >0.05], biparietal diameter [controls, 114 (10) mm; twins, 116 (8) mm; P >0.05], or transverse cerebellum diameter [controls, 15.4 (0.7) mm; twins, 15.6 (0.9) mm; P >0.05]. Similarly, we found no significant differences in these parameters between group A and group B twins (P >0.05 for all).

S100B concentrations in amniotic fluid were higher in the twins [0.62 (0.46) μg/L] than in the controls [0.53 (0.22) μg/L; P <0.001]. Similarly, S100B concentrations in amniotic fluid were significantly higher in group A twins [1.06 (0.21) μg/L] than in group B [0.51 (0.25) μg/L] or the controls [0.53 (0.22) μg/L; P <0.001 for all; Fig. 1A]. We found no statistical difference between group B twins and controls (P >0.05).

We found significant correlations between S100B in amniotic fluid and gestational age in both monoamniotic/diamniotic twins (r = 0.56 and 0.52, respectively; Fig. 1B) and controls (r = 0.23; P <0.001 for all)

Our results for the correlation between gestational age at sampling and S100B protein concentrations fit previous observations, offering additional support for normality reference curves in uncomplicated singleton and multiple pregnancies. The results for S100B concentrations in the amniotic fluid of controls also offer support for previously reported S100B values in the amniotic fluid of pregnant women with uncomplicated pregnancies and for the correlation between the protein concentrations and gestational age at sampling (5).

Our findings showing a higher (approximately double) concentration of S100B in amniotic fluid in physiologic monoamniotic pregnancies than in diamniotic twin or singleton pregnancies appear interesting. The data are consistent with a neurotrophic role for the protein (1–5) and support the hypothesis that each fetus releases a physiologically defined amount of the protein, in accordance with the role of S100B as a cytokine (1, 2), that appears to be more concentrated in relation to the volume of the amniotic sac, which is not usually twice as voluminous in twins as in singleton pregnancies. The source of

much of the S100B present in the amniotic fluid could be the fetal nervous system, where the protein has been shown to be present at the ages investigated, albeit not at mature concentrations (9–12). In this respect, we cannot exclude the possibility that at least a part of the S100B present in monoamniotic twins is a consequence of compression of the fetuses in the sac.

Nonetheless, because different placental tissues contain S100B at concentrations that vary in relation to gestational age (13, 14), the possibility that the protein derives partly from the placenta must be taken into account. However, the higher concentrations of S100B in monoamniotic fetuses than in diamniotic and control fetuses seem to suggest a fetal rather than a placental origin for the protein in amniotic fluid, although the possibility that placental tissues produce a higher amount of neurotrophic S100B in cases of multiple pregnancies cannot be ruled out.

The possibility that S100B may be released, at least in part, from other sites in which it is concentrated, such as adipose tissue (15), could also be considered, although data on the presence of the protein in adipocytes at this stage of maturation are not available.

In conclusion, the present study offers a clue for the investigation of S100B dynamics in vivo, with special reference to a possible role of the protein as a cytokine involved in fetal brain maturation in both singleton and multiple pregnancies.
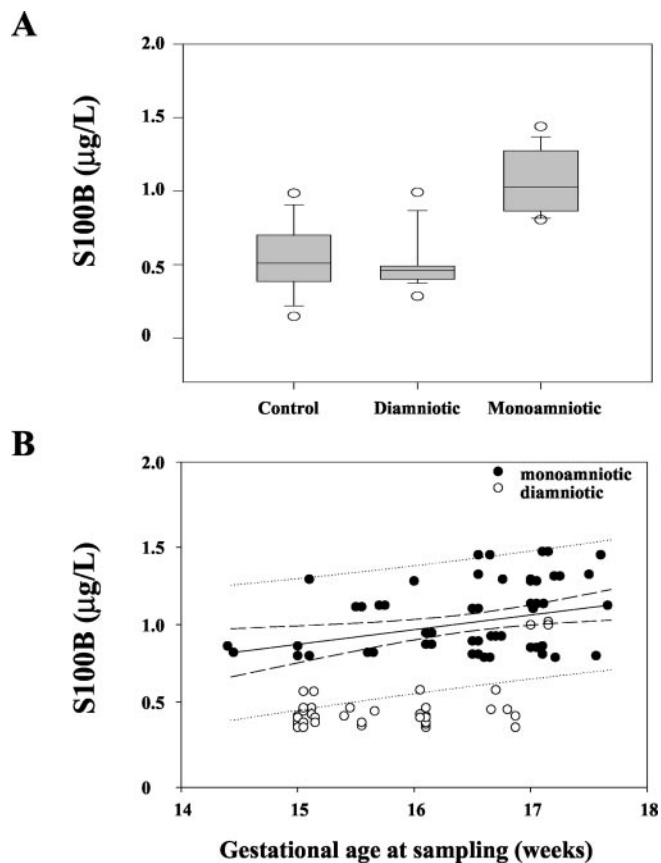
Fig. 1. S100B protein concentrations in amniotic fluid (μg/L) in controls and in monoamniotic and diamniotic twins (*A*), and correlation with gestational age (*B*).

(*A*), *boxes* indicate the interquartile ranges, with the *horizontal line* indicating the *median*. The *lower* and *upper horizontal bars* represent the 10th and 90th percentiles, and ○ indicate the 5th and the 95th percentiles. S100B values were significantly higher in monoamniotic twins than in diamniotic twins or controls (*P* <0.001 for both). (*B*), correlations are for the 14th–19th week of sampling in healthy monoamniotic (●) and diamniotic twin (○) fetuses. Values are expressed as median (*solid line*) and interquartile range (*dashed lines*) in the monoamniotic twins group. The *lower* and *upper dashed lines* represent the 3rd and 97th percentiles, respectively. The *dotted lines* represent the 25th and 75th percentiles. There was a positive significant correlation ($r = 0.56$; P <0.001).

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