the exception of one patient), albumin, and α1M. α1M dominated in comparison with albumin, so that the renal function of these patients was rated as restricted tubulo-interstitial reabsorption (one patient with glomerulopathy and restricted tubulo-interstitial reabsorption). The restricted tubular reabsorption may lead to the appearance of cTnT and cTnl in urine. Restricted tubular reabsorption may occur as a result of tubulus ischemic damage or an overload of the tubular reabsorption capacity. A decrease in the glomerular filtration rate to <70 mL/min will cause an overload of the tubular reabsorption capacity for α1M (10), and the α1M concentration in the plasma will increase with increasingly restricted filtration.

Group C patients presented with massive, combined glomerular and tubular renal damage (albumin >638 mg/g of creatinine and α1M >895 mg/g of creatinine). We found cTnT in the plasma and urine of all eight of the patients, but we found cTnl at very low concentrations, in the plasma of only two cases, and no cTnl in the urine of any of the cases.

The calculated protein ratios in urine and plasma (Fig. 1D) considered the initial plasma concentration of the proteins, and a direct comparison between the troponin ratios with α1M and albumin ratios was possible. The α1M and albumin ratios suggested a dependence of the molecular weight and the degree of renal impairment. In group A, troponins were not detectable. In group B, the troponin ratios were in the order of magnitude of α1M and albumin, and the cTnT ratios were higher in group C than in group B. The cTnl ratios for group C could not be calculated because cTnl was not detectable in this group, as is frequently seen in these patients.

Although increased cTnT values are an important prognostic factor for cardiovascular disease in hemodialysis patients, the frequently observed differences in the values between cTnT and cTnl are not clear. The lack of cTnl in these patients could be the result of changes in the molecular structure of antigenic regions caused by degradation, oxidation, phosphorylation, or nonenzymatic glycation.

On the basis of the demonstrated parallels between the troponin and the α1M and albumin results, we can not exclude an influence of the kidney on troponin kinetics. Usually, a complex of troponin I and troponin C (cTnC complex) is the dominant form in blood, but under uremic conditions, the portion of free cTnl could increase and produce alterations in the glomerular filtration compared with cTnT. Other studies could not confirm an influence of kidney function on plasma troponin concentrations (11, 12). Hannemann-Pohl et al. (13), however, found differences in the plasma concentration of myoglobin in patients with renal failure depending on the degree of renal impairment. On the basis of our data, kidney function seems to contribute to the elimination of troponins.

References


Cell-free Fetal DNA in Maternal Circulation after Amniocentesis, Osamu Samura,* Norio Mihara, Maki Hyodo, Hiroshi Honda, Yoko Ohashi, Nao Honda, Tetsuaki Hara, and Koso Ohama (Department of Obstetrics and Gynecology, Hiroshima University Faculty of Medicine, 1-2-3 Kasumi Minami-ku, Hiroshima 734-8551, Japan; * author for correspondence: fax 81-82-257-5264, e-mail osamura@hiroshima-u.ac.jp)

After amniocentesis, 5–20% of patients have evidence of fetal-maternal hemorrhage as indicated by increases in maternal serum α-fetoprotein (1–5) or by the Betke-Kleihauer test (6–8). The Betke-Kleihauer test can differentiate fetal from maternal erythrocytes by the relative resistance of hemoglobin F-containing cells to acid elution, and it is the most popular method of diagnosing and assessing the severity of fetal-maternal hemorrhage (9). The reliability of this test has been questioned, however, because numerous sources of error are associated with it (10). These sources of error possibly contribute to the wide variation in the reported incidence of fetal-maternal hemorrhage; a more accurate method of assessing fetal-maternal hemorrhage is therefore required in the clinical setting of rhesus D-negative pregnant women.

The discovery of cell-free fetal DNA in maternal serum and plasma has opened a new avenue for noninvasive prenatal diagnosis and has provided a useful marker of complicated pregnancies (11–16). The analysis of fetal DNA in maternal serum or plasma has afforded diagnoses of fetal rhesus D status (12) and single-gene disor-
ders (13), as well as the determination of fetal gender (14, 15). Cell-free DNA may be liberated directly from the fetal-placental interface into the maternal circulation (16), or it may be transferred into the maternal circulation as a result of destruction of fetal cells in that milieu; a combination of both of these processes may also be involved. The concentration of fetal DNA in maternal blood may be a more specific and accurate quantitative marker of a fetal-maternal hemorrhage. Accurate quantification of subclinical fetal-maternal hemorrhage would facilitate the study of placental disturbance induced by amniocentesis.

Twenty-nine healthy pregnant women (gestational age, 15–17 weeks) attending the Department of Obstetrics and Gynecology at Hiroshima University Hospital for amniocentesis provided written, informed consent to participate in the study, which was approved by the Research Ethics Committee of Hiroshima University. At the time of sampling, none of the women had manifested pregnancy-related complications, such as hypertension or threatened abortion. Using simultaneous real-time ultrasound, amniocentesis was performed once with a 22-gauge needle. Two 6-mL samples of maternal peripheral blood were collected before and 10 min after amniocentesis into plain Vacutainer Tubes without anticoagulant for serum separation. None of the women had manifested pregnancy-related complications, such as hypertension or threatened abortion. Using simultaneous real-time ultrasound, amniocentesis was performed once with a 22-gauge needle. Two 6-mL samples of maternal peripheral blood were collected before and 10 min after amniocentesis into plain Vacutainer Tubes without anticoagulant for serum separation and were then stored at −20°C as described (16). None of the pregnancies was disrupted within the 4 weeks after the amniocentesis, and none of the amniotic fluid samples was visibly contaminated with blood.

When the karyotype of each fetus was confirmed from cytogenetic findings after amniocentesis, we selected 24 and 5 women who were carrying single male and single female fetuses, respectively. The 24 male and 5 female karyotypes were 46,XY and 46,XX, respectively.

We extracted DNA from 1.2-mL serum samples with use of a QIAamp DNA Blood Mini Kit (Qiagen) according to the “blood and body fluid spin protocol” with minor modifications. The extracted DNA was concentrated in 70 μL of water, and 15 μL was used as a template for each reaction. The SRY gene on the Y chromosome was used as a marker for male fetuses, and the concentration of the fetal SRY sequence was measured by a real-time quantitative PCR assay and an ABI PRISM 7700 Sequence Detector (Applied Biosystems) as described by Ohashi et al. (16). We analyzed all samples in duplicate and determined the mean concentrations.

Handling of all liquids with aerosol-resistant pipette tips (ART; Molecular Bio-Products) prevented contamination during PCR. DNA extraction, preparation of amplification mixtures, actual amplification reactions, and PCR product detection were performed in different areas. All manipulations except product detection were performed under a laminar flow hood. Several negative water blanks were included in each real-time quantitative PCR analysis. A female staff member performed all procedures. Changes in the concentration of the amplified SRY gene sequence before and after amniocentesis were compared by the Wilcoxon signed-rank test. Data were analyzed statistically with StatView software (Ver. 5.0; SAS Institute).

The quantitative SRY data from maternal serum are shown in Fig. 1. Amplification products of the SRY gene were undetectable in control fetuses with the 46,XX karyotype. In 46,XY fetuses, the mean concentration of the SRY gene was 7.2 copies/mL (range, 0.5–26 copies/mL) before amniocentesis and 11.9 copies/mL (range, 2.5–57 copies/mL) after the procedure (P = 0.0066). The concentration of SRY increased in 19 and decreased in 5 of 24 samples (21%) after amniocentesis.

The assessment of fetal-maternal hemorrhage is difficult. The Betke–Kleihauer test and flow cytometry are widely used, but these tests have limited ability to detect fetal-maternal hemorrhage because of the rarity of fetal cells in the maternal circulation (10). Lau et al. (17) found that an external cephalic version performed near term increased the concentration of cell-free fetal DNA in the maternal plasma. To our knowledge, this is the first effort to quantify fetal-maternal hemorrhage after amniocentesis by use of real-time quantitative PCR.

The origin of the increase in cell-free fetal DNA in maternal serum after amniocentesis requires further investigation. High concentrations of cell-free fetal DNA are present in amniotic fluid (18). Excess cell-free fetal DNA in maternal serum after amniocentesis might be caused by leakage across the placenta or leakage of cell-free fetal DNA in amniotic fluid across the myometrium.

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**Fig. 1. Concentrations of SRY sequence in maternal serum before and after amniocentesis in mothers carrying fetuses with a 46,XY karyotype.**
The present study showed that amniocentesis is associated with a significant increase in the fetal DNA concentrations, representing a transfer of either fetal cells or fetal DNA to the maternal circulation. However, in 21% of our study participants, the concentration of the SRY sequence decreased after amniocentesis, which could be associated with uterine contraction in response to the procedure or may reflect assay imprecision. Frequent uterine contraction may inhibit fetal DNA transfer into the maternal circulation for a few minutes because fetal DNA is cleared from the maternal circulation, with a mean half-life of 16.3 min (19).

The limitation of the current method in assessing subclinical fetal-maternal hemorrhage is that only women carrying a male fetus can provide useful information because the fetal origin of the DNA is based on the presence of the SRY gene on the Y chromosome. A system that uses polymorphic markers outside the Y chromosome (20, 21) or epigenetic markers (22) must be developed before this technique can be applied to mothers carrying female fetuses.

In conclusion, amniocentesis significantly disturbs the maternal-placental interface; further studies are needed to determine whether fetal DNA is a sensitive marker for fetal-maternal hemorrhage or whether its increase after amniocentesis reflects transfer of DNA from amniotic fluid to the maternal circulation.

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References


The Transcobalamin (TC) Codon 259 Genetic Polymorphism Influences Holo-TC Concentration in Cerebrospinal Fluid from Patients with Alzheimer Disease, Henrik Zetterberg,1* Ebba Nexè,2 Björn Regland,3 Lennart Mithlon,4 Roberta Boson,4 Mona Palmér,1 Lars Rymo,1 and Kay Bielawska1,3 (1 Department of Clinical Chemistry and Transfusion Medicine, 2 Institute of Clinical Neuroscience, Psychiatry Section, and 3 Institute of Clinical Neuroscience, Department of Experimental Neuroscience, Sahlgrenska University Hospital, Göteborg University, S-413 45 Gothenburg, Sweden; 4 Department of Clinical Biochemistry, AKH, Aarhus University Hospital, DK 8000 Aarhus C, Denmark; * Neurropsychiatric Clinic, Malmö University Hospital, S-205 02 Malmö, Sweden; author for correspondence: fax 46-31-828458, e-mail henrik.zetterberg@clinchem.gu.se) Two proteins bind vitamin B12 in plasma: haptocorrin (transcobalamin I) and transcobalamin (transcobalamin II; TC). The latter is the critical transporter that delivers vitamin B12 to peripheral tissues. TC carries one-third of the circulating B12 (holo-TC), but most TC is unsaturated (apo-TC) (I, 2). Polyacrylamide gel electrophoresis has revealed two common TC isoforms, M and X, and two rare variants, S and F (3, 4), that may influence the cellular availability of vitamin B12 (5, 6). The phenotypic variability is a multifactorial phenomenon that probably includes cell-type-specific processing of translated TC (5), but the substitution of proline (P) for arginine (R) at codon 259 of the TC gene is the major determinant of the TC variability, at least in Caucasians (5, 7), and affects TC concentrations