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 cTnI 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 cTnI, at very low concentrations, in the plasma of only two cases, and no cTnI 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 cTnI ratios for group C could not be calculated because cTnI 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 cTnI are not clear. The lack of cTnI 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 (cTnI-C complex) is the dominant form in blood, but under uremic conditions, the portion of free cTnI 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

1. Dierkes J, Domröse U, Westphal S, Ambrosch A, Bosselmann HP. Cardiac troponin T predicts mortality in patients with end-stage renal disease. Circulation 2000;102:1964–9.
2. Ooi DS, Zimmermann D, Graham J, Wells GA. Cardiac troponin T predicts long-term outcomes in hemodialysis patients. Clin Chem 2001;47:412–7.
3. Wu AHB, Feng YJ, Moore R, Apple FS, McPherson PH, Buechler KF, et al. Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. American Association for Clinical Chemistry Subcommittee on cTnI Standardization. Clin Chem 1998;44:1198–208.
4. Gerhardt W, Jjungdahl L. Troponin T: a sensitive and specific diagnostic and prognostic marker of myocardia damage. Clin Chim Acta 1998;272:47–57.
5. Richiutti V, Voss EM, Ney A, Odland M, Anderson PAW, Apple FS. Cardiac troponin T isoforms expressed in renal diseased skeletal muscle will not cause false-positive results by the second generation cardiac troponin T assay by Boehringer Mannheim. Clin Chem 1998;44:1919–24.
6. Hofmann W, Guder WG. A diagnostic programme for quantitative analysis of proteinuria. J Clin Chem Clin Biochem 1989;27:589–600.
7. Apple FS, Wu AHB. Myocardial infarction redefined: role of cardiac troponin testing. Clin Chem 2001;47:377–9.
8. Thygesen K, Alpert JS, Antman E, Bassand JP. Myocardial infarction redefined—a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36:959–69.
9. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41.
10. Weber MH, Scholz P, Stibbe W, Scheler F. α-1-Mikroglobulin in urin und serum bei proteinurie und niereninsuffizienz. Klin Wochenschr 1991;63:711–7.
11. Aviles RJ, Askari AT, Lindahl B, Wallentin L, Jia G, Ohman EM, et al. Troponin T levels in patients with acute coronary syndrome, with or without renal dysfunction. N Engl J Med 2002;27:2047–52.
12. Ellis K, Dreisbach AW, Lertora JJL. Plasma elimination of cardiac troponin I In end-stage renal disease. South Med J 2001;94:993–6.
13. Hannemann-Pohl K, Glöer G, Kampf SG. Myoglobin: diagnosis of acute myocardial infarction and check of successful reopening during lysis therapy. J Lab Med 1996;20:16–28.

Cell-free Fetal DNA in Maternal Circulation after Amniocentesis, *Osamu Samura,* Norio Miharu, 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 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 fetal 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.

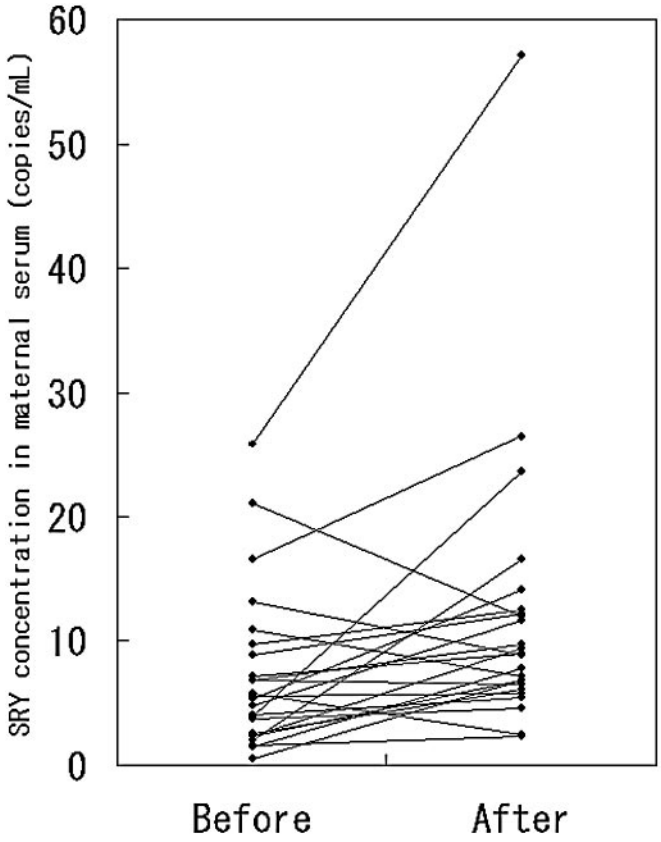

Fig. 1. Concentrations of *SRY* sequence in maternal serum before and after amniocentesis in mothers carrying fetuses with a 46,XY karyotype.

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

1. Hay DL, Barrie JU, Davison GB, Buttery BW, Horacek I, Pepperell RJ, et al. The relation between maternal serum α-fetoprotein levels and fetomaternal haemorrhage. Br J Obstet Gynecol 1979;86:516–20.
2. Dallaire L, Belanger L, Smith CJP. Origin of amniocentesis induced rises of α-fetoprotein concentration in maternal serum. Br J Obstet Gynecol 1980; 87:856–9.
3. Lele AS, Carmody PJ, Hurd ME, OLeary JA. Fetomaternal bleeding following diagnostic amniocentesis. Obstet Gynecol 1982;60:60–4.
4. Mennuti MT, DiGaetano A, McDonnell A. Fetal-maternal bleeding associated with genetic amniocentesis: real-time versus static ultrasound. Obstet Gynecol 1983;62:26–30.
5. Thomsen SG, Isager-Sally L, Lange AP, Saurbrey N. Elevated maternal serum α-fetoprotein caused by midtrimester amniocentesis: a prognostic factor. Obstet Gynecol 1983;62:297–300.
6. Lachman E, Hingley SM, Bates G, Ward AM, Stewart CR, Duncan S. Detection and measurement of fetomaternal haemorrhage: serum α-fetoprotein and the Kleihauer technique. Br Med J 1977;1:1377–9.
7. Mennuti MT, Brummond W, Crombelholme WR, Schwarz RH, Arvan DA. Fetal-maternal bleeding associated with genetic amniocentesis. Obstet Gynecol 1980;55:48–54.
8. Tabor A, Bang J, Norgaard-Pedersen B. Feto-maternal haemorrhage associated with genetic amniocentesis: results of a randomized trial. Br J Obstet Gynecol 1987;94:528–34.
9. Lenke RR, Ashwood ER, Cyr DR, Gravett M, Smith JR, Stenchever MA. Genetic amniocentesis: significance of intraamniotic bleeding and placental location. Obstet Gynecol 1985;65:798–801.
10. Johnson PRE, Tait RC, Austin EB, Shwe KH, Lee D. Flow cytometry in diagnosis and management of large fetomaternal haemorrhage. J Clin Pathol 1995;48:1005–8.
11. Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CWG, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350: 485–7.
12. Faas BH, Beuling EA, Christiaence GC, von den Borne AE, van der Schoot CE. Detection of fetal RH-D specific sequences in maternal plasma. Lancet 1998;352:1196.
13. Saito H, Sekizawa A, Morimoto T, Suzuki M, Yanaihara T. Prenatal DNA diagnosis of a single-gene disorder from maternal plasma. Lancet 2000; 356:1170.
14. Honda H, Miharu N, Ohashi Y, Ohama K. Successful diagnosis of fetal gender using conventional PCR analysis of maternal serum. Clin Chem 2001;47:41–6.
15. Honda H, Miharu N, Ohashi Y, Samura O, Kinutani M, Tetsuaki H, et al. Fetal gender determination in early pregnancy through qualitative and quantitative analysis of fetal DNA in maternal serum. Hum Genet 2002;110:75–9.
16. Ohashi Y, Miharu N, Honda H, Samura O, Ohama K. Correlation of fetal DNA and human chorionic gonadotropin concentration in second-trimester maternal serum. Clin Chem 2002;48:386–8.
17. Lau TK, Lo KWK, Chan LYS, Leung TY, Lo YMD. Cell-free fetal deoxyribonucleic acid in maternal circulation as a marker of fetal-maternal hemorrhage in patients undergoing external cephalic version near term. Am J Obstet Gynecol 2000;183:712–6.
18. Bianchi DW, Leshane ES, Cowan JM. Large amounts of cell-free fetal DNA are present in amniotic fluid. Clin Chem 2001;47:1867–9.
19. Lo YMD, Zhang J, Leung TN, Lau TK, Chang AMZ, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma Am J Hum Genet 1999;64:218–24.
20. Tang NL, Leung TN, Zhang J, Lau TK, Lo YMD. Detection of fetal-derived paternally inherited X-chromosome polymorphisms in maternal plasma. Clin Chem 1999;45:2033–5.
21. Pertl B, Sekizawa A, Samura O, Orescovic I, Rahaim PT, Bianchi DW. Detection of male and female fetal DNA in maternal plasma by multiplex fluorescent polymerase chain reaction amplification of short tandem repeats. Hum Genet 2000;106:45–9.
22. Poon LLM, Leung TN, Lau TK, Chow KCK, Lo YMD. Differential DNA methylation between fetus and mother as a strategy for detecting fetal DNA in maternal plasma. Clin Chem 2002;48:35–41.

The Transcobalamin (TC) Codon 259 Genetic Polymorphism Influences Holo-TC Concentration in Cerebrospinal Fluid from Patients with Alzheimer Disease, *Henrik Zetterberg,[1*] Ebba Nexö,[2] Björn Regland,[3] Lennart Minthon,[4] Roberta Boson,[4] Mona Palmér,[1] Lars Rymo,[1] and Kaj Blennow[1,5]* ([1] Department of Clinical Chemistry and Transfusion Medicine, [3] Institute of Clinical Neuroscience, Psychiatry Section, and [5] Institute of Clinical Neuroscience, Department of Experimental Neuroscience, Sahlgrenska University Hospital, Göteborg University, S-413 45 Gothenburg, Sweden; [2] Department of Clinical Biochemistry, AKH, Aarhus University Hospital, DK 8000 Aarhus C, Denmark; [4] Neuropsychiatric 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 B_{12} in plasma: haptocorrin (transcobalamin I) and transcobalamin (transcobalamin II; TC). The latter is the critical transporter that delivers vitamin B_{12} to peripheral tissues. TC carries one-third of the circulating B_{12} (holo-TC), but most TC is unsaturated (apo-TC) *(1, 2)*. Polyacrylamide gel electrophoresis has revealed two common TC isotypes, M and X, and two rare variants, S and F *(3, 4)*, that may influence the cellular availability of vitamin B_{12} *(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