and future needs of regional adult congenital heart disease centers in Japan. *Circ J* 2011; **75:** 2220–2227.
6. Hunter S, Robson SC. Adaptation of the maternal heart in pregnancy. *Br Heart J* 1992; **68:** 540–543.
7. Robson SC, Hunter S, Boys RJ, Dunlop W. Serial study of factors influencing changes in cardiac output during human pregnancy. *Am J Physiol* 1989; **256:** H1060–H1065.
8. Mabie WC, DiSessa TG, Crocker LG, Sibai BM, Arheart KL. A longitudinal study of cardiac output in normal human pregnancy. *Am J Obstet Gynecol* 1994; **170:** 849–856.
9. Robson SC, Dunlop W, Boys RJ, Hunter S. Cardiac output during labour. *Br Med J* 1987; **295:** 1169–1172.
10. Robson SC, Hunter S, Moore M, Dunlop W. Haemodynamic changes during the puerperium: A Doppler and M-mode echocardiographic study. *Br J Obstet Gynaecol* 1987; **94:** 1028–1039.
11. Siu SC, Sermer M, Colman JM, Alvarez AN, Mercier LA, Morton BC, et al; Cardiac Disease in Pregnancy (CARPREG) Investigators. Prospective multicenter study of pregnancy outcomes in women with heart disease. *Circulation* 2001; **104:** 515–521.
12. Lui GK, Silversides CK, Khairy P, Fernandes SM, Valente AM, Nickolaus MJ, et al; Alliance for Adult Research in Congenital Cardiology (AARCC). Heart rate response during exercise and pregnancy outcome in women with congenital heart disease. *Circulation* 2011; **123:** 242–248.
13. Ohuchi H, Nakajima T, Kawade M, Matsuda M, Kamiya T. Measurement and validity of the ventilatory threshold in patients with congenital heart disease. *Pediatr Cardiol* 1996; **17:** 7–14.
14. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* 1986; **60:** 2020–2027.
15. Waien SA, Liu PP, Ross BL, Williams WG, Webb GD, McLaughlin PR. Serial follow-up of adults with repaired tetralogy of Fallot. *J Am Coll Cardiol* 1992; **20:** 295–300.
16. JCS Joint Working Group. Guidelines for indication and management of pregnancy and delivery in women with heart disease (JCS 2010): Digest version. *Circ J* 2012; **76:** 240–260.
17. Franciosa JA, Park M, Levine TB. Lack of correlation between exercise capacity and indexes of resting left ventricular performance in heart failure. *Am J Cardiol* 1981; **47:** 33–39.
18. Harrison DA, Liu P, Walters JE, Goodman JM, Siu SC, Webb GD, et al. Cardiopulmonary function in adult patients late after Fontan repair. *J Am Coll Cardiol* 1995; **26:** 1016–1021.
19. Vasapollo B, Valensise H, Novelli GP, Larciprete G, Di Pierro G, Altomare F, et al. Abnormal maternal cardiac function and morphology in pregnancies complicated by intrauterine fetal growth restriction. *Ultrasound Obstet Gynecol* 2002; **20:** 452–457.
20. Ohuchi H, Takasugi H, Ohashi H, Okada Y, Yamada O, Ono Y, et al. Stratification of pediatric heart failure on the basis of neurohormonal and cardiac autonomic nervous activities in patients with congenital heart disease. *Circulation* 2003; **108:** 2368–2376.
21. Wasserman K, Hansen JE, Sue DY, Whipp BJ, Casaburi R. Principles in exercise testing and interpretation. Philadelphia: Lea & Febiger, 1994.
22. Peleg D, Kennedy CM, Hunter SK. Intrauterine growth restriction: Identification and management. *Am Fam Physician* 1998; **58:** 453–460, 466–467.
23. Cogswell ME, Yip R. The influence of fetal and maternal factors on the distribution of birthweight. *Semin Perinatol* 1995; **19:** 222–240.
24. Brooks AA, Johnson MR, Steer PJ, Pawson ME, Abdalla HI. Birth weight: Nature or nurture? *Early Hum Dev* 1995; **42:** 29–35.
25. Cogswell ME, Parvanta I, Ickes L, Yip R, Brittenham GM. Iron supplementation during pregnancy, anemia, and birth weight: A randomized controlled trial. *Am J Clin Nutr* 2003; **78:** 773–781.

Forensic Science International xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Case report

Fatal amniotic fluid embolism with typical pathohistological, histochemical and clinical features

Wakako Hikiji [a,*], Naoaki Tamura [b], Akio Shigeta [a,1], Naohiro Kanayama [b], Tatsushige Fukunaga [a,1]

[a] Tokyo Medical Examiner's Office, Tokyo Metropolitan Government, 4-21-18 Otsuka, Bunkyo-ku, Tokyo 112-0012, Japan
[b] Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, Japan

ARTICLE INFO

Article history:
Received 17 August 2012
Received in revised form 27 October 2012
Accepted 2 December 2012
Available online xxx

Keywords:
Amniotic fluid embolism
Maternal death
Autopsy

ABSTRACT

Despite the decrease in maternal mortality rate, amniotic fluid embolism (AFE) is still one of the most feared complications of pregnancy due to the high rate of mortality in Japan. The authors present a fatal case of a healthy 39-year-old woman who died during delivery after a normal 40-week second pregnancy. Shortly after the arrival at hospital, an abrupt drop of foetal heart rate was observed, followed by deterioration of consciousness and cardiac arrest of the patient. Prompt cardiopulmonary resuscitation (CPR) was performed but the patient died about an hour and a half after her arrival at hospital. Forensic autopsy confirmed the pathohistological diagnosis of amniotic fluid embolism supported by histochemical analysis results and excluded other possible causes of death. This paper stresses the fundamental importance of autopsy in an unexpected maternal death in conjunction with the significance of data accumulation on maternal death.

1. Introduction

Despite earlier recognition and aggressive treatment, morbidity and mortality rates of amniotic fluid embolism (AFE) remain high. An estimated 5–15% of all maternal deaths in Western countries are due to AFE [1]. The reported maternal mortality rates for AFE range from 37% to over 80%, with one report stating that 25–50% of deaths occur within the first hour of diagnosis [2–4]. Diagnosis of AFE has historically been based on autopsy, revealing amniotic components in maternal pulmonary vasculature. Some recent studies introduced zinc coproporphyrin I (ZnCP-I) and sialyl-Tn (STN), both characteristic components in meconium, as less invasive, diagnostic markers for AFE [5,6]. Presented in this paper is a fatal AFE case in which the typical features were observed in pathohistological, histochemical and clinical findings.

2. Case history

The patient was a 39-year-old multiparous woman without any medical history or eventful course of pregnancy at 40-week gestation. She had no known allergies and was not taking any medication. Shortly after the membrane rupture at home, she was brought to the labour and delivery unit of the hospital by her husband's car, in active labour. Her cervix was dilated 4 cm and contraction occurred every 1.5 min on admission. Although the foetal heart rate (FHR) was 140–160 bpm, meconium staining was already observed at this stage. About 12 min later the FHR dropped to less than 120 bpm, and it became undetectable by a few minutes after the initial drop. An intravenous infusion of tocolytic agent was started to weaken labour pains, but the patient deteriorated, becoming unconscious, displaying the signs of cardiovascular collapse. Cardiopulmonary resuscitation (CPR) was begun immediately but the patient was pronounced dead about an hour and a half after her arrival at hospital. Because of the abrupt onset of symptoms and intensive CPR, an emergency caesarian section could not be carried out to deliver the foetus. The estimated blood loss was very little.

3. Autopsy findings

Forensic autopsy was performed approximately 18 h postmortem. The decedent was 162 cm in height and weighed 63 kg. No significant findings at external examination of body were present. All organs were congested. The weights of lungs were 442 g left and 535 g right, both strongly oedematous. The heart was of normal size, weighing 336 g, and the coronary arteries were free of atherosclerosis. No clots were found in the heart blood and a number of petechiae were seen in bilateral palpebral conjunctiva and epiglottis. A male foetus without remarkable anomaly was in

* Corresponding author. Tel.: +81 3 3944 1481; fax: +81 3 3944 7585.
E-mail address: hikiji@kyudai.jp (W. Hikiji).
[1] Tel.: +81 3 3944 1481; fax: +81 3 3944 7585.

0379-0738/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.forsciint.2012.12.008

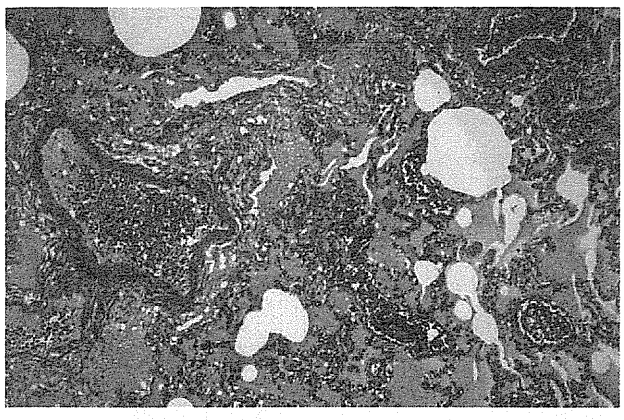

Fig. 1. Pathohistological section of the lung: blood congestion in capillaries (haematoxylin-and-eosin stain, original magnification 100×).

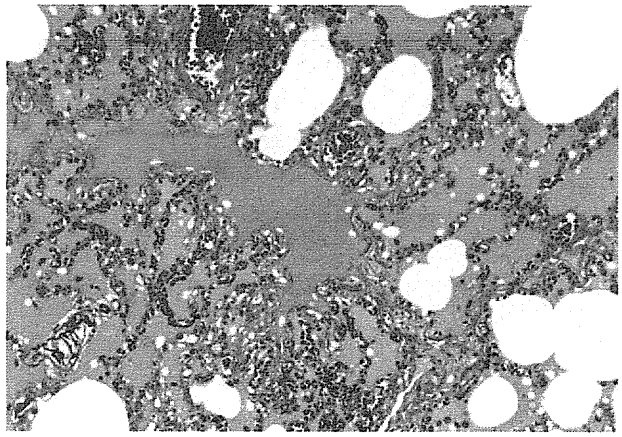

Fig. 3. Pathohistological section of the lung: blood congestion in capillaries (Azan stain, original magnification 100×).

the uterus, weighing 3800 g. The placenta was intact, and the umbilical cord was also normal. A small tear was observed on the posterior wall of cervix. Biological sample from heart blood was reserved but no urine could be obtained for toxicological investigation. Microscopic examination revealed extensive blood congestion in the pulmonary vasculature and microthromboemboli in the uterine microvasculature by haematoxylin-and-eosin and azan staining (Figs. 1–4). Furthermore, amniotic components were also detected inside the pulmonary vessels by alcian blue and ZnCP-I staining (Figs. 5 and 6). Immunohistochemical staining for C5a receptor (C5aR) was positive in stromal cells around the pulmonary capillaries and inflammatory cells in alveolus (Fig. 7). No remarkable pathological changes were observed in placenta. Alcohol and drugs were not detected by routine toxicological analysis. Concentrations of ZnCP-I and STN were 72.5 pmol/mL (normal: <1.6 pmol/mL) and 2630 U/mL (normal: <45 U/mL), respectively. These findings confirmed AFE as cause of death of the patient.

4. Discussion

The Japanese maternal mortality rate (number of maternal deaths per 100,000 live births) has been declining since the 1970s, being stable around 5 for the past decade [7]. Nevertheless, maternal death occurs on occasion, and the most frequently reported causes today include AFE, complications of pregnancy induced hypertension (PIH), pulmonary embolism, haemorrhage and infectious diseases [8–11]. The AFE syndrome was first described by Meyer in 1926 [12] and numerous case reports have been published from various countries to date. Despite its long, worldwide recognition, it is still challenging to save AFE patients due to the fulminant onset of symptoms and rapid clinical course [13].

AFE is generally characterized by a rapidly progressive clinical course with dyspnoea, hypoxaemia, hypotension and foetal bradycardia with subsequent and acute cardiorespiratory collapse, disseminated intravascular coagulopathy (DIC), neurological compromise, maternal and foetal death [14,15]. Since not all of these symptoms are evident on presentation, the differential diagnosis for AFE is broad and includes anaphylactic or haemorrhagic shock, eclampsia, cerebrovascular diseases and pulmonary embolism [16,17]. There are no universal diagnostic criteria to confirm AFE but some countries have their own for the national registry, including the United States of America, the United Kingdom and Japan [2,3,18]. A reliable diagnosis can be made only upon pathohistological examination, by the proof of amniotic fluid elements such as epithelial squamous cells, lanugo hair, and fat from vernix or infantile mucin in the pulmonary vascular bed of the mother [19]. These components of amniotic fluid could be identified in routine haematoxylin-and-eosin-stained sections, but the use of immunohistochemistry permits a more reliable assessment of the dimension of AFE. Special stains that have been used to demonstrate amniotic fluid include alcian blue stain to

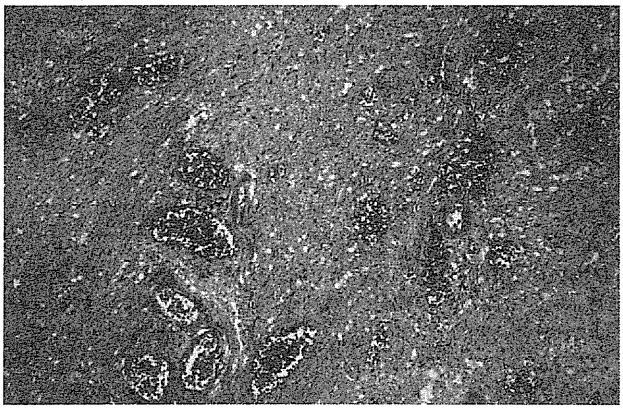

Fig. 2. Pathohistological section of the uterus: emboli in capillaries (haematoxylin-and-eosin stain, original magnification 100×).

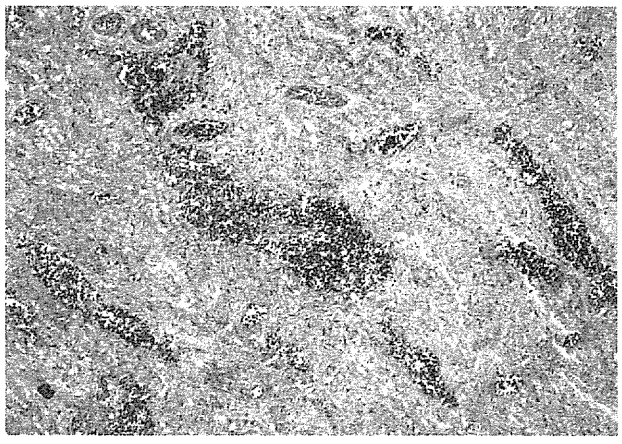

Fig. 4. Pathohistological section of the uterus: emboli in capillaries (Azan stain, original magnification 100×).

Please cite this article in press as: W. Hikiji, et al., Fatal amniotic fluid embolism with typical pathohistological, histochemical and clinical features, Forensic Sci. Int. (2012), http://dx.doi.org/10.1016/j.forsciint.2012.12.008

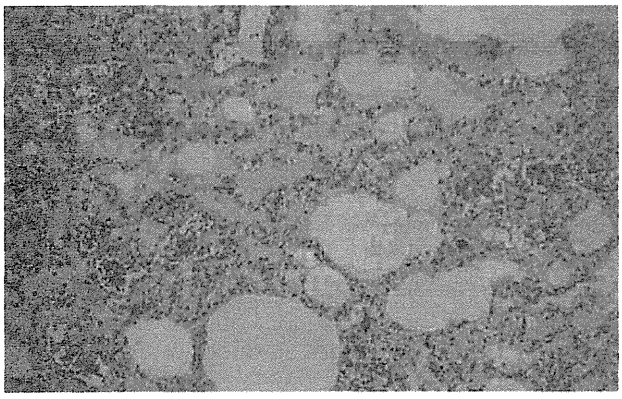

Fig. 5. Pathohistological section of the lung: positive staining in vessels (Alcian blue stain, original magnification 200×).

detect mucin, cytokeratin AE1/AE3 to detect foetal squamous cells, and ZnCP-I stain to detect meconium [20,21]. A recent study by Töro et al. introduced C5aR stain as a helpful technique to prove the complement activation and anaphylatoxin formation [22]. It is suggested that in the presented case that meconium components had entered the maternal circulation, as the interior space of pulmonary vessels and uterine capillaries being positively stained with haematoxylin-and-eosin, alcian blue and ZnCP-I. Additionally, it is likely that an anaphylaxis had been provoked as positive C5aR staining was distinctly observed in the interstitial space between the capillaries of lungs and uterus.

Two main pathophysiological mechanisms have been proposed to be associated with death in AFE. Elements of an embolus may inflict mechanical obstruction of the pulmonary vasculature and cause pulmonary vasospasm, leading to haemodynamic changes [19,23–25]. Moreover, it has been suggested that the amniotic fluid constituents can provoke an anaphylaxis or anaphylactoid reaction which may be fatal depending on the severity [25–27]. The results of histopathological findings in conjunction with immunohistochemical staining suggest that both mechanisms have contributed significantly to the presented case. Furthermore, the marked elevation of AFE diagnostic markers, ZnCP-I and STN, as well as the fulminant clinical course, which had taken only about an hour from the onset of the symptoms until death, support the diagnosis of AFE. The absence of clots in cardiac blood and the presence of petechiae in palpebral conjunctiva and epiglottis are both compatible with the characteristics of sudden death. It could be said that the presented case is typical in pathohistology, histochemistry and symptomatology of AFE.

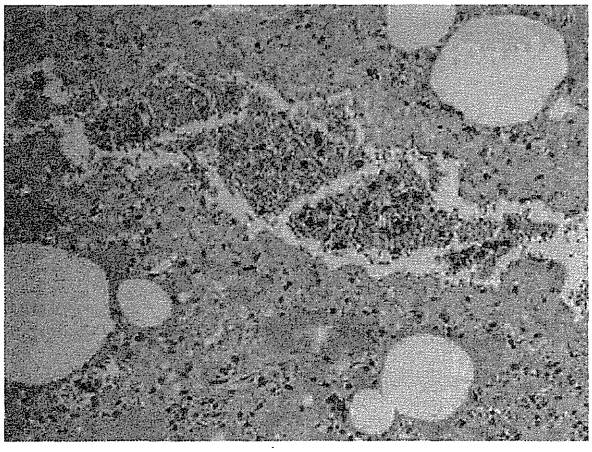

Fig. 6. Pathohistological section of the lung: positive staining in vessels (ZnCP-I stain, original magnification 200×).

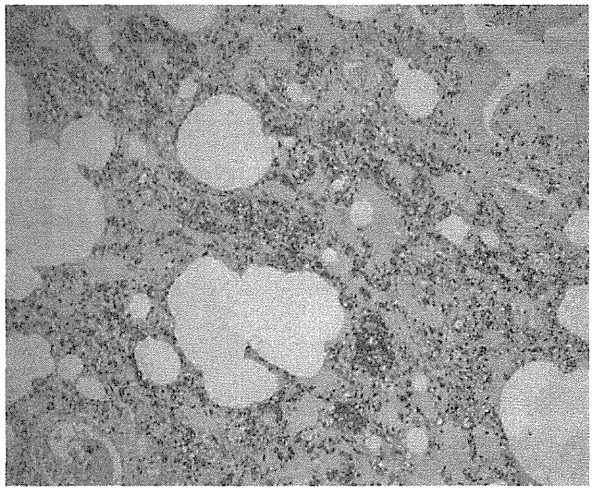

Fig. 7. Pathohistological section of the lung: positive staining in stromal cells around the pulmonary capillaries and inflammatory cells in alveolus (C5a receptor stain, original magnification 200×).

Several possible risk factors for AFE have been identified to date, which include maternal age of 35 years or older, multigravida, polyhydramnios, placental abruption, tumultuous labour or delivery with uterine hypercontractility and caesarean section [5,15,28,29]. Of the patients 41% had a medical history of allergy or autopsy [2]. The increased maternal age, multigravida and uterine hypercontractility could have been predisposing factors in the presented case. It is also speculated that a small tear in the posterior cervix during labour caused amniotic fluid to slowly infiltrate the maternal circulation.

In Japan, it is encouraged to report all maternal mortality cases to the Japan Association of Obstetricians and Gynaecologists, but there is no legal responsibility for physicians to do so. The available data and the conclusions that can be drawn depend on the depth and precision of the cases reported voluntary. Nevertheless, it is speculated that there were 60–70 maternal deaths in Japan in 2010, but only about half of the cases had been brought to either forensic or clinical autopsy [30]. Needless to say, thorough postmortem investigation, including autopsy, is essential for the accurate cause of death determination in any case, and it is possible that AFE would be underestimated in Japan, due to the low autopsy rate. Accumulation of cases nationwide would allow the establishment of wide and reliable database and possibly the identification and standardization of treatments for the diseases associated with maternal death. The elevation of autopsy rate, especially in the maternal death cases, is strongly desired not only from the aspects of forensic science but also of obstetrics and preventive medicine. Whilst on the other hand, it is encouraged for both forensic and clinical pathologists to continuously disclose the information derived from postmortem investigation.

5. Conclusion

Postmortem histopathologic and histochemical analyses further support the clinical diagnosis of AFE. The authors emphasize the significance of postmortem examinations in cases of maternal death, including the cases of suspected AFE.

References

[1] A. Conde-Agudelo, R. Romero, Amniotic fluid embolism: an evidence based review, Am. J. Obstet. Gynecol. 201 (2009), 445e1–e13.

Please cite this article in press as: W. Hikiji, et al., Fatal amniotic fluid embolism with typical pathohistological, histochemical and clinical features, Forensic Sci. Int. (2012), http://dx.doi.org/10.1016/j.forsciint.2012.12.008

[2] S.L. Clark, G.D. Hankins, D.A. Dudley, G.A. Dildy, T.F. Porter, Amniotic fluid embolism: analysis of the national registry, Am. J. Obstet. Gynecol. 172 (1995) 1158–1169.
[3] D.J. Tuffnell, United Kingdom amniotic fluid embolism register, BJOG 112 (2005) 1625–1629.
[4] S.L. Clark, F.J. Montz, J.P. Phelan, Hemodynamic alterations associated with amniotic fluid embolism: a reappraisal, Am. J. Obstet. Gynecol. 151 (1985) 617–621.
[5] N. Kanayama, T. Yamazaki, H. Naruse, K. Sumimoto, K. Horiuchi, T. Terao, Determining zinc coproporphyrin in maternal plasma: a new method for diagnosing amniotic fluid embolism, Clin. Chem. 38 (1992) 526–529.
[6] H. Oi, H. Kobayashi, Y. Hirayama, T. Yamazaki, T. Kobayashi, T. Terao, Serological immunohistochemical diagnosis of amniotic fluid embolism, Semin. Thromb. Hemost. 24 (1998) 479–484.
[7] I. Kaneda (Ed.), Maternal Child Health Statistics in Japan, Mother's and Children's Health and Welfare Association, Japan, 2009, pp. 78–80.
[8] B. Boedker, L. Hvidman, T. Weber, M. Moeller, A. Aarre, K.M. Nielsen, J.L. Soerensen, Maternal deaths in Denmark 2002–2006, Acta. Obstet. Gynecol. Scand. 88 (2009) 556–562.
[9] M.E. Akar, E.G. Eyi, E.S. Yilmaz, B. Yuksel, Z. Yilmaz, Maternal deaths and their causes in Ankara, Turkey, 1982–2001, J. Health. Popul. Nutr. 22 (2004) 420–428.
[10] S. Alexander, K. Wildman, W. Zhang, M. Langer, C. Vutuc, G. Lindmark, Maternal health outcomes in Europe, Eur. J. Obstet. Gynecol. Reprod. Biol. 111 (2003) S78–S87.
[11] N. Kanayama, J. Inori, H. Ishibashi-Ueda, M. Takeuchi, M. Nakayama, S. Kimura, Y. Matsuda, J. Yoshimatsu, T. Ikeda, Maternal death analysis from the Japanese autopsy registry for recent 16 years: significance of amniotic fluid embolism, J. Obstet. Gynaecol. Res. 1 (2011) 58–63.
[12] J. Meyer, Embolia pulmonar amnio caseosa, Bras. Med. 2 (1926) 301.
[13] R.S. Gist, I.P. Stafford, A.B. Leibowitz, Y. Beilin, Amniotic fluid embolism, Anesth. Analg. 108 (2009) 1599–1602.
[14] H.A. Abenhaim, L. Azoulay, M.S. Kramer, L. Leduc, Incidence and risk factors amniotic fluid embolisms: a population-based study on 3 million births in the United States, Am. J. Obstet. Gynecol. 199 (2008) 1–8.
[15] M.S. Kramer, J. Rouleau, T.F. Baskett, K.S. Joseph, Maternal Health Study Group of The Canadian Perinatal Surveillance System, Amniotic-fluid embolism and medical induction of labour: a retrospective, population based cohort study, Lancet 368 (2006) 1444–1448.
[16] E. Turillazzi, R. Gambassi, M. Gherardi, V. Fineschi, The diagnosis of amniotic-fluid embolism: an immunohistochemical study for the quantification of pulmonary mast cell tryptase, Int. J. Legal Med. 111 (1998) 238–243.
[17] I. Stafford, J. Sheffield, Amniotic fluid embolism, Obstet. Gynecol. Clin. North Am. 34 (2007) 545–553.
[18] H. Oi, K. Naruse, T. Noguchi, T. Sado, S. Kimura, N. Kanayama N, T. Terao, H. Kobayashi, Fatal factors of clinical manifestations and laboratory testing in patients with amniotic fluid embolism, Gynecol. Obstet. Invest. 70 (2010) 138–144.
[19] G. Lau, Amniotic fluid embolism as a cause of sudden maternal death, Med. Sci. Law 34 (1994) 213–220.
[20] B.J. Marcus, K.A. Collins, R.A. Harley, Ancillary studies in amniotic fluid embolism, Am. J. Forensic Med. Pathol. 26 (2005) 92–95.
[21] N. Furuta, C. Yaguchi, H. Itoh, Y. Morishima, N. Tamura, M. Kato, T. Uchida, K. Suzuki, K. Sugihara, Y. Kawabata, N. Suzuki, T. Sasaki, K. Horiuchi, N. Kanayama, Immunohistochemical detection of meconium in the fetal membrane, placenta and umbilical cord, Placenta. 33 (2012) 24–30.
[22] K. Töro, K. Borka, M. Kardos, I. Kristóf, P. Sótonyi, Expression and function of C5a receptor in a fatal anaphylaxis after honey bee sting, J. Forensic Sci. 56 (2011) 526–528.
[23] P. Girard, H. Mal, J.P. Laine, P. Petitpretz, B. Rain, P. Duroux, Left heart failure in amniotic fluid embolism, Anesthesiology 64 (1986) 262–265.
[24] S.L. Clark, D.B. Cotton, B. Gonik, J. Greenspoon, J.P. Phelan, Central hemodynamic alterations in amniotic fluid embolism, Am. J. Obstet. Gynecol. 158 (1988) 1124–1126.
[25] G.J. Locksmith, Amniotic fluid embolism, Obstet. Gynecol. Clin. North Am. 26 (1999) 435–444.
[26] M.D. Benson, R.E. Lindberg, Amniotic fluid embolism, anaphylaxis, and tryptase, Am. J. Obstet. Gynecol. 175 (1996) 737.
[27] M.D. Benson, H. Kobayashi, R.K. Silver, H. Oi, P.A. Greenberger, T. Terao, Immunologic studies in presumed amniotic fluid embolism, Obstet. Gynecol. 97 (2001) 510–514.
[28] M.P. Margarson, Delayed amniotic embolism following caeserean section under spinal anaesthesia, Anaesthesia 50 (1995) 804–806.
[29] P. Malhotra, R. Agarwal, A.A.D.A.S. Awasthi, D. Behera, Delayed presentation of amniotic fluid embolism: lessons from a case diagnosed at autopsy, Respirology 12 (2007) 148–150.
[30] M. Takeuchi, J. Yoshimatsu, N. Kanayama, T. Ikeda, Pathology and Clinical Medicine 30 (special edition) (2012) 62–68.

Please cite this article in press as: W. Hikiji, et al., Fatal amniotic fluid embolism with typical pathohistological, histochemical and clinical features, Forensic Sci. Int. (2012), http://dx.doi.org/10.1016/j.forsciint.2012.12.008

Clin Physiol Funct Imaging (2013) 33, pp109–116 doi: 10.1111/cpf.12001

Cerebral oxygen saturation evaluated by near-infrared time-resolved spectroscopy (TRS) in pregnant women during caesarean section – a promising new method of maternal monitoring

Kaori Yamazaki[1†], Kazunao Suzuki[1†], Hiroaki Itoh[1], Keiko Muramatsu[1], Kotomi Nagahashi[1], Naoaki Tamura[1], Toshiyuki Uchida[1], Kazuhiro Sugihara[1], Hideki Maeda[2] and Naohiro Kanayama[1]

[1]Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, and [2]Hamamatsu Photonics K.K., Hamamatsu, Japan

Summary

Time-resolved spectroscopy (TRS-20) measures tissue oxygen saturation (%) by evaluating the absolute concentrations of oxygenated, deoxygenated and total haemoglobin based on measurement of the transit time of individual photons through a tissue of interest. We measured tissue oxygen saturation in the prefrontal lobes of the brain by TRS-20 in eighteen pregnant women during caesarean section. In a case of placenta previa, massive bleeding immediately decreased cerebral oxygen saturation from 67·2% to 54·2%, but did not alter peripheral tissue oxygenation as measured by pulse oximetry. Four cases of pre-eclampsia revealed chronic changes in elevated base levels of cerebral oxygen saturation, though peripheral oxygen saturation was similar to that in normotensive pregnant women. Average cerebral oxygen saturation in the cases of pre-eclampsia before the introduction of anaesthesia was 73·6 ± 4·4 (SD)% (n = 4), significantly higher than in normotensive pregnant women, 67·2 ± 4·3% (n = 13, P<0·05). Z-scores of cerebral oxygen saturation prior to anaesthesia positively correlated with those of systolic or diastolic blood pressure. TRS-20 could detect acute as well as chronic changes in brain oxygen saturation in response to pregnancy-associated complications.

Correspondence

Hiroaki Itoh, Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 341-3192, Japan

E-mail: hitou-endo@umin.ac.jp

†First two authors equally contributed to the study.

Accepted for publication

Received 07 March 2012;
accepted 20 August 2012

Key words

brain; caesarean section; near-infrared spectroscopy; oxygenation; pregnancy

Introduction

Caesarean section is the most frequently performed laparotomy in modern medicine. However, during the procedure, pregnancy-specific complications can suddenly affect maternal brain circulation, such as hypertensive encephalopathy associated with eclampsia (Marik, 2009), intracerebral haemorrhage (Marik, 2009), cardiopulmonary shock caused by amniotic fluid embolism (Lee *et al.*, 2010), disseminated intravascular coagulation, massive blood loss as a result of uterine atonic bleeding or operative procedure of placenta previa or accreta (Nisar & Sohoo, 2009), etc., in addition to compilations from the anaesthesia, such as hypotension, desaturation, anaphylaxis, etc. (Chau-in *et al.*, 2010; Chow *et al.*, 2011). These complications can occur suddenly even in low-risk pregnancies. Indeed, maternal brain damage during caesarean section has been reported (Roopnarinesingh *et al.*, 1991). Moreover, it is recommended that a perimortem caesarean delivery be performed within 4 min of maternal cardiac arrest, which could improve maternal as well as neonatal outcomes (Katz *et al.*, 1986, 2005). Therefore, appropriate monitoring of the oxygenation of organs critical to maternal mortality and motility, that is, the brain, is needed to prepare for any sudden deterioration in maternal cerebral circulation.

The monitoring of brain oxygenation in perioperative care has undergone radical change in recent years owing to the introduction of non-invasive measurement techniques, particularly near-infrared spectroscopy (NIRS) (Jobsis, 1977) and its subsequent application to the assessment of tissue oxygenation during anaesthesia (Lovell *et al.*, 1997a,b; (Madsen & Secher, 1999). In NIRS, the different absorptive properties of oxygenated and deoxygenated haemoglobin are used to evaluate oxygen metabolism. NIRS has been utilized in various clinical fields all over the world (Mathieu & Mani, 2007; Vardi & Nini, 2008). A number of approaches to measuring tissue oxygenation using several types of NIRS have been proposed; that is, continuous wave near-infrared spectroscopy (Miura *et al.*, 2000) by which only relative values of tissue oxygenation can be measured; phase-modulated spectroscopy (Franceschini *et al.*, 2002) by which amplitude signals for

phase, intensity and depth of modulation after passage can be measured; and tissue oxygen index, by which we successfully assessed the relative oxygenation of the human and porcine placenta in normal and pathological states, such as foetal growth restriction (Kawamura *et al.*, 2007; Kakogawa *et al.*, 2010a), placental chorangiosis (Suzuki *et al.*, 2009), acute foetal hypoxia (Suzuki *et al.*, 2012) and pregnancy-induced hypertension (Kakogawa *et al.*, 2010b). With these methods, for converting the absorption of light to absolute concentrations of total haemoglobin and oxyhaemoglobin, it is necessary to measure the path length of light from the source to detector, because path length has been shown to be affected by factors such as skin and subcutaneous fat (McCully & Hamaoka, 2000). Therefore, the absolute concentration of oxygenated and deoxygenated haemoglobin cannot be accurately determined and the relative status of tissue oxygenation assessed, because path length cannot be measured.

By contrast, TRS-20, a new near-infrared time-resolved spectroscopic system, has high data acquisition and can calculate tissue oxygen saturation by evaluating the absolute concentrations of oxygenated, deoxygenated and total haemoglobin through measuring the transit time of photons through a tissue of interest (Oda *et al.*, 1999, 2000; Hamaoka *et al.*, 2000; Ijichi *et al.*, 2005a,b; Yamada *et al.*, 2008).

In this study, it was hypothesized that measurements of tissue oxygen saturation by TRS-20 in the prefrontal lobes of pregnant women undergoing a caesarean section are promising for the conventional assessment of changes in brain oxygenation in response to acute and/or chronic pregnancy-associated complications. To test the hypothesis, we measured tissue oxygen saturation in the brains of eighteen pregnant women during caesarean deliveries.

Materials and methods

Subjects

Eighteen pregnant women 23–42 years old (mean age 33·4 ± 5·7 years) who underwent a caesarean section at Hamamatsu University Hospital from May 2010 to January 2011 were enrolled (Table 1). Fourteen of the women were normotensive and underwent a caesarean delivery because of a previous caesarean section (nine cases), foetal growth restriction (two cases), twins (one case) and placenta previa (two cases). One case of placenta previa, in which the patient suffered massive bleeding (3750 ml), was assessed independently of the other thirteen normotensive cases (no more than 1760 ml blood loss) (Table 1). Four pregnancies were complicated with pre-eclampsia diagnosed according to the Report of the National High Blood Pressure Education Programme Working Group on High Blood Pressure in Pregnancy (High Blood Pressure Education Program Working Group, 2000) (Table 1). Informed consent was obtained after full explanation of the study.

Table 1 Clinical features of the subjects.

Group	*n*	Age	Weeks of gestation	Total bleeding (ml)
Normotensive	13	31·5 ± 11·5	36·4 ± 8·4	668·9 ± 721·1 (280–1760)
Normotensive with massive bleeding	1	35	36	3750
Pre-eclampsia	4	33·8 ± 8·8	36·6 ± 5·5	711·3 ± 221·3 (490–850)

Fluid loading (ml kg^{-1}) was illustrated in the middle panel of each Figure. After initial use of 500 ml SALINHES fluid solution 6%® (Furesenius Kabi Japan, Tokyo, Japan; Table 2A), BICARBON® (AJINOMOTO Pharmaceutical Co., Ltd, Tokyo Japan; Table 2B) was used through the operation (Figs 1 and 3). Saviozol Injection ® (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan, Table 2C) was used in cases of massive bleeding (Fig. 2). The procedures of anaesthesia and operation and/or administration of phenylephrine were described in the lower panel of each Figure.

Measurements

Regional cerebral blood flow was estimated by measuring oxygenated haemoglobin, deoxygenated haemoglobin and total haemoglobin concentrations in the prefrontal lobes using TRS-20 (Hamamatsu Photonics K.K., Japan) (Oda *et al.*, 1999, 2000). Two fibre optic bundles (optodes), emitting and collecting near-infrared pulsed laser light, were fixed on both sides of the forehead of pregnant women with an interoptode distance of 4 cm. TRS-20 was used to evaluate the oxygenation of the prefrontal lobes, because hair could affect the transit time of each photon. Brain tissue oxygenation was calculated and expressed as a percentage by evaluating absolute

Table 2 Fluids used during the operation.

Ingredient	In 500 ml (g)
(A) SALINHES fluid solution 6%® (Furesenius Kabi Japan)	
Hydroxyethylated Starch 70000	30·00
Sodium chloride	4·5
(B) BICARBON® (AJINOMOTO Pharmaceutical Co., Ltd)	
Sodium chloride	3·07
Potassium chloride	0·15
Calcium chloride hydrate	0·11
Magnesium chloride	0·051
Sodium hydrogen carbonate	1·05
Sodium citrate hydrate	0·245
(C) Saviozol Injection® (Otsuka Pharmaceutical Co., Ltd)	
Dextran40	15·00
Calcium chloride hydrate	0·10
Potassium chloride	0·15
Sodium chloride	3·00
L-Lactate$^-$	1·55

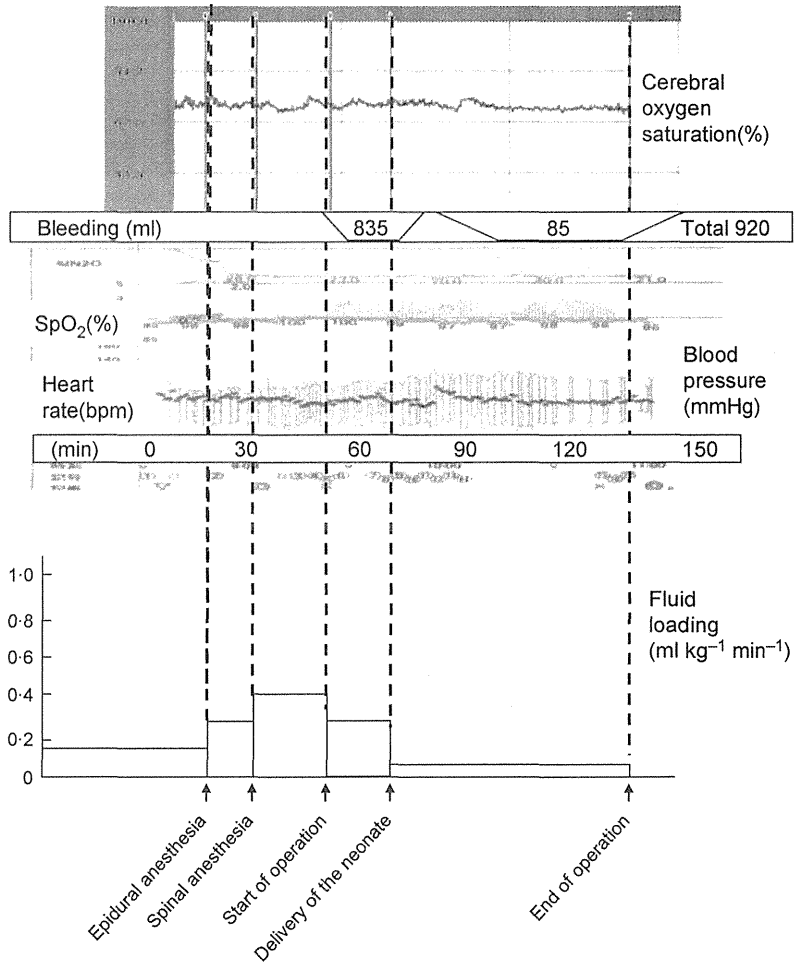

Figure 1 Changes in cerebral tissue oxygen saturation during a caesarean section in a normotensive pregnant woman with ordinary bleeding.

concentrations of oxygenated haemoglobin, deoxygenated haemoglobin and total haemoglobin. In brief, the light source is a graded index type single fibre with a numerical aperture (NA) of 0·25 and a core 200 μm in diameter, and the light detector is a bundle of fibre with a diameter of 3 mm and NA of 0·21. Finally, a set of histograms of photon flight time, known as a re-emission profile, was recorded. One temporal re-emission profile includes 1024 time channels spanning about 10 ns in steps of about 10 ps. In this study, the emerging light was collected over a period of 2 s to exceed at least one thousand counts of photons at the peak channel of the re-emission profiles. The instrumental response was measured with an input fibre placed opposite the receiving fibre through a neutral density filter. The instrumental response of the TRS-20 system was around 150 ps FWHM at each wavelength. The mean optical path length was calculated from the difference between the centre of gravity of the measured re-emission profile and that of the instrumental response. Changes in oxygenated haemoglobin, deoxygenated haemoglobin, total haemoglobin and the sum of oxygenated haemoglobin and deoxygenated haemoglobin were calculated using photon intensity and mean path length.

The oxygenation of maternal peripheral haemoglobin was measured by pulse oximetry at the fingertips (IntelliVue X2; PHILIPS Electronics Japan, Tokyo, Japan). A cuff was placed around the upper arm and blood pressure was measured intermittently (IntelliVue X2; PHILIPS Electronics Japan). The measurements started before the introduction of anaesthesia and continued until the end of the caesarean section.

Approval

The Ethics Committee of the Hamamatsu University School of Medicine approved the use of an optimal density of near-infrared rays up to 200 mW cm^{-2} for assessing foetuses by the transabdominal approach. We used TRS-20 in this study, because the optimal density of TRS-20 is <25 mW cm^{-2}, much lower than 200 mW cm^{-2}.

Statistical analysis

Data are expressed as means ± SDs, and the statistical significance of differences between two mean values was assessed using the Student's t-test or Mann–Whitney U-test, as

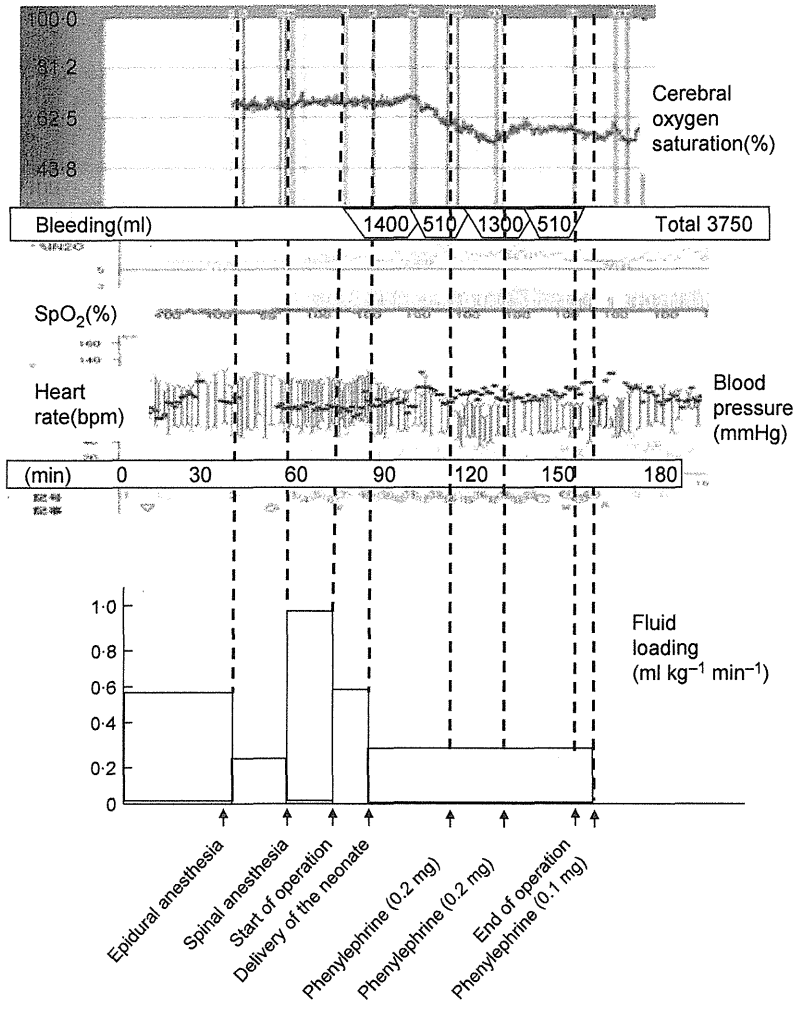

Figure 2 Changes in cerebral tissue oxygen saturation during a caesarean section in a normotensive pregnant woman with massive (3750 ml) bleeding. The blood transfusion was carried out after the operation.

appropriate. Z-scores were calculated using the formula [(data − mean of the population)/(standard deviation of the population)] (Larsen, 2000). Spearman's rank correlation coefficient was calculated between z-scores of two different parameters. A P value of $<0{\cdot}05$ was regarded as statistically significant.

Results

Cerebral oxygen saturation in normotensive women with ordinary bleeding during a caesarean section

Figure 1 shows representative changes of tissue oxygen saturation in the prefrontal lobes of normotensive pregnant women. Average cerebral tissue oxygenation was $67{\cdot}2 \pm 4{\cdot}3$ (SD)% (n = 13) before the anaesthesia and $66{\cdot}3 \pm 4{\cdot}2$% (n = 13) after the operation. This decrease was not significant. A baseline ripple was observed in cerebral tissue oxygen saturation throughout, with no remarkable changes observed during the operative procedures (Fig. 1). By contrast, a rather stable baseline was observed in peripheral tissue oxygen saturation by pulse oximetry (97–100%) (Fig. 1).

Change of cerebral oxygen saturation in a normotensive pregnant woman with massive bleeding during a caesarean section

Figure 2 shows changes of tissue oxygen saturation in the prefrontal lobes of a pregnant woman with placenta previa who suffered massive atonic bleeding after closing the uterine suture. In accordance with the rapid increase in atonic bleeding, cerebral tissue oxygen saturation began to decrease from 67·2% to 54·2% and remained low until the end of the operation (Fig. 2). Interestingly, the increased bleeding did not affect peripheral tissue oxygen saturation determined by pulse oximetry (99–100%) (Fig. 2).

Cerebral oxygen saturation in women with pre-eclampsia during a caesarean section

Figure 3 shows representative changes of tissue oxygen saturation in the prefrontal lobes of pregnant women with pre-eclampsia. Average cerebral oxygen saturation before the anaesthesia was $73{\cdot}6 \pm 4{\cdot}4$ (SD)% (n = 4), significantly

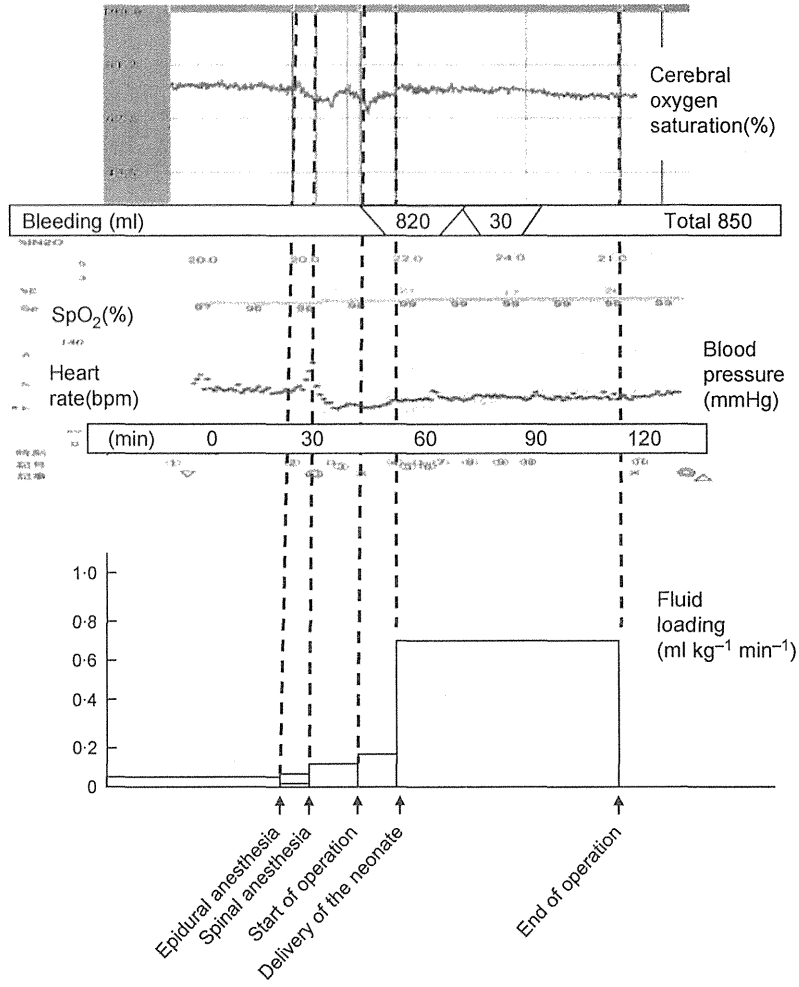

Figure 3 Changes in cerebral tissue oxygen saturation during caesarean section in a pregnant woman complicated with pre-eclampsia.

higher than in normotensive pregnant women, 67·2 ± 4·3% (n = 13, P<0·05). Average cerebral oxygen saturation decreased slightly from the introduction of anaesthesia, 73·6 ± 4·4% (n = 4), to the end of the operation, 72·7 ± 5·1% (n = 4), but not significantly. Transient decreases were observed in the cerebral tissue oxygen saturation approximately in accordance with the transient decrease in blood pressure (Fig. 2). By contrast, a rather stable baseline (98–100%) was observed in peripheral tissue oxygen saturation by pulse oximetry (Fig. 3).

Correlation between z-scores of cerebral tissue oxygen saturation and of systolic or diastolic blood pressure in all the pregnant women enrolled

Z-scores of systolic blood pressure positively correlated with those of cerebral tissue oxygenation (P<0·05; Fig. 4a). Z-scores of diastolic blood pressure positively correlated with those of cerebral tissue oxygenation (P<0·05; Fig. 4b). There was no significant correlation between z-scores of systolic or diastolic pressure and those of peripheral tissue oxygenation by pulse oximetry (data not shown).

Discussion

In the present study, we successfully measured tissue oxygen saturation in the prefrontal lobes of pregnant women using TRS-20 during the perioperative period of caesarean deliveries. Both acute and chronic changes in maternal circulatory status affected cerebral oxygenation. Acute massive bleeding rapidly decreased cerebral oxygen saturation (Fig. 2) and chronic pre-eclampsia significantly elevated the baseline of oxygenation in parallel with an increase in blood pressure (Figs 3 and 4a,b). Cerebral oxygen saturation decreased soon after the progress of massive bleeding, while it was not associated with the changes of fluid loading, administration of phenylephrine, nor procedures of anaesthesia and operation (Fig. 2). Nevertheless, these critical changes did not affect maternal peripheral oxygen saturation measured by pulse oximetry (Figs 1–3), frequently used by anaesthesiologists to

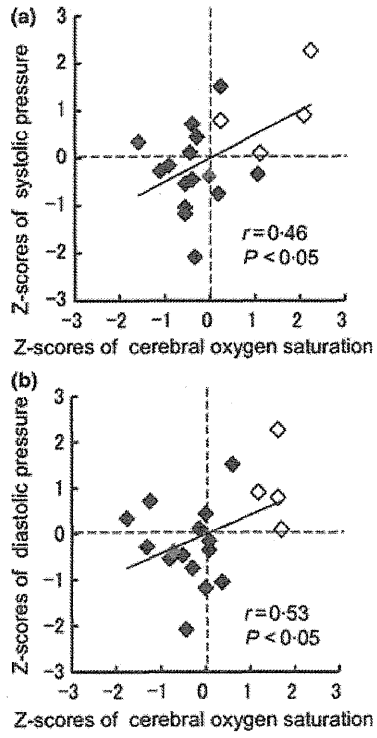

Figure 4 Correlation between z-scores of cerebral tissue oxygen saturation and those of systolic (a) and diastolic (b) blood pressure before introduction of anaesthesia in all the pregnant women enrolled. Black lozenges indicate normotensive pregnant women with ordinary bleeding. The grey lozenge indicates a normotensive pregnant woman with massive bleeding (3750 ml). White lozenges indicate pregnant women with pre-eclampsia.

obtain information on maternal circulation (Pedersen *et al.*, 2009) in addition to the cardiovascular information from continuous electrocardiography and intermittent/continuous measurements of blood pressure. Indeed, a systemic review of the literature shows that pulse oximetry can detect hypoxaemia and related events but cannot improve the outcome of patients in surgery (Bardenheuer & Brack, 1996). One reason for this could be that information on the peripheral oxygenation of maternal haemoglobin dose not always represent the tissue oxygenation of critical and sensitive organs, such as the brain and splanchnic organs (Wilson & Shapiro, 2001). As TRS-20 could detect a rapid decrease in cerebral tissue oxygenation in response to massive bleeding during a caesarean section even though peripheral oxygenation did not change, it would provide the timely as well as appropriate information for the clinical intervention to prevent brain damage even in cases of unexpected sudden maternal deterioration. A large-scale cohort study is necessary to clarify the sensitivity and specificity of TRS-20 in response to the amount of bleeding during caesarean section. It was reported that TRS-20 could be a promising sensor in the assessment of vasospasm in subarachnoid haemorrhage (Yokose *et al.*, 2010) and the effect of treatment for cerebral arteriovenous fistula (Hoshino *et al.*, 2010).

It is reasonable that the acute decrease in circulating blood caused by massive blood loss decreased cerebral tissue oxygenation (Fig. 2); however, it was a paradox that pre-eclampsia elevated the baseline of cerebral tissue oxygen saturation (Fig. 3). NIRS revealed that cerebral oxygenation decreases with brain ischaemia (Pennekamp *et al.*, 2009) but could increase in cerebral hyperperfusion syndrome (Ogasawara *et al.*, 2003; Komoribayashi *et al.*, 2006; Pennekamp *et al.*, 2009). Interestingly, increasing evidence has revealed the possible involvement of cerebral hyperperfusion in pre-eclampsia/eclampsia, especially when complicated by posterior reversible encephalopathy syndrome (PRES) (Takeuchi *et al.*, 2005; Bartynski, 2008a,b). Recently, it was hypothesized that maternal hypertension leads to failed vascular autoregulation, and subsequent hyperperfusion, with endothelial injury/vasogenic oedema in PRES (Bartynski, 2008b). Currently, this hypothesis is more popular than the classical one that vasoconstriction and hypoperfusion lead to brain ischaemia and subsequent vasogenic oedema in PRES (Bartynski, 2008b). As there were few clinical symptoms of eclampsia or encephalopathy associated with PRES in the present four cases of pre-eclampsia, we did not carry out brain MRI and so no information is available regarding the presence or absence of brain oedema. Nevertheless, the high baseline of cerebral tissue oxygen saturation suggests that a tendency for cerebral hyperperfusion might exist in the greater part of pre-eclampsia even without apparent clinical symptoms of encephalopathy. As the increase in cerebral tissue oxygen saturation paralleled the rise in blood pressure (Fig. 4a,b), there might be a dose-dependent causative association between elevated blood pressure and possible cerebral hyperperfusion in women. A larger cohort is necessary to test this speculation.

The present results should be interpreted with caution, because two fibre optic bundles (optodes) were fixed on the forehead of pregnant women to evaluate oxygenation saturation in the prefrontal lobes considering that hair could affect the transit time of each photon and that it would be convenient for routine monitoring in caesarean section. However, the oedema usually predominates in the parietal and occipital regions of the brain in PRES (Bartynski, 2008a). Therefore, a different study design is preferable to clarify further the possible association between elevated cerebral tissue oxygenation and PRES or its preceding changes.

In conclusion, we applied TRS-20 to eighteen pregnant women during caesarean deliveries and successfully measured oxygenation saturation in the prefrontal lobes of the brain. Massive bleeding immediately and markedly decreased cerebral oxygen saturation, while peripheral oxygen saturation remained stable. Four cases of pre-eclampsia revealed chronic changes in the baseline of cerebral oxygen saturation, while peripheral oxygen saturation was similar to that in normotensive pregnant women. As TRS-20 can detect both acute and chronic changes of brain oxygen saturation in response to pregnancy-associated complications, it is a promising new tool for maternal monitoring in caesarean deliveries.

Acknowledgments

The authors thank Mrs Nahoko Hakamada, Mrs Yumiko Yamamoto, Mrs Naoko Kondo, Mrs Miuta Sawai, Mrs Kazuko Sugiyama and Mrs Yoriko Morishima for secretarial or technical assistance. This study was supported in part by Grants-in-aid for Scientific Research from the Ministry of Education, Science, Culture and Sports, Japan (No. 22591820, No. 21390322 and No. 23659534) and grants from the Kanzawa Medical Research Foundation, Society for Women's Health Science Research, The Food Science Institute Foundation, and Hamamatsu Foundation for Science and Technology Promotion.

Conflict of interest

The authors declare no conflict of interest.

References

Bardenheuer HJ, Brack A. Problems and limits in noninvasive oxygen monitoring. *Anaesthesist* (1996); **45**: 614–625.

Bartynski WS. Posterior reversible encephalopathy syndrome, part 1: fundamental imaging and clinical features. *AJNR Am J Neuroradiol* (2008a); **29**: 1036–1042.

Bartynski WS. Posterior reversible encephalopathy syndrome, part 2: controversies surrounding pathophysiology of vasogenic edema. *AJNR Am J Neuroradiol* (2008b); **29**: 1043–1049.

Chau-in W, Hintong T, Rodanant O, Lekprasert V, Punjasawadwong Y, Charuluxananan S, Tanudsintum S. Anesthesia-related complications of caesarean delivery in Thailand: 16,697 cases from the Thai Anaesthesia Incidents Study. *J Med Assoc Thai* (2010); **93**: 1274–1283.

Chow L, Farber MK, Camann WR. Anesthesia in the pregnant patient with hematologic disorders. *Hematol Oncol Clin North Am* (2011); **25**: 425–443.

Franceschini MA, Boas DA, Zourabian A, Diamond SG, Nadgir S, Lin DW, Moore JB, Fantini S. Near-infrared spiroximetry: noninvasive measurements of venous saturation in piglets and human subjects. *J Appl Physiol* (2002); **92**: 372–384.

Hamaoka T, Katsumura T, Murase N, Nishio S, Osada T, Sako T, Higuchi H, Kurosawa Y, Shimomitsu T, Miwa M, Chance B. Quantification of ischemic muscle deoxygenation by near infrared time-resolved spectroscopy. *J Biomed Opt* (2000); **5**: 102–105.

High Blood Pressure Education Program Working Group. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol* (2000); **183**: S1–S22.

Hoshino T, Sakatani K, Yokose N, Awano T, Nakamura S, Murata Y, Kano T, Katayama Y. Changes in cerebral blood oxygenation and hemodynamics after endovascular treatment of vascular malformation measured by time-resolved spectroscopy. *Adv Exp Med Biol* (2010); **662**: 491–496.

Ijichi S, Kusaka T, Isobe K, Islam F, Okubo K, Okada H, Namba M, Kawada K, Imai T, Itoh S. Quantification of cerebral hemoglobin as a function of oxygenation using near-infrared time-resolved spectroscopy in a piglet model of hypoxia. *J Biomed Opt* (2005a); **10**: 024026.

Ijichi S, Kusaka T, Isobe K, Okubo K, Kawada K, Namba M, Okada H, Nishida T, Imai T, Itoh S. Developmental changes of optical properties in neonates determined by near-infrared time-resolved spectroscopy. *Pediatr Res* (2005b); **58**: 568–573.

Jobsis FF. Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science* (1977); **198**: 1264–1267.

Kakogawa J, Sumimoto K, Kawamura T, Minoura S, Kanayama N. Noninvasive monitoring of placental oxygenation by near-infrared spectroscopy. *Am J Perinatol* (2010a); **27**: 463–468.

Kakogawa J, Sumimoto K, Kawamura T, Minoura S, Kanayama N. Transabdominal measurement of placental oxygenation by near-infrared spectroscopy. *Am J Perinatol* (2010b); **27**: 25–29.

Katz VL, Dotters DJ, Droegemueller W. Perimortem cesarean delivery. *Obstet Gynecol* (1986); **68**: 571–576.

Katz V, Balderston K, DeFreest M. Perimortem cesarean delivery: were our assumptions correct? *Am J Obstet Gynecol* (2005); **192**: 1916–1920, discussion 1920–1921.

Kawamura T, Kakogawa J, Takeuchi Y, Takani S, Kimura S, Nishiguchi T, Sugimura M, Sumimoto K, Kanayama N. Measurement of placental oxygenation by transabdominal near-infrared spectroscopy. *Am J Perinatol* (2007); **24**: 161–166.

Komoribayashi N, Ogasawara K, Kobayashi M, Saitoh H, Terasaki K, Inoue T, Ogawa A. Cerebral hyperperfusion after carotid endarterectomy is associated with preoperative hemodynamic impairment and intraoperative cerebral ischemia. *J Cereb Blood Flow Metab* (2006); **26**: 878–884.

Larsen RJ, Marx ML. *An Introduction to Mathematical Statistics and Its Applications*, 3rd edn. (2000). Prentice Hall, Upper Saddle River.

Lee JH, Yang HJ, Kim JH, Lee SY, Gill HJ, Kim BK, Kim MG. Amniotic fluid embolism that took place during an emergent Cesarean section -A case report. *Korean J Anesthesiol* (2010); **59**(Suppl): S158–S162.

Lovell AT, Owen-Reece H, Elwell CE, Smith M, Goldstone JC. Continuous measurement of cerebral oxygenation by NIRS during induction of anaesthesia. *Adv Exp Med Biol* (1997a); **428**: 213–218.

Lovell AT, Owen-Reece H, Elwell CE, Smith M, Goldstone JC. Predicting oscillation in arterial saturation from cardiorespiratory variables. Implications for the measurement of cerebral blood flow with NIRS during anaesthesia. *Adv Exp Med Biol* (1997b); **428**: 629–638.

Madsen PL, Secher NH. Near-infrared oximetry of the brain. *Prog Neurobiol* (1999); **58**: 541–560.

Marik PE. Hypertensive disorders of pregnancy. *Postgrad Med* (2009); **121**: 69–76.

Mathieu D, Mani R. A review of the clinical significance of tissue hypoxia measurements in lower extremity wound management. *Int J Low Extrem Wounds* (2007); **6**: 273–283.

McCully KK, Hamaoka T. Near-infrared spectroscopy: what can it tell us about oxygen saturation in skeletal muscle? *Exerc Sport Sci Rev* (2000); **28**: 123–127.

Miura H, Araki H, Matoba H, Kitagawa K. Relationship among oxygenation, myoelectric activity, and lactic acid accumulation in vastus lateralis muscle during exercise with constant work rate. *Int J Sports Med* (2000); **21**: 180–184.

Nisar N, Sohoo NA. Emergency peripartum hysterectomy: frequency, indications and maternal outcome. *J Ayub Med Coll Abbottabad* (2009); **21**: 48–51.

Oda M, Yamashita Y, Nakano T, Suzuki A, Shimizu K, Hirano I, Shimomura F, Ohmae E, Suzuki T, Tsuchiya Y. Near infrared time-resolved spectroscopy system for tissue oxygenation monitor oxygenation monitor. *SPIE* (1999); **3597**: 611–617.

Oda M, Yamashita Y, Nakano T, Suzuki A, Shimizu K, Hirano I, Shimomura F, Ohmae E, Suzuki T, Tsuchiya Y. Near infrared time-resolved spectroscopy system for tissue oxy-

genation monitor. *SPIE* (2000); **4160**: 204–210.

Ogasawara K, Konno H, Yukawa H, Endo H, Inoue T, Ogawa A. Transcranial regional cerebral tissue oxygen saturation monitoring during carotid endarterectomy as a predictor of postoperative hyperperfusion. *Neurosurgery* (2003); **53**: 309–314, discussion 314–315.

Pedersen T, Dyrlund Pedersen B, Moller AM. Pulse oximetry for perioperative monitoring. *Cochrane Database Syst Rev* (2009); CD002013.

Pennekamp CW, Bots ML, Kappelle LJ, Moll FL, de Borst GJ. The value of near-infrared spectroscopy measured cerebral oximetry during carotid endarterectomy in perioperative stroke prevention. A review. *Eur J Vasc Endovasc Surg* (2009); **38**: 539–545.

Roopnarinesingh S, Ramoutar P, Bassaw B. Maternal mortality at Mount Hope Women's Hospital, Trinidad. *West Indian Med J* (1991); **40**: 139–141.

Suzuki K, Itoh H, Kimura S, Sugihara K, Yaguchi C, Kobayashi Y, Hirai K, Takeuchi K, Sugimura M, Kanayama N. Chorangiosis and placental oxygenation. *Congenit Anom (Kyoto)* (2009); **49**: 71–76.

Suzuki K, Itoh H, Muramatsu K, Yamazaki K, Furuta N, Nagahashi K, Tamura N, Makoto K, Uchida T, Sugihara K, Sumimoto K, Kanayama N. Transient ligation of umbilical vessels elevates placental tissue oxygen index (TOI) values measured by near-infrared spectroscopy (NIRS) in clawn miniature pig animal model. *Clin Exp Obstet Gynecol* (2012); In press.

Takeuchi M, Matsuzaki K, Harada M, Nishitani H, Matsuda T. Cerebral hyperperfusion in a patient with eclampsia with perfusion-weighted magnetic resonance imaging. *Radiat Med* (2005); **23**: 376–379.

Vardi M, Nini A. Near-infrared spectroscopy for evaluation of peripheral vascular disease. A systematic review of literature. *Eur J Vasc Endovasc Surg* (2008); **35**: 68–74.

Wilson WC, Shapiro B. Perioperative hypoxia. The clinical spectrum and current oxygen monitoring methodology. *Anesthesiol Clin North America* (2001); **19**: 769–812.

Yamada E, Kusaka T, Arima N, Isobe K, Yamamoto T, Itoh S. Relationship between muscle oxygenation and electromyography activity during sustained isometric contraction. *Clin Physiol Funct Imaging* (2008); **28**: 216–221.

Yokose N, Sakatani K, Murata Y, Awano T, Igarashi T, Nakamura S, Hoshino T, Kano T, Yoshino A, Katayama Y, Ohmae E, Suzuki T, Oda M, Yamashita Y. Bedside assessment of cerebral vasospasms after subarachnoid hemorrhage by near infrared time-resolved spectroscopy. *Adv Exp Med Biol* (2010); **662**: 505–511.

Successful Living Donor Liver Transplantation for Fulminant Hepatic Failure That Manifested Immediately After Cesarean Delivery

Yoshimasa Horikoshi,* Hiroaki Itoh,* Suguru Kikuchi,* Toshiyuki Uchida,* Kazunao Suzuki,* Kazuhiro Sugihara,* Naohiro Kanayama,* Akira Mori,† and Shinji Uemoto†

A 31-year-old pregnant woman was diagnosed as having acute hepatitis of unknown etiology and conservatively treated. An emergency cesarean delivery was performed 5 days later at 33 weeks and 3 days of gestation because of a gradual deterioration in liver function. Two days after the cesarean delivery, she lost consciousness in the evening (Glasgow coma scale [GCS] = 9) because of hepatic encephalopathy and was diagnosed as having fulminant hepatic failure (FHF). Five days after the cesarean delivery, the patient (blood type B) underwent a successful left lobe with caudate lobe (S1+2+3+4) liver transplantation from her father (blood type AB), an ABO-incompatible donor. At 1 year follow-up, she and her baby are in good medical condition. The drastic deterioration in hepatic function, despite intensive plasmapheresis and continuous hemodiafiltration, during the early postpartum period suggested a possible causative association between the termination of pregnancy and progression of FHF from acute hepatitis of unknown etiology. *ASAIO Journal* 2012; 58:174–176.

Fulminant hepatic failure (FHF) has been reported at various points during pregnancy.[1,2] A gold standard for treatment has not yet been established, especially concerning the timing of termination of pregnancy, because the condition can have serious consequences not only for the mother but also for the fetus.[3] There is limited experience in liver transplantation during pregnancy or the early postpartum period.[4] We here report a case of acute hepatitis that progressed to FHF 2 days after cesarean delivery in the third trimester of pregnancy. Intensive plasmapheresis and continuous hemodiafiltration according to Japanese style of artificial liver support (ALS) did not improve her hepatic coma but gave her a greater chance of undergoing successful living donor transplantation at a different clinical institution located in another prefecture.

From the *Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, Hamamatsu, Japan; and †Department of Transplantation, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Submitted for consideration August 2011; accepted for publication in revised form November 2011.

Disclosure: The authors have no conflicts of interest to report.

Reprint Requests: Hiroaki Itoh, MD, DMedSci, Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 341-3192, Japan. Email: hitou-endo@umin.ac.jp.

DOI: 10.1097/MAT.0b013e3182444ed4

Case Report

A 31-year-old gravida 1 and parous 1 woman became pregnant. Neither the patient nor her family had any history of hepatic disease. At 32 weeks and 6 days of gestation, she complained of epigastric pain and consulted a local outpatient clinic. She was transferred to Hamamatsu University Hospital because of elevated liver enzyme levels. On physical examination, she presented with severe jaundice, a mild fever of 37.8°C, and tachycardia of 116 beats/min, although her blood pressure and urinalysis were normal. Mild tenderness was observed in the right hypochondriac area; however, abdominal ultrasonography of the liver was unremarkable. Laboratory evaluation showed alanine transaminase (ALT) 930 unit/L, aspartate aminotransferase (AST) 1553 unit/L, platelet count 299,000/μl, prothrombin time (PT) (international normalized ratio [INR] 1.01, 97%), blood urea nitrogen (BUN) 6.0 mg/dl, negative serologic tests for viral hepatitis A, B, C, and E, as well as Epstein-Barr (EB), herpes, and cytomegaloviruses. Electric fetal heart rate monitoring showed a reassuring fetal status. She was diagnosed as having acute hepatitis of unknown etiology and conservatively treated.

However, her liver function gradually deteriorated as shown in **Figure 1**, and an emergency cesarean delivery was performed 5 days after admission at 33 weeks and 3 days of gestation. An immature, male baby weighing 2,268 g was born with an Apgar score of 6 (1 minute) and subjected to a resuscitation procedure.

Two days after the cesarean delivery, she lost consciousness in the evening (Glasgow coma scale [GCS] = 9) because of hepatic encephalopathy and was diagnosed as having FHF, *i.e.*, PT (INR 1.86, 30%), BUN 2.3 mg/dl, and ammonia (NH_3) 235 μg/dl. Plasmapheresis and continuous hemodiafiltration were immediately performed in accordance with Japanese style of artificial liver support (ALS)[5] (**Figure 1**).

The ALS did not achieve recovery from coma in the patient, but provided more time for us to assess the indications for liver transplantation according to the Liver Transplantation Guideline published by the Acute Liver Failure Study Group of Japan (*i.e.*, 11 days from onset of hepatitis to hepatic coma, low percentage of PT, total bilirubin level more than 10 mg/dl, low ratio of direct to total bilirubin).[6] She was transferred to Kyoto University Hospital by helicopter 5 days after the cesarean delivery. On the same day, the patient (blood type B) underwent a successful left lobe with caudate lobe (S1+2+3+4) liver transplantation from her father (blood type AB), an ABO-incompatible donor. Rituximab 300 mg was administered during surgery. She regained

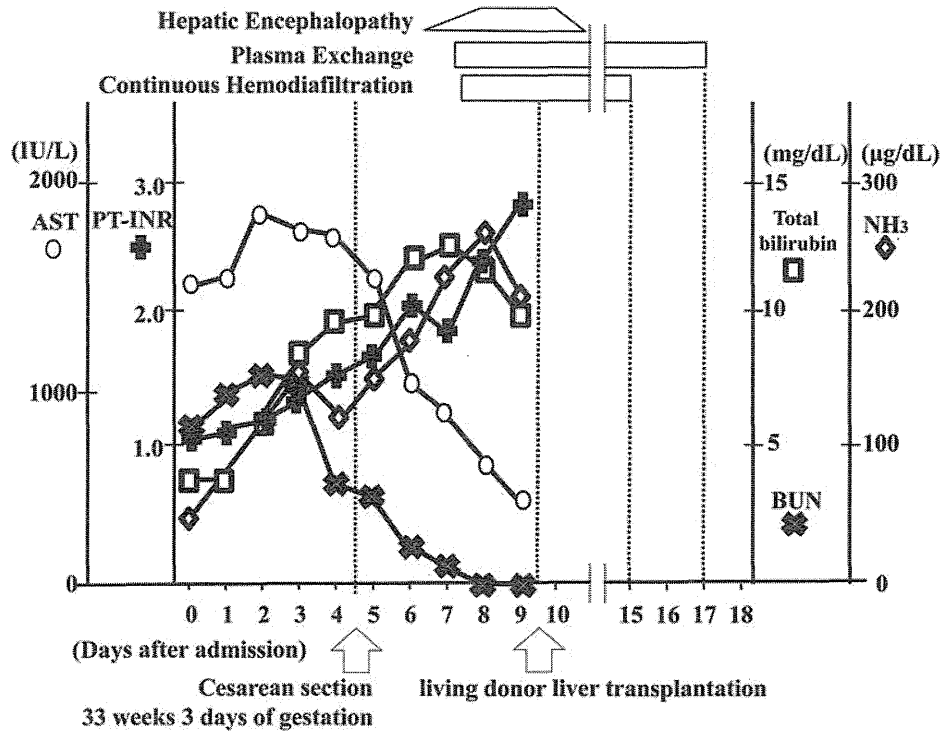

Figure 1. Longitudinal changes of the parameters associated with liver function. AST, aspartate aminotransferase; BUN, blood urea nitrogen; PT, prothrombin time; INR, international normalized ratio.

consciousness 1 day after the operation (**Figure 1**). Plasmapheresis and hemodiafiltration were continued until 8 and 6 days after the operation, respectively (**Figure 1**). Histology showed massive hepatic necrosis. She was discharged 64 days after transplantation. One year on, she and her baby are in good medical condition, although she demonstrated transient ascites 4 months after delivery because of acute cellular rejection.

Discussion

Some cases of FHF during pregnancy are based on diseases unique to pregnancy, such as hemolytic anemia, elevated liver enzymes and low platelet count (HELLP) syndrome, acute fatty liver of pregnancy, and preeclampsia, in which the hepatic dysfunction begins to resolve postpartum.[7,8] Other cases are usually regarded as similar to those seen in nonpregnant women.[9] However, it has been suggested that hepatitis B virus (HBV)-related FHF in pregnancy sometimes improves with delivery to a point suitable for surgical treatment[1,4,10] on modification of the biohumoral substrate[1,4] or stabilization of the viral parameters of hepatic function.[10]

In this case, however, acute hepatitis progressed to FHF 2 days after cesarean delivery (**Figure 1**). The ALS could not improve her coma but effectively reduced total bilirubin levels with a tendency to decrease NH_3 levels (**Figure 1**), which contributed to sustenance of the patient until living donor liver transplantation became available. We cannot deny the possibility that ALS could not remove some toxins.

As far as we know, there have been five case reports, including ours, of FHF rescued by liver transplantation early postpartum during the third trimester of pregnancy (**Figure 1**, **Table 1**).[11-14] These reports suggest that early termination of pregnancy may not always improve FHF. In view of the fact that a better prognosis for newborns is expected in the third trimester of pregnancy than in the second trimester, termination of pregnancy would be one of the therapeutic choices in cases of acute severe deterioration of maternal hepatic function especially in the third trimester. In such situations, it would be worth

Table 1. Reports of Liver Transplantation Soon After Delivery in the Third Trimester of Pregnancy

Reference (year)	Etiology	ALS	Mode of Delivery (w.g.)	Onset of FHF (d.a.d.)	LT (d.a.d.)	Maternal (Fetal) Outcome
Bourliere *et al.*[11]	Drug-induced	NS	CS (30)	1	1	Survived (survived)
Remiszewski *et al.*[12]	AFLP	BT	VD (33)	10	10	Survived (survived)
Gill *et al.*[13]	Drug-induced AFLP	BT	VD (33)	3	3	Survived (fetal death)
Ockner *et al.*[14]	AFLP	BT	CS (37)	1	3	Survived (survived)
This case	FH of unknown etiology	BT and AT	CS (32)	2	5	Survived (survived)

AFLP, acute fatty liver of pregnancy; CS, cesarean section, VD, vaginal delivery; FH, fulminant hepatitis; FHF, fulminant hepatic failure; LT, liver transplantation; w.g., weeks of gestation; d.a.d., days after delivery; ALS, artificial liver support; NS, no statement; BT, before transplantation (no statement after transplantation); and BT and AT, before and after transplantation.

considering a possible manifestation of FHF immediately after delivery, although the exact causative relationship between the onset of FHF and termination of pregnancy is unclear.

The early postpartum period is characterized by a dramatic withdrawal of placental steroid hormones, being associated with enormous changes in almost all maternal physiologic processes, such as cardiovascular, endocrine, immune, nutrient, and energy-metabolic functions.[15] It is plausible that these drastic physiologic changes after delivery might be associated with, at least partly, the acute deterioration of FHF in this case.

Conclusion

In conclusion, we successfully carried out living donor liver transplantation for a patient developing FHF 2 days after cesarean delivery in the third trimester of pregnancy. The drastic deterioration of hepatic function during the early postpartum period suggested that early termination of pregnancy may not always lead to the resolution of FHF.

Author Contributions

YH, HI, AM, and NK contributed toward writing the manuscript; YH, HI, SK, TU, and NK were responsible for the obstetric care of the patient; KS contributed toward image interpretation; and AM and SU performed the liver transplantation.

References

1. Jayanthi V, Udayakumar N: Acute liver failure in pregnancy: an overview. *Minerva Gastroenterol Dietol* 54: 75–84, 2008.
2. Paternoster DM, Gerace PF, Manganelli F, Boccagni P, Cillo U, D'Amico D: Acute hepatic failure in pregnancy. *Eur J Obstet Gynecol Reprod Biol* 112: 230–232, 2004.
3. Patra S, Kumar A, Trivedi SS, Puri M, Sarin SK: Maternal and fetal outcomes in pregnant women with acute hepatitis E virus infection. *Ann Intern Med* 147: 28–33, 2007.
4. Toti L, Manzia TM, Romano P, *et al*: Successful management of a same-day emergency delivery and liver transplant in a 27 weeks pregnant woman with fulminant hepatic failure. *Transpl Int* 23: 114–115, 2010.
5. Inoue K, Watanabe T, Maruoka N, Kuroki Y, Takahashi H, Yoshiba M: Japanese-style intensive medical care improves prognosis for acute liver failure and the perioperative management of liver transplantation. *Transplant Proc* 42: 4109–4112, 2010.
6. Mochida S, Nakayama N, Matsui A, Nagoshi S, Fujiwara K: Re-evaluation of the Guideline published by the Acute Liver Failure Study Group of Japan in 1996 to determine the indications of liver transplantation in patients with fulminant hepatitis. *Hepatol Res* 38: 970–979, 2008.
7. Rahman TM, Wendon J: Severe hepatic dysfunction in pregnancy. *QJM* 95: 343–357, 2002.
8. Cunningham FG, Leveno KJ, Bloom SL, Hauth SL, Rouse DJ, Spong CY (eds): Hepatic, gallbladder, and pancreatic disorders, in *Williams Obstetrics*. 23rd ed. New York, McGraw-Hill, 2010:1063–1078.
9. Jalan R: Acute liver failure: current management and future prospects. *J Hepatol* 42(suppl): S115–S123, 2005.
10. De Gasperi A: [Fulminant liver failure: intensive care, extracorporeal treatment and liver transplantation]. *G Ital Nefrol* 23(suppl 36): S61–S68, 2006.
11. Bourliere M, Le Treut YP, Manelli JC, *et al*: Chlormezanone-induced fulminant hepatitis in a pregnant woman: successful delivery and liver transplantation. *J Gastroenterol Hepatol* 7: 339–341, 1992.
12. Remiszewski P, Pawlak J, Skwarek A, *et al*: Orthotopic liver transplantation for acute liver failure resulting from "acute fatty liver of pregnancy". *Ann Transplant* 8: 8–11, 2003.
13. Gill EJ, Contos MJ, Peng TC: Acute fatty liver of pregnancy and acetaminophen toxicity leading to liver failure and postpartum liver transplantation. A case report. *J Reprod Med* 47: 584–586, 2002.
14. Ockner SA, Brunt EM, Cohn SM, Krul ES, Hanto DW, Peters MG: Fulminant hepatic failure caused by acute fatty liver of pregnancy treated by orthotopic liver transplantation. *Hepatology* 11: 59–64, 1990.
15. Cunningham FG, Leveno KJ, Bloom SL, Hauth SL, Rouse DJ, Spong CY (eds): Labor and delivery, in *Williams Obstetrics*. 23rd ed. New York, McGraw-Hill, 2010:374–576.

Thrombosis Research 129 (2012) e161–e163

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Letter to the Editors-in-Chief

The first report of uncontrollable subchorionic and retroplacental haemorrhage inducing preterm labour in complete PAI-1 deficiency in a human

Dear Editors,

Fig. 1

Plasminogen activator inhibitor-1 (PAI-1) is a member of the serine protease inhibitor (SERPIN) superfamily and is the primary physiological regulator of urokinase-type plasminogen activator and tissue-type plasminogen activator [1]. Although a number of studies have indicated that elevated levels of PAI-1 are associated with several pathological states such as arterial thrombotic events [2] and poor prognosis in cancer patients [3], our knowledge of the consequences of PAI-1 deficiency is still limited due to the rarity of this condition. We recently reported a case of genetically identified complete PAI-1 deficiency in a human [4]. The patient showed a tendency for massive bleeding, which was also observed in a PAI-1-deficient patient in a previous report [5].

Genetic alterations that lead to a predisposition for bleeding are associated with clinical complications during pregnancy. The most vivid example of this association is congenital afibrinogenemia and congenital coagulation factor XIII (FXIII) deficiency, which result in genital bleeding and spontaneous miscarriage in the first 6–8 weeks of gestation if left untreated [6,7].

The patient with complete PAI-1 deficiency we described recently [4] fell pregnant 3 times, at the age of 26, 27, and 29 years. Although we were unable to identify the reason for her increased bleeding tendency at the time, we attempted to manage her pregnancies. In this report, we describe the clinical course of these pregnancies. This is the first report describing the clinical course of pregnancies in a completely PAI-1-deficient human.

Case report

The patient was a 47-year-old woman who had experienced multiple episodes of major bleeding, as described previously [4]. The first pregnancy occurred at the age of 26 years. The pregnancy course was uneventful and foetal growth was normal until 16 weeks' gestation. At the end of 16 weeks' gestation, a small amount of genital bleeding was observed, and she was hospitalised. Her prothrombin time, activated partial thromboplastin time, and plasma fibrinogen levels were within the normal limits (13.0s, 34.9s, and 165 mg/dL, respectively); however, plasma D-dimer levels were slightly elevated (2.4 μg/mL, a normal range is<0.5 μg/mL). Fresh frozen plasma (FFP) was injected twice a week to control the genital bleeding and maintain her pregnancy. Plasma D-dimer levels were suddenly elevated at the end of 18 weeks' gestation, and massive genital bleeding was observed at 19 weeks' gestation. Although a retro-placental echo-free space was not observed, the foetal heart beat had stopped and the cervix of the patient's uterus was found to be fully opened. Plasma D-dimer levels were decreased and genital bleeding had stopped 1 week after removal of the foetus (220 g). The aborted foetus was male and his appearance was normal.

The second pregnancy occurred at the age of 27 years. Immediately after the confirmation of pregnancy at 7 weeks' gestation, she was admitted to our hospital. Although a small amount of genital bleeding was observed at 8 weeks' gestation, this pregnancy was uneventful until 11 weeks' gestation. A continuous but small amount of genital bleeding was observed after the end of the 11th week of gestation; therefore, FFP administration 2–3 times per week was initiated. Although the genital bleeding had stopped at 20 weeks' gestation, FFP administration was continued to stabilise the pregnancy. Because plasma D-dimer levels were slightly elevated at 28 weeks' gestation, the frequency of FFP administration was changed from 2–3 times per week to once a day. However, plasma D-dimer levels continued to be elevated, reaching 128 μg/mL at 32 weeks' gestation, accompanied by uncontrollable uterine contractions with grade 2 placental abruption diagnosed by ultrasonography. Hence, an emergency caesarean section was carried out; the patient delivered a live 1736-g female infant. Perioperative blood loss amounted to 4500 mL, which was controlled by the administration of 42 U FFP.

The third pregnancy occurred at the age of 29 years. Based on the successful management of the previous pregnancy, the patient was hospitalised at 8 weeks' gestation and continuous FFP administration was initiated. FFP was administered twice a week until 16 weeks' gestation, which was gradually increased until 19 weeks' gestation. After 20 weeks' gestation, FFP administration was performed every day. Although the pregnancy was stabilised until 24 weeks' gestation, plasma D-dimer levels were continuously elevated from 25 weeks' gestation, reaching 57.9 μg/mL at 27 weeks' gestation, accompanied by uncontrollable uterine contractions with placental abruption. An emergency caesarean section was again carried out; the patient delivered a live 978-g female infant. Perioperative blood loss amounted to 1037 mL. In both cases, the blood loss was calculated to measure the weight of the blood absorbed by gauze and/or sanitary napkins. These daughters were healthy and did not have any symptoms.

Discussion

We have intensively investigated the relationship between coagulation disorders and spontaneous miscarriage. Indeed, we have previously reported that fibrinogen and FXIII are critical factors for stabilising placental attachment to the uterus [6,8]. Pregnancies in patients with both afibrinogenemia and congenital FXIII deficiency result in spontaneous miscarriage at around 7–8 weeks of gestation in humans [6,8]. We have successfully managed such pregnancies with suitable supplemental therapies [9,10].

Coagulation factor deficiencies are very rare, and thus, it is difficult to investigate the mechanisms underlying the associated increased bleeding tendency during pregnancy. In order to overcome this difficulty, fibrinogen-deficient mice [11,12] and FXIII-deficient mice [13] were generated by gene manipulation methods. Afibrinogenemia in

0049-3848/$ – see front matter
doi:10.1016/j.thromres.2011.10.008

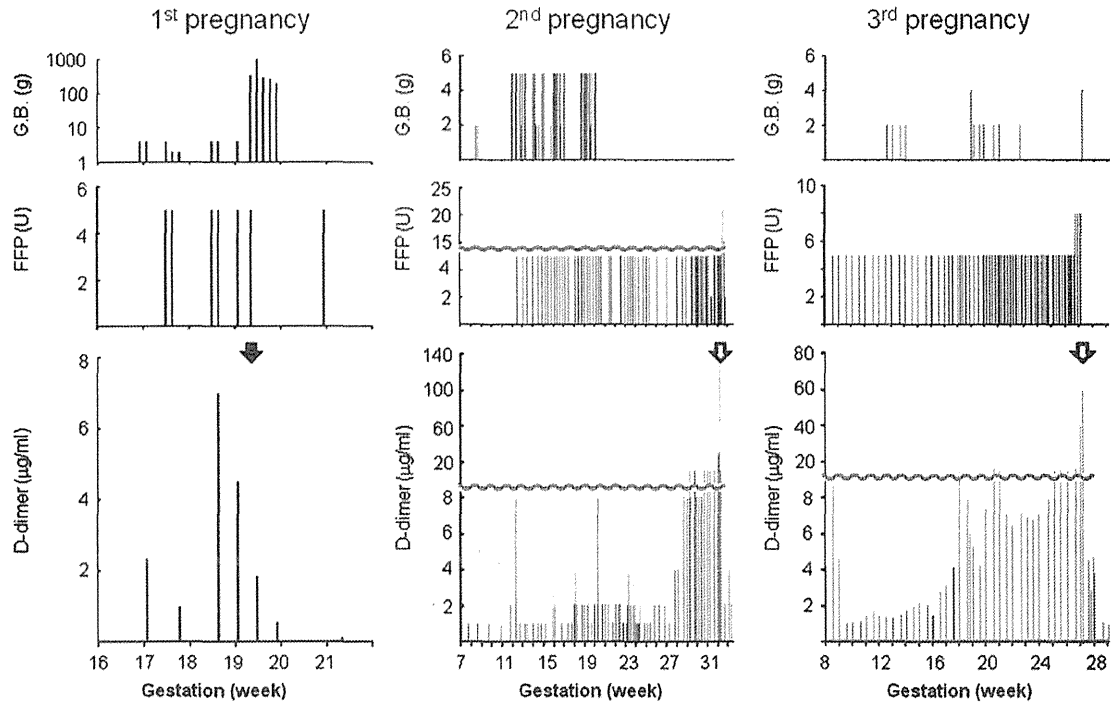

Fig. 1. The amount of genital bleeding (GB) (g), fresh frozen plasma (FFP) administered (U: 1 U = plasma separated from 200 mL blood), and plasma D-dimmer levels (μg/mL) observed during the course of the patient's 3 pregnancies. The black arrow indicates the day of the miscarriage. The white arrows indicate when the emergency caesarean sections were performed.

mice was found always to result in spontaneous miscarriage [11], but supplemental fibrinogen could rescue these pregnancies [14]. Spontaneous miscarriages were also observed in FXIII-deficient mice [13]. It must be emphasised, however, that not all of the latter mice showed spontaneous miscarriage, and some normal deliveries were observed. These results suggest that further stabilisation of the fibrin matrix in the fibrinoid layer through crosslinking is preferable, but not essential, for a successful pregnancy in mice. These mice are reliable tools for mimicking human diseases; however, it should be noted that some phenotypical discrepancies do exist between humans and mice even when the same genes are deficient.

In the case of complete PAI-1 deficiency, such discrepancies must be further emphasised. Several reports have described that PAI-1 deficiency leads to bleeding diathesis in humans [15]. However, *Serpine1* $^{-/-}$ mice do not show an increase in bleeding tendency [16], and pregnancies in *Serpine1*-deficient mice were found to be completely uneventful. Indeed, deficient male and female mice are being successfully crossbred to maintain *Serpine1*-deficient colonies. On the other hand, the PAI-1-deficient patient reported herein experienced a spontaneous miscarriage due to massive genital bleeding in the 19th week of her first pregnancy. The genital bleeding began in the 16th week of gestation in the absence of supplemental therapies. When comparing PAI-1 deficiency to congenital afibrinogenemia or FXIII deficiency, it becomes clear that genital bleeding begins much later in complete PAI-1 deficiency. In this case, the patient was given 5 U FFP twice a week after genital bleeding was observed. Each administration could theoretically restore approximately 20% of plasma coagulation factors. Indeed, the concentration of PAI-1 in FFP is almost equivalent to that in fresh plasma [17]. However, the half-life of active PAI-1 in vivo is extremely short (about 6 min) [18]. Therefore, the supplementation of PAI-1 via FFP administration did not appear to fulfil the demand for this factor in this pregnancy, resulting in further genital bleeding and miscarriage. The next 2 pregnancies were successfully maintained by the administration of large amounts of FFP; however, despite this increased treatment, it was very difficult to maintain the pregnancies in the 2nd trimester. The demand for PAI-1 during pregnancy appears to continuously increase, but the amount of PAI-1 that can be delivered via FFP administration is limited. Thus, the fragile supply and demand balance was broken midway through the last 2 pregnancies. Indeed, uncontrollable uterine contractions with elevated plasma D-dimer levels, which indicate massive genital bleeding in the uterus, especially the foeto-maternal surface, triggered preterm labour, resulting in the need for caesarean section for safe delivery. These findings indicate that PAI-1 plays an important role in the maintenance of pregnancy in humans. It may therefore be assumed that low levels of PAI-1 may be a risk factor for spontaneous miscarriage and/or preterm labour in humans.

In summary, our findings show the following: (1) FFP administration is not sufficient for rescuing PAI-1-deficient patients from massive bleeding after 25 weeks' gestation, and purified and/or recombinant PAI-1 is therefore necessary. (2) Such interventions are not required in mice, because of a lack of such a bleeding tendency. Based on studies in *Serpine1* $^{-/-}$ mice, several PAI-1 inhibitors have been synthesised for use in clinical trials to treat thrombotic events such as stroke and coronary ischaemia. However, the phenotypical manifestations of PAI-1 deficiency in humans are quite different to those in mice. Thus, care must be taken when translating research findings from the mouse to the human in this field.

Authors' roles

Contributions: T.I., K.N., and K.U. analysed results and made the figure; T.K., N.K., and T.T. took clinical care of the proband and her family; T.I. and N.K. designed the research and wrote the paper.

Conflict of Interest Statement

The authors declare no competing financial interests.

Acknowledgments

We thank to the proband and her family for their cooperation to this study. This work was supported in part by Japan Society for the Promotion of Science (JSPS) KAKENHI 20890093 and 22790247 (to T.I.), and the Uehara Memorial Foundation (to T.I.).

References

[1] Loskutoff DJ, Sawdey M, Mimuro J. Type 1 plasminogen activator inhibitor. Prog Hemost Thromb 1989;9:87–115.
[2] Meltzer ME, Doggen CJ, de Groot PG, Rosendaal FR, Lisman T. The impact of the fibrinolytic system on the risk of venous and arterial thrombosis. Semin Thromb Hemost 2009;35:468–77.
[3] Binder BR, Mihaly J. The plasminogen activator inhibitor "paradox" in cancer. Immunol Lett 2008;118:116–24.
[4] Iwaki T, Tanaka A, Miyawaki Y, Suzuki A, Kobayashi T, Takamatsu J, et al. Life-threatening hemorrhage and prolonged wound healing are remarkable phenotypes manifested by complete plasminogen activator inhibitor-1 deficiency in humans. J Thromb Haemost 2011;9:1200–6.
[5] Fay WP, Shapiro AD, Shih JL, Schleef RR, Ginsburg D. Brief report: complete deficiency of plasminogen-activator inhibitor type 1 due to a frame-shift mutation. N Engl J Med 1992;327:1729–33.
[6] Iwaki T, Castellino FJ. Maternal fibrinogen is necessary for embryonic development. Curr Drug Targets 2005;6:535–9.
[7] Asahina T, Kobayashi T, Takeuchi K, Kanayama N. Congenital blood coagulation factor XIII deficiency and successful deliveries: a review of the literature. Obstet Gynecol Surv 2007;62:255–60.
[8] Ichinose A, Asahina T, Kobayashi T. Congenital blood coagulation factor XIII deficiency and perinatal management. Curr Drug Targets 2005;6:541–9.
[9] Kobayashi T, Terao T, Kojima T, Takamatsu J, Kamiya T, Saito H. Congenital factor XIII deficiency with treatment of factor XIII concentrate and normal vaginal delivery. Gynecol Obstet Invest 1990;29:235–8.
[10] Kobayashi T, Kanayama N, Tokunaga N, Asahina T, Terao T. Prenatal and peripartum management of congenital afibrinogenaemia. Br J Haematol 2000;109:364–6.
[11] Suh TT, Holmback K, Jensen NJ, Daugherty CC, Small K, Simon DI, et al. Resolution of spontaneous bleeding events but failure of pregnancy in fibrinogen-deficient mice. Genes Dev 1995;9:2020–33.
[12] Ploplis VA, Wilberding J, McLennan L, Liang Z, Cornelissen I, DeFord ME, et al. A total fibrinogen deficiency is compatible with the development of pulmonary fibrosis in mice. Am J Pathol 2000;157:703–8.
[13] Koseki-Kuno S, Yamakawa M, Dickneite G, Ichinose A. Factor XIII A subunit-deficient mice developed severe uterine bleeding events and subsequent spontaneous miscarriages. Blood 2003;102:4410–2.
[14] Iwaki T, Sandoval-Cooper MJ, Paiva M, Kobayashi T, Ploplis VA, Castellino FJ. Fibrinogen stabilizes placental-maternal attachment during embryonic development in the mouse. Am J Pathol 2002;160:1021–34.
[15] Mehta R, Shapiro AD. Plasminogen activator inhibitor type 1 deficiency. Haemophilia 2008;14:1255–60.
[16] Carmeliet P, Stassen JM, Schoonjans L, Ream B, van den Oord JJ, De Mol M, et al. Plasminogen activator inhibitor-1 gene-deficient mice. II. Effects on hemostasis, thrombosis, and thrombolysis. J Clin Invest 1993;92:2756–60.
[17] Nielsen HJ, Reimert C, Pedersen AN, Dybkjoer E, Brunner N, Alsbjorn B, et al. Leucocyte-derived bioactive substances in fresh frozen plasma. Br J Anaesth 1997;78:548–52.
[18] Vaughan DE, Declerck PJ, Van Houtte E, De Mol M, Collen D. Studies of recombinant plasminogen activator inhibitor-1 in rabbits. Pharmacokinetics and evidence for reactivation of latent plasminogen activator inhibitor-1 in vivo. Circ Res 1990;67:1281–6.

Takayuki Iwaki
Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu, Japan
Corresponding author at: The Department of Pharmacology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashiku, Hamamatsu Shizuoka, Japan. Tel.: +81 53 435 2271; fax: +81 53 435 2270.
E-mail address: tiwaki@hama-med.ac.jp.

Kotomi Nagahashi
Department of Gynecology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Takao Kobayashi
Hamamatsu Medical Center, Hamamatsu, Japan

Kazuo Umemura
Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Toshihiko Terao
Department of Gynecology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Naohiro Kanayama
Department of Gynecology, Hamamatsu University School of Medicine, Hamamatsu, Japan

22 July 2011

FULL PAPER

BJC

British Journal of Cancer (2013) 108, 395–401 | doi: 10.1038/bjc.2012.572

Keywords: REG; biomarker; chemotherapy; prognosis; apoptosis; gastric cancer

REG Iα is a biomarker for predicting response to chemotherapy with S-1 plus cisplatin in patients with unresectable stage IV gastric cancer

A Sekikawa[1,5], H Fukui[*,2,5], X Zhang[2], T Maruo[1], T Tsumura[1], Y Okabe[1], T Wakasa[3], Y Osaki[1], T Chiba[4], T Tomita[2], T Oshima[2], J Watari[2] and H Miwa[2]

[1]Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Osaka, Japan; [2]Division of Upper Gastroenterology, Department of Internal Medicine, Hyogo College of Medicine, I-1, Mukogawa, Nishinomiya 663-8501, Japan; [3]Department of Pathology, Osaka Red Cross Hospital, Osaka, Japan and [4]Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, Kyoto, Japan

Background: The regenerating gene Iα (REG Iα) is involved in gastric carcinogenesis as an antiapoptotic factor. Therefore, we investigated whether REG Iα confers resistance to chemotherapeutic drugs in gastric cancer (GC) cells and whether REG Iα expression is useful for predicting the response to chemotherapy and outcome in patients with GC.

Methods: A total of 70 patients with unresectable stage IV GC received first-line chemotherapy with S-1 and cisplatin (S-1/CDDP). The expression of REG Iα was evaluated immunohistochemically using biopsy samples obtained before chemotherapy, and its relationship to clinicopathological parameters was analysed statistically. The effects of *REG Iα* gene induction on resistance to 5-FU or CDDP treatment were examined by cell survival assay and flow cytometry.

Results: Of the 70 patients with unresectable stage IV GC, 19 (27%) were positive for REG Iα expression. The expression of REG Iα was independently predictive of poorer progression-free and overall survival in such patients (hazard ratio (HR) 2.46; $P=0.002$ and HR 1.89; $P=0.037$, respectively). The gene induction of *REG Iα* conferred resistance to cell death induced by 5-FU or CDDP in GC cells.

Conclusion: In patients with stage IV GC, REG Iα, which confers resistance to chemotherapeutic drugs in GC cells, is a potential biomarker for predicting resistance to S-1/CDDP treatment.

Gastric cancer (GC) is a major cause of cancer-related death worldwide (Kamangar *et al*, 2006; Ferlay *et al*, 2010), and the outcome of patients with unresectable GC is very poor (Chau *et al*, 2004; Lee *et al*, 2007). Recently, advances in chemotherapy have considerably improved the prognosis of patients with unresectable GC, and subsequently the combination of S-1 (comprising a prodrug of 5-fluorouracil, 5-chloro-2,4-dihydropyrimidine, and potassium oxonate) with cisplatin (S-1/CDDP) has been accepted as a first-line therapy for such patients in Japan (Boku, 2008; Koizumi *et al*, 2008; Ohtsu, 2008). However, the response to chemotherapy is known to differ widely among such patients, and in fact GC patients who show a poor response to first-line chemotherapy are considered to have a dismal prognosis (Matsubara *et al*, 2008; Choi *et al*, 2011; Park *et al*, 2011). In this

*Correspondence: Dr H Fukui; E-mail: hfukui@hyo-med.ac.jp
[5]These authors contributed equally to this work.

Received 11 September 2012; revised 2 November 2012; accepted 21 November 2012; published online 15 January 2013