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生体肝移植後のC型肝炎再発予防を目指したステロイド剤不使用による免疫抑制療法に関する研究

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研究要旨

C型肝硬変患者に対する生体肝移植後の肝炎の再発率は高く、その治療戦略の確立が望まれている。本研究では、肝移植後の肝炎再発の実態を解明すると共に、肝炎再発防止を目指した、ステロイド剤を使用しない、新しい免疫抑制療法に関する無作為比較試験を行い、その効果を判定した。移植後の肝炎再発率は極めて高く、移植前に血中 HCVRNA が陽性であった症例では移植後全例 HCVRNA の高度な上昇を認め、また肝炎再発によると思われる胆汁うっ滞によるビリルビン値の急激な上昇を認めたものが 35 例中 6 例あった。無作為試験の効果判定は全体症例の分析に委ねることとなるが、ステロイド剤不使用による重篤な有害事象は特に認めなかった。

A. 研究目的

C 型肝炎ウィルス(HCV)感染による肝硬変は、近年、国内外において肝移植の適応疾患の第一位となっている。しかし、肝移植後の HCV 肝炎再発が移植肝の予後を左右する重要な問題となっており、肝炎再発に関連するグラフト機能不全のために他の疾患に比べて 5 年以降の長期予後が有意に不良であることが示されている。さらに、移植後早期に急激な血清ビリルビン値の上昇をきたすいわゆる FCH (Fibrosing cholestatic hepatitis) により肝不全に至る症例も認められ、そ治療戦略の確立が望まれている。

肝移植後のHCV肝炎再発の特徴として、ウィルス量が肝移植後に急速に上昇し、その値は移植前に比べて非常に高くなること、慢性肝炎から肝硬変への進展が早い、すなわち肝の線維化速度が速いことなどが挙げられ、その原因として移植後免疫抑制療法の影響が考えられている。特に、ステロイド剤は HCV の増殖を促進すると言われ、移植後 HCV 肝炎再発防止のためにはこれまでのステロイドを中心とした免疫抑制療法の見直しが必要であると考えられた。

本研究の目的は、１）当施設で実施した C 型肝炎への生体肝移植後の肝炎再発

について、再発の形式および再発に対する治療成績を検討することにより、その実態を明らかにする、２）生体肝移植後ステロイドフリーの免疫抑制療法を従来法と比較検討し、肝炎再発防止を目指した、新しい免疫抑制プロトコールの確立をめざす。

B. 研究方法

１）平成12年9月から、熊本大学小児外科・移植外科で生体肝移植を受けたHCV肝硬変患者について、再発形式をウィルス学的及び病理組織学的に検討するとともに、再発症例に対する肝炎治療の成績を分析することにより、移植後 HCV 肝炎の再発の実態を解明した。

２）HCV 関連肝硬変患者の生体肝移植後肝炎再発防止を目指した新しい免疫抑制療法の開発として、従来のタクロリムスとステロイド剤による免疫抑制療法を行う群（A群）と、ステロイド剤を一切使用せずミコフェノール酸モフェチル（MMF）とタクロリムスを使う新しい免疫抑制療法を行う群（B群）の２群における前向きの無作為比較試験を開始した。この臨床試験に関する倫理面への配慮については、本学の倫理委員会の審議を経てその指針を受けている。

B. 研究結果

１）当科にてこれまでC型肝炎、肝硬変にて肝移植を受けた症例は35例であるが、そのうち術前からHCVRNAが陰性であった4例においては、移植後もRNAは陰性であったが、陽性であった残り31例ではRNA量は移植直後一時的に低下するものの、その後は術前より遙かに高いRNA量に至ることが解った。組織学的には移植後肝生検が可能であった26例中これまでのところ22例（85%）で、F1A1以上の肝炎の再発を認めている。また、6例が移植後比較的早期（2ー13ヶ月）に肝炎再発によると思われる急激なビリルビンの上昇を来した。２例は肝炎に対する治療を行う機会を逸し、死亡した。もう１例はインターフェロン（INF）などの肝炎治療を行ったが、その進行を止めることが出来ず肝不全にて失った。残りの３例はインターフェロン＋リバビリン療法に反応し、救命できた。これらは、HCV肝炎再発による、CH（cholestatic hepatitis）ともいうべき病態であろうと考えられた。

HCV肝炎に対する移植後生存例28例のうち肝炎再発に対してIFN＋リバビリン療法を施行した症例は現在まで14例あり、そのうちHCVRNAが陰性化した症例が7例あるが、癌再発を来したためIFN治療を中止した1例を除き、6例は今も抗ウィルス療法を継続しており、いずれもSVR（sustained viral response）を確認するまでには至っていない。

２）平成16年9月から本無作為比較試験に参加しており、登録した合計症例はステロイド使用群が３例、ステロイド非使用MMF投与群が３例であった。そのうち２例は術前よりHCVRANAの定性が陰性であったので効果判定は困難であった。

別の１例は免疫抑制剤の変更で脱落し、もう１例の MMF 投与症例は難治性の下痢のため、MMF を中止せざるをえなかった。2 群間の HCV 肝炎再発予防効果の比較については、全体症例の検討に委ねたいと考えている。

D. 考察

本研究期間においては、移植後 HCV 肝炎再発により肝硬変に至った症例は幸い経験しなかったが、移植後全例において、ウィルス学的な再発が認められ、それも術前の値とは格段に高い値となること、組織学的にも 8 割以上に再発を認めたこと、さらには、移植後 FCH ともいうべき、肝炎再発に起因すると思われる急激なビリルビンの上昇を来した症例を６例認め、治療に難渋したことから、HCV 肝炎に対する肝移植後には、早期より再発に対しての厳密な監視が必要であると考えられた。移植後の抗ウィルス療法の開始については、組織学的所見と、RNA 量やトランスアミナーゼ値の変動を考慮して行ったが、副作用もあり、その開始時期については議論のあるところである。一方、免疫抑制療法下における抗ウィルス療法の効果はそれほど良好とは言えず、インターフェロン＋リバビリン療法を施行した生存例 14 例中、HCVRNA が陰性化したのは 7 例（50%）で、1 例を除き治療継続中であり、これまでに SVR を確認できた症例はまだない、汎血球減少症などのために治療を後退、中断せざるを得ない症例もあった。残念ながら、今までのところ、本臨床試験への参加症例は 6 例と少なく、脱落症例もあり、その効果を判定するには至っていないが、京都大学や他施設での症例を加えた分析結果に期待するものである。ステロイドフリーによる重篤な有害事象は特に認めなかった。

E. 結論

多施設共同の無作為比較試験として立ち上げられた本研究に参加した。肝移植後のＣ型肝炎の再発の克服は依然として大きな課題であった。

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A DIFFERENT AMYLOID FORMATION MECHANISM: DE NOVO OCULOLEPTOMENINGEAL AMYLOID DEPOSITS AFTER LIVER TRANSPLANTATION

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Background. **Liver transplantation has served as a treatment for patients with familial amyloidotic polyneuropathy (FAP) because variant transthyretin (TTR), the pathogenic protein of FAP, is predominantly produced by the liver. However, the effect on amyloid formation of TTR that is synthesised by the retina and the choroid plexus remains to be elucidated in FAP patients with liver transplants.**

Objective. **To investigate changes in ocular tissues and the central nervous system (CNS) of FAP patients after liver transplantation.**

Design. **Clinical study.**

Setting. **Graduate School of Medical Sciences, Kumamoto University, Japan.**

Intervention. **Transplantation of livers from cadaveric or living donors.**

Measurements. **Preoperative measures and postoperative (16–108 months) follow-up of clinical data, including routine ophthalmologic, neurologic, and laboratory evaluations.**

Results. **In 22 patients with FAP related to the amyloidogenic TTR (ATTR) Val30Met and 3 patients with FAP ATTR Tyr114Cys, after liver transplantation, 3 patients began to show evidence of de novo glaucoma, and 1 had vitreous opacity that was caused by the variant TTR. Another three patients showed new amyloid deposits in the pupillary margin, which could lead to glaucoma and vitreous opacity. As for changes in the CNS and levels of total protein and TTR in cerebrospinal fluid (CSF), after liver transplantation, two FAP ATTR Tyr114Cys patients exhibited de novo amyloid deposition in the leptomeninges, and total protein and TTR levels in CSF were significantly increased.**

Conclusions. **Oculoleptomeningeal involvement in FAP was not prevented by liver transplantation because variant TTR produced by the retina and the choroid plexus forms amyloid fibrils in situ.**

Since 1990, liver transplantation has been used to treat familial amyloidotic polyneuropathy (FAP) (*1*) because amyloidogenic transthyretin (ATTR), the pathogenic protein of FAP, is predominantly synthesised by the liver. By the end of 2000, more than 500 FAP patients had undergone the surgery, with approximately 80% of the patients surviving (*2*). Liver transplantation is now considered a promising therapy for prevention of deterioration of neurologic complications of FAP patients (*1*, *3–7*). After transplantation, variant TTR levels in serum have decreased to below 1% of those before transplantation, and clinical findings have improved, or at least the patients' conditions did not deteriorate (*8*). However, TTR is also synthesised by the choroid plexus and the retina (*9*, *10*), and the role of TTR synthesised by these tissues in FAP patients remains to be elucidated. Moreover, ocular manifestations are common in patients with FAP ATTR Val30Met (*11*, *12*), and these symptoms may become serious problems after liver transplantation.

Advances in molecular genetics and protein chemistry techniques have resulted in more than 80 different points of mutation in TTR being reported (*13*). Among the hereditary systemic amyloidoses, the type FAP ATTR Val30Met is the most common (*14–16*). FAP ATTR Val30Met is characterized by progressive sensorimotor peripheral neuropathy in addition to symptoms in the gastrointestinal tract, heart, kidney, and autonomic nervous system (*14*, *15*). Liver transplantation has been reported to halt the progression of these clinical manifestations (*1*, *3–7*). However, changes in manifestations in the eye and central nervous system (CNS) have not been carefully evaluated, and no precise or long-term follow-up documentation is available.

In the Kumamoto district, from 1994 to the end of 2002, 25 FAP patients underwent liver transplantation. All patients are still alive, and 84% have resumed their normal daily activities. Neurologic and laboratory examinations revealed that therapy has thus far prevented progression of systemic symptoms of FAP, and autonomic dysfunction partially improved after surgery (*5*, *17*). For this report, we evaluated posttransplantation changes in clinical and laboratory data related to ocular and CNS symptoms.

PATIENTS AND METHODS

Subjects

All FAP patients included in the study had a definite diagnosis of FAP on the basis of genetic investigations. The patients had a diagnosis

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of FAP ATTR Val30Met (*18*) or FAP Tyr114Cys (*19*); there were 13 men and 12 women, aged 28 to 59 (average age 38.26) years (Table 1).

Ophthalmologic Examinations

Routine ophthalmologic examinations for the FAP patients who had had liver transplantations were performed before and every 1 to 6 months after transplantation. Patients were carefully evaluated for keratoconjunctivitis sicca, glaucoma, pupillary disorders, and vitreous opacity (*11*).

Analysis of the Vitreous Amyloid Sample

Amyloid fibrils were collected by centrifugation of the vitrectomized corpus vitreum from an FAP patient (no. 3 in Table 1). The fibrils were washed three times with 1 mL of saline and distilled water and were centrifuged at 9,000*g* for 5 minutes. The collected amyloid fibrils were ultrasonicated (Branson 1200, Shelton, CT) for 1 hour after addition of 20 μL of 6 M guanidine hydrochloride and 10 μL of 2.7 mM dithiothreitol (DTT). After ultrasonication, 500 μL of 50 mM Tris-HCl and 100 μL of polyclonal anti-TTR antibody (Dako, Dakopatts, Glostrup, Denmark) were added, and the sample was incubated overnight (final concentrations 225 mM guanidine hydrochloride and 0.06 mM DTT). The resulting precipitate was centrifuged at 9,000*g* for 5 minutes and was washed two times with 100 μL of saline and 100 μL of water, respectively, at 4°C. The precipitate was dissolved in 50 mL of 4% acetic acid and 4% acetonitrile in water, and the solution was passed through a 1,000 kDa centrifugal concentrator to separate the dissociated TTR from the antibody and to constitute a pass-through fraction. The centrifugal concentrators were washed with 3×100 μL with the same solution before use to clean the membrane surface (*20*).

Mass Spectrometry

The extracted amyloid samples were analyzed by using a Bruker Reflex mass spectrometer (Bruker Franzen Analytik GmbH, Bremen, Germany) operated at a wavelength of 337 nm. The best spectra of TTR were obtained at an ion accelerating voltage of 27.5 kV and a reflectron voltage of 30 kV. The spectra were calculated by using external calibration with $[M+H]^-$ ions produced from horse cytochrome *c* (m/z 12360.08) and horse myoglobin (m/z 16951.46). The matrix was a saturated solution of sinapinic acid in 1:2 acetonitrile:water containing 0.1% trifluoroacetic acid (TFA). The samples were deposited onto the sample probe assembly (*21*).

Two-Dimensional Electrophoresis

Before electrophoresis, an 11 cm Immobiline DryStrip gel (gel strip) (Pharmacia Biotech, Uppsala, Sweden) was hydrated overnight in the vitreous sample dissolved in 8 M urea, 1% Triton X, 65 mM DTT, 2% Pharmalyte (pH 4–7), and 0.025% bromphenol blue in a reswelling cassette (Pharmacia). Electrode wicks (Pharmacia) were oriented on the proper sides of the isoelectric focusing electrode strips (Pharmacia), and the gel strip was placed on an aligner (Pharmacia). Electrophoresis was carried out at 3,500 V for 5 hours (gradient mode, V×hours=15,000) at 15°C on a water-cooled Multiphor II (Pharmacia). After electrophoresis, the gel strip was soaked in 0.05 M Tris-HCl (pH 8.8), 6 M urea, 30% glycerol, and 2% sodium dodecyl sulphate (SDS) (i.e., equilibrating buffer) containing 50 mM DTT for 15 minutes, after which it was soaked in equilibrating buffer containing 135 mM iodoacetamide for 15 minutes. After the processes of reduction and alkylation, the gel strip was subjected to a second electrophoresis step (SDS-polyacrylamide gel electrophoresis). The strip was placed on a 10% acrylamide gel containing 0.375 M Tris-HCl (pH 8.8), 0.1% SDS, 0.03% ammonium persulphate, and 0.05% TEMED, and electrophoresis proceeded at 30 mA loading in a buffer consisting of 250 mM Tris base, 1.92 M glycine, and 1% SDS.

The acrylamide gel was transferred onto polyvinylidene fluoride membrane and was subjected to Western blot analysis. Anti-human TTR antibody from rabbit (diluted in 1:1,000) and horseradish peroxidase-conjugated anti-rabbit antibody from goat (diluted 1:1000)

TABLE 1. Patients' characteristics

No	Age	Sex	Duration	Condition	Date	University	Other	Mutation
1	37	M	3	Full time	Feb, 1994	Sweden		Val30Met
2	40	M	4	Full time	Dec, 1994	Sweden		Val30Met
3	48	F	4	Full time	Jan, 1995	Sweden		Val30Met
4	41	M	5	Full time	Jul, 1995			Val30Met
5	54	F	5	Full time	Sep, 1995			Val30Met
6	41	F	4	Full time	Jan, 1996			Val30Met
7	36	F	1	Full time	Feb, 1996			Val30Met
8	51	M	5	Full time	Mar, 1996			Val30Met
9	30	M	2	Full time	May, 1997	Sweden	Domino	Val30Met
10	40	F	1	Full time	Nov, 1998	Sweden	Domino	Val30Met
11	33	M	2	Half time	Dec, 1998	Sweden		Val30Met
12	33	M	2	Full time	Dec, 1998	Sweden		Val30Met
13	35	M	2	Full time	Dec, 1998	Sweden		Val30Met
14	32	M	2	Full time	Jan, 1999	Kyushu Univ.	Partial LT	Val30Met
15	38	F	1	Full time	Jan, 1999	Kumamoto Univ.	Partial LT	Val30Met
16	61	M	1	Full time	Jul, 1999	Kyoto Univ.	Partial LT, Domino	Val30Met
17	52	F	4	Stay home	Jul, 1999	Kyushu Univ.	Partial LT, Domino	Tyr114Cys
18	37	F	2	Full time	Jul, 1999	Kumamoto Univ.	Partial LT	Tyr114Cys
19	34	M	1	Full time	Nov, 1999	Kumamoto Univ.	Partial LT	Val30Met
20	38	F	2	Full time	Nov, 2000	Australia		Val30Met
21	37	F	1	Half time	Nov, 2000	Australia		Val30Met
22	47	M	4	Full time	Dec, 2000	Kumamoto Univ.	Partial LT, Domino	Val30Met
23	35	F	3	Full time	Apr, 2001	Australia		Val30Met
24	40	M	3	Stay home	Sep, 2001	Kumamoto Univ.	Partial LT, Domino	Tyr114Cys
25	32	F	1	Full time	Dec, 2001	Kumamoto Univ.	Partial LT, Domino	Val30Met

The patients with clinical score (17) below 40 received liver transplantation.

Duration, duration after the onset of the disease; date, the transplant date; place, the transplant place; condition, daily activity; full time, works full time; half time, works half time; stay home, stay at home; LT, liver transplantation.

were used as the primary antibody and the secondary antibody, respectively. Electrogenerated chemiluminescence (Pharmacia) was adjusted according to the manual and mounted on the membrane for 1 minute, and radiographic film was exposed for 10 to 60 seconds. Chemiluminescence was quantified from scanner images by using Multi-Analyst version 1.0.2 (Bio-Rad, Marnes, France).

Neurologic Examinations

Routine neurologic examinations were performed before and every 1 to 6 months after liver transplantation. Several patients had lumbar puncture (nos. 1, 2, 3, 6, 7, 13, 17, 18, 21, and 24 in Table 1) and magnetic resonance imaging (MRI) studies (nos. 1, 3, 6, 13, 17, 18, 21, and 24 in Table 1) in addition to the clinical neurologic examinations before and after liver transplantation.

RESULTS

Ophthalmologic Examinations

Ophthalmologic examinations after liver transplantation revealed de novo pupillary disorders, including keratoconjunctivitis sicca, glaucoma, and vitreous opacity, in 3, 3, and 1 FAP patients, respectively (Table 2). A 46-year-old woman (no. 3 in Tables 1 and 2) underwent vitrectomy 5 years after liver transplantation, and matrix-assisted laser desorption-ionization/time-of-flight mass spectrometry (MALDI/TOF-MS) of the purified amyloid fibrils from the vitreous sample revealed that the variant TTR peak was predominant (peak labeled a in Figure 1A). The ratio was confirmed by two-dimensional electrophoresis (Fig. 1B), and the chemiluminescence intensity of the normal TTR and the variant TTR was 12% and 88%, respectively.

Neurologic Examinations

Neurologic examinations of patients with FAP ATTR Val30Met revealed no CNS disorders that appeared to be related to de novo leptomeningeal amyloidosis. Examination of two patients with FAP ATTR Tyr114Cys, however, showed the presence of CNS disorders after liver transplantation. Fifteen months after liver transplantation, a 50-year-old female patient with FAP ATTR Tyr114Cys (no. 17 in Table 1) experienced sudden numbness and muscle weakness in the right upper extremity and dysarthria that continued for approximately 5 hours. In a 38-year-old male patient with FAP ATTR Tyr114Cys (no. 24 in Table 1), transient depression and disorientation occurred 8 months after transplantation. In these two patients, cerebrospinal fluid (CSF) total protein and TTR levels increased after the surgery: CSF total protein levels before and 15 months after liver transplantation in the first patient were 235 mg/dL and 365 mg/dL, respectively; corresponding TTR levels were 1.6 mg/dL and 2.7 mg/dL. For the second patient, CSF total protein levels before and 8 months after the surgery were 123 mg/dL and 219 mg/dL, respectively; corresponding wild-type TTR and ATTR Tyr114Cys were 1.3 and 0.3 mg/dL and 1.8 and 0.4 mg/dL. In these two patients, de novo amyloid deposition in the leptomeninges below the cervical lesions was confirmed by MRI study (Fig. 2).

DISCUSSION

It has been widely accepted that liver transplantation can halt the worsening of systemic clinical symptoms of FAP. However, a few reports have appeared of vitreous opacity occurring in FAP patients after liver transplantation (*22*). In our 25 FAP patients who had liver transplantation, 7 (20%) patients and 2 (8%) patients showed new ocular changes and leptomeningeal amyloid deposits, respectively, after the surgery. These findings suggest that ocular tissues and CNS may have different amyloid formation mechanisms. Because the transplantation resulted in negligible serum levels of variant TTR in these patients and a cessation of the TTR supply from the liver to the ocular tissues and CSF (*8*), it was natural to consider that the amyloid deposits in both ocular tissues and leptomeninges may be induced by TTR produced by the retina and the choroid plexus (*9*, *10*). In fact, we reported that a significant amount of ATTR Val30Met was detected in the aqueous humor of FAP ATTR Val30Met patients receiving transplants (*23*). In addition, MALDI/TOF-MS and two-dimensional electrophoresis revealed that amyloid fibrils in the vitreous body from an FAP patient who had received a liver transplantation consisted predominantly of variant TTR (Fig. 1). This finding indicated that the variant TTR produced by the retina plays an important role in amyloid formation in the vitreous body (*22*). In our experience with FAP patients who have received a liver transplantation, the occurrence of ocular complaints increases yearly, and this problem may become more common in other types of FAP as well as in FAP ATTR Val30Met.

TABLE 2. Changes in ocular manifestations

No.	Age	Sex	Point of mutation	Before				After			
				P	K	G	V	P	K	G	V
1	35	M	Val30Met	−	−	−	−	−	−	−	−
2	38	M	Val30Met	−	+	−	−	+	+	−	−
3	46	F	Val30Met	−	−	−	−	+	−	+	+
5	52	F	Val30Met	−	+	−	−	+	−	+	+
8	49	M	Val30Met	−	+	−	−	−	+	−	−
10	38	F	Val30Met	−	+	−	−	−	+	−	−
12	33	M	Val30Met	−	−	−	−	−	−	−	−
13	31	F	Val30Met	−	−	−	−	−	−	+	−
15	38	F	Val30Met	−	+	−	−	−	+	−	−
16	61	M	Val30Met	+	+	−	−	++	+	−	−
17	50	F	Tyr114Cys	+	−	+	+	+	−	++	+
18	35	F	Try114Cys	−	−	−	+	−	−	−	++
25	31	F	Val30Met	−	+	−	−	−	+	−	−

P, papillary disorders; K, keratoconjunctivitis sicca; G, glaucoma; V, vitreous opacity.

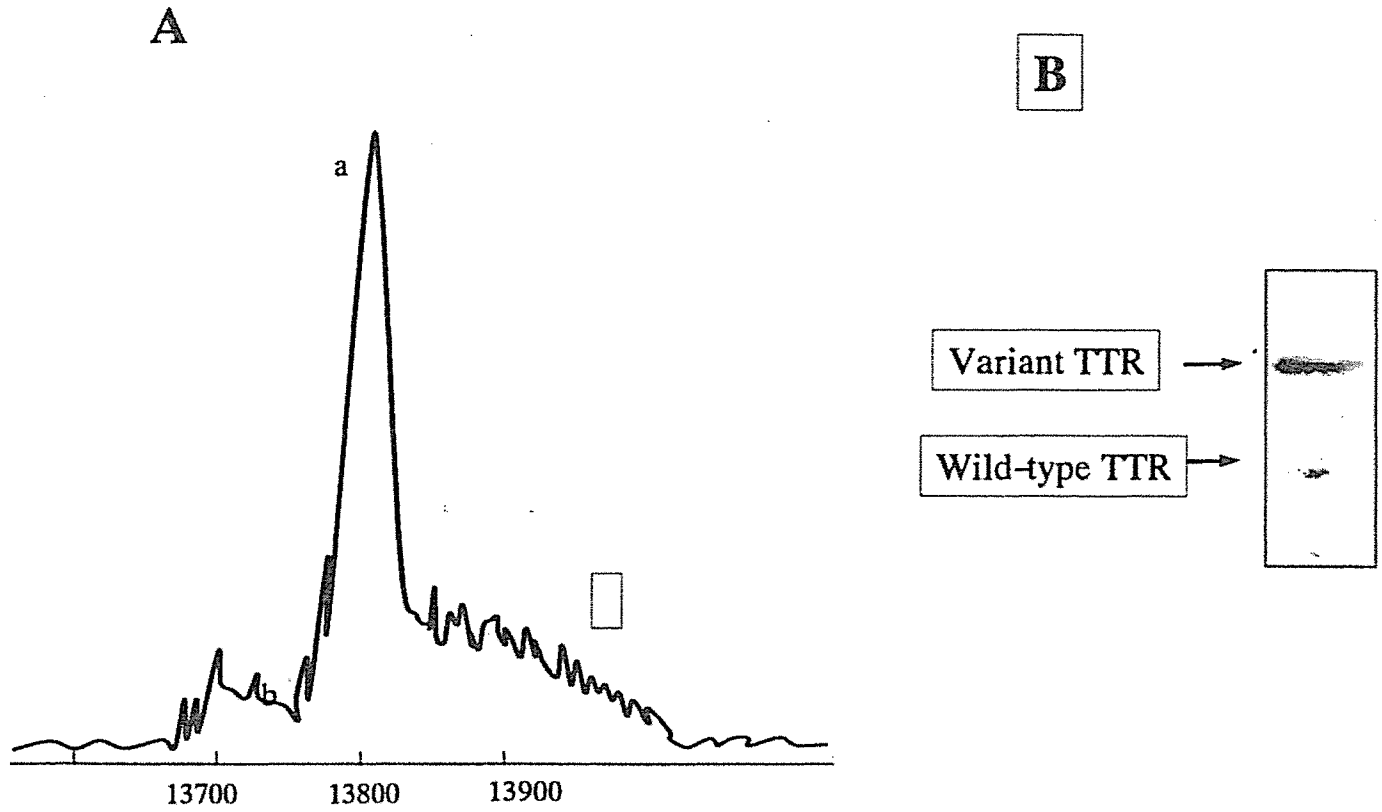

FIGURE 1. **Transthyretin (TTR) in the vitreous body. The vitrectomized sample obtained from a 46-year-old female patient with familial amyloidotic polyneuropathy (FAP) amyloidogenic (A)TTR Val30Met was analyzed by matrix-assisted laser desorption-ionization/time-of-flight mass spectrometry (MALDI/TOF-MS) (A) and two-dimensional electrophoresis (B).**

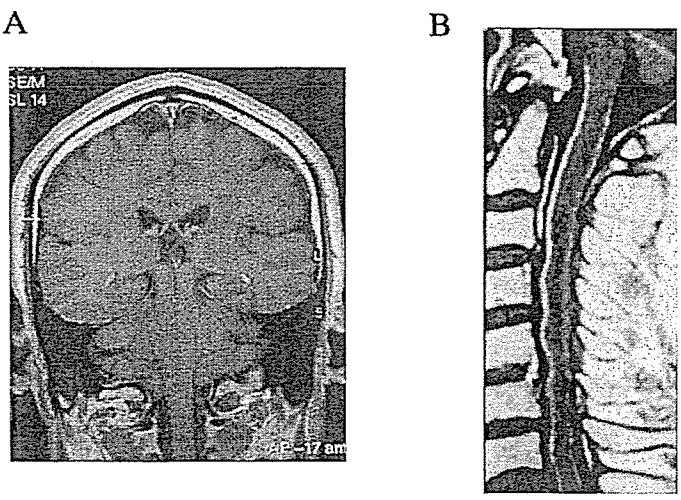

FIGURE 2. **Changes in the leptomeninges as evidenced by magnetic resonance imaging (MRI) before and after liver transplantation. Gadlinium enhanced MRI (T_1) before (A) and 1.5 years after liver transplantation (B) in a 50-year-old female FAP patient (no. 17).**

Our study demonstrated that liver transplantation could not prevent posttransplantation leptomeningeal amyloid deposition. It has been reported that amyloid deposition in the leptomeninges is often recognized in autopsied materials from patients with FAP ATTR Val30Met, and a few FAP patients have shown amyloid angiopathy and CNS disorders (*24–26*). As for amyloid formation in the CNS, little is known about the role of TTR produced by the choroid plexus (*27, 28*). The TTR may play an important role in CSF and CNS because TTR is the second-most abundant protein in the CSF, and it may be indispensable for metabolism in the brain or CSF.

In our 22 FAP ATTR Val30Met patients, no CNS disorders were observed, and TTR levels in the CSF before liver transplantation did not change after the surgery (data not shown). MRI studies of seven FAP ATTR Val30Met patients revealed no amyloid deposition in the leptomeninges after transplantation. In contrast, FAP ATTR Tyr114Cys patients showed de novo amyloid deposition after the transplantation, which was confirmed by MRI. In addition, one of the patients had a transient ischemia attack 1.5 years after liver transplantation. Recently, we reported a different amyloid formation mechanism in ATTR Tyr114Cys (*29*), which may facilitate leptomeningeal amyloid deposits more than in ATTR Val30Met.

Although FAP ATTR Tyr114Cys is classified as an oculoleptomeningeal amyloidosis (*13*), there is a possibility that long-term follow-up after liver transplantation may show that leptomeningeal amyloidosis and other unknown symptoms may also become serious consequences of FAP ATTR Val30Met as well as other types of FAP. Before the use of liver transplantation as treatment of FAP, the life span of patients with FAP ATTR Val30Met was usually approximately 10 years after the onset of the disease. It is possible that leptomeningeal amyloidosis may not show itself as a

serious problem in such a short time period. However, most FAP patients now undergo liver transplantation and live much longer. In this situation, ATTR produced from the choroid plexus and retina may induce ocular amyloidosis and leptomeningeal amyloidosis in FAP ATTR Val30Met as well as in other types of FAP.

It has been well documented that cardiac amyloid deposits progressed even after liver transplantation because wild-type TTR participated in amyloid fibril formation, mainly in non-FAP ATTR Val30Met-type patients (*30*, *31*). In addition, as reported here, ocular manifestations such as vitreous opacity and glaucoma restrict the daily life of patients. Because these ocular disorders can be addressed surgically, precise and long-term follow-up evaluations for ocular manifestations before and after liver transplantation are needed to ensure a good quality of life for these FAP patients. With regard to leptomeningeal amyloidosis, we cannot predict when and what types of clinical manifestations may occur and become serious problems in FAP ATTR Val30Met. In FAP ATTR Tyr114 Cys, leptomeningeal amyloidosis does have clinical importance, as demonstrated in this report. To answer these questions, we must continue radiologic and neurologic follow-up over the long term.

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Early graft failure due to a veno-occlusive disease after a pediatric living donor liver transplantation

Izaki T, Inomata Y, Asonuma K, Okajima H, Ohshiro H, Ueno M, Hamamoto R, Iyama K, Tanaka K. Early graft failure due to a veno-occlusive disease after a pediatric living donor liver transplantation. Pediatr Transplantation 2004: 8: 301–304. © 2004 Blackwell Munksgaard

Abstract: A 10-month-old boy with biliary atresia after Kasai procedure underwent a living donor liver transplantation (LDLT). Five days after the LDLT, high fever and increased ascites followed by poor bile drainage was accompanied by elevation of serum liver enzymes. Liver biopsy showed occlusion of the central veins by fibro-edematous endothelium and submassive necrosis of the parenchyma. Veno-occlusive disease (VOD) was suspected, and re-LDLT was urgently performed because of deterioration of hepatic failure. There are few cases of VOD after liver transplantation and this is the first one in an infant after LDLT.

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Key words: veno-occlusive disease – complication – rejection – living donor liver transplantation

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VOD of the liver was first described by Bras et al. (1). In the transplantation field, most cases of VOD have been reported after preconditioning treatment for bone marrow transplantation, or immunosuppression associated with renal transplantation (2, 3). Clinical symptoms like jaundice, body weight gain or ascites, and painful hepatomegaly, have been the diagnostic clues. A limited number of cases with VOD after liver transplantation have been reported, and the outcome is quite poor (4). Conversely, the incidence of early graft failure is quite low after LDLT except for technical or infectious complications. We present a case of infantile VOD, which occurred only 5 days after LDLT, and the patient survivor with a re-LDLT.

Abbreviations: LDLT, living donor liver transplantation; VOD, veno-occlusive disease; UW, University of Wisconsin; MPD, methylpredonisolone; CDC, complement dependent cytotoxicity; ALT, alanine amino-transferase; AST, aspartate transaminase; Tbil, total bilirubin.

Case report

A LDLT was performed in a 10-month-old boy following a failed Kasai procedure. The donor was his ABO-identical father. Preoperative lymphocyte cross-match using the CDC method was negative. Donor surgery was uneventful. The left lateral segment graft was 240 g in weight. Graft weight per recipient's body weight ratio was 3.9%. The graft was preserved in the UW solution until transplanted orthotopically, with the usual technique (5). The hepatic artery was reconstructed by a microsurgical technique. The cold and the warm ischemia time were 54 and 34 min, respectively. After the completion of vascular reconstruction, doppler ultrasonography (US) comfirmed excellent flow in the hepatic vein, portal vein, and hepatic artery. A transanastomotic biliary stent was passed through the Roux-en-Y loop via a Witzel-type enterostomy and conducted to the outside of the abdomen. Good blood flow in all reconstructed vessels was again confirmed just before and after the closure of the abdomen. Operation time was 8 h and

Table 1. Correlation of lab data, tacrolimus level, fever, and volume of drained ascites in the post-operative days

POD	AST (IU/L)	ALT (IU/L)	Tbil. (MG/DL)	Platelet ($\times 10^4/mm^3$)	Tac level (ng/mL)	BT max. (C)	Ascites (mL/day)
1	712	1240	5.6	12.0	2.5	39.9	356
2	279	879	4.0	12.1	13.9	36.6	341
3	464	1614	4.1	12.1	8.2	37.0	181
4	124	995	3.9	9.1	6.3	37.9	584
5	51	502	6.1	8.4	3.4	38.6	554
6	167	451	6.7	8.9	3.0	39.2	689
7	590	881	6.5	1.9	3.6	38.0	812
8	909	1064	13.4	5.4	7.5	37.8	931

33 min. Immunosuppressants consisted of oral tacrolimus and low dose steroids. Biopsy of the donor liver at the time of transplantation (zero biopsy) did not show any specific pathology.

Post-operatively, the peak transaminase (ALT) level was over 1600 IU/L on day 3 (Table 1). This peak ALT was unusually high in comparison with other age-matched cases in our experience, but the exact cause was unknown. The patient was awake and extubated the day after the transplantation. He was clinically stable until 5 days when he developed a high fever, with increased volume of drained ascites and a pulmonary effusion. The trough level of tacrolimus was 13.9 ng/mL on day 3. Considering the high transaminase level reflecting the poor metabolic function of the liver, we transiently stopped. The level decreased to 3.4 ng/mL on day 5, when high fever was initially noted. We did not re-start the tacrolimus because we thought the patient was infected, although a focus was not apparent. From the evening of day 6, we re-started tacrolimus because the transaminase level which had decreased started to increase, although the patient still had a high fever. From day 7, color of the drained bile became pale. Hepatic blood circulation frequently checked by doppler US did not show any abnormality either of the hepatic artery or the hepatic vein. However, the portal vein flow decreased on day 7. The graft blood flow was excellent and any infection focus was not defined by repeated pan-culture of various specimens, including arterial blood. At this time, we assumed that the series of the events was because of an acute cellular rejection that occurred under the low level of tacrolimus. Steroid pulse therapy by bolus injection of MPD was started on day 7 without biopsy confirmation. On day 8, thrombocytopenia and a rapid increase of the transaminase level with deterioration of consciousness were recognized. We did a liver biopsy on day 8 and performed an exchange transfusion to improve the level of consciousness and coagulation function. Liver biopsy findings were available on day 9 and showed centrilobular hemorrhagic necrosis (Fig. 1), fibro-edematous thickening of intima of the central veins (Fig. 2), associated with moderate cellular infiltration (Fig. 1) and endothelitis of the portal area. On day 9, the patient became comatose with poor coagulation function. Urgent re-LDLT from his blood type compatible mother was performed, and the patient subsequently recovered uneventfully. Explanted liver was 310 g (1.7 times larger than the original graft weight), and more than 90% was histologically necrotic. All three reconstructed vessels were macroscopically patent without any sign of thrombosis. Correlating the clinical and histological findings, we diagnosed that the event

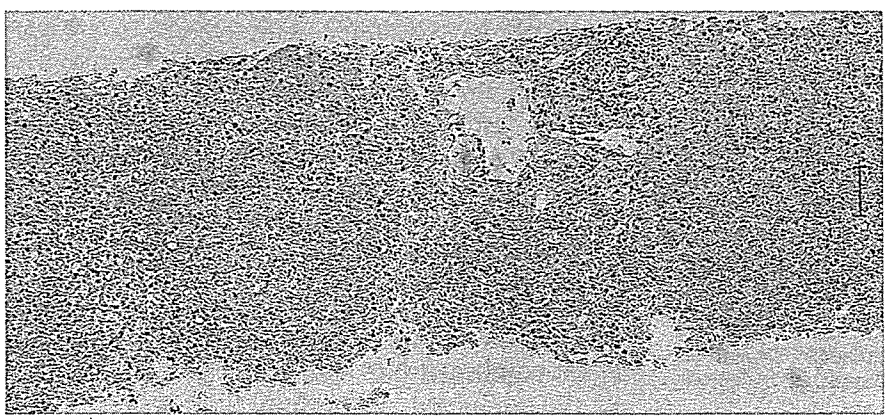

Fig. 1. Cell infiltration in the portal area and hemorrhagic necrosis of the graft (needle biopsy on day 8, H&E, ×100).

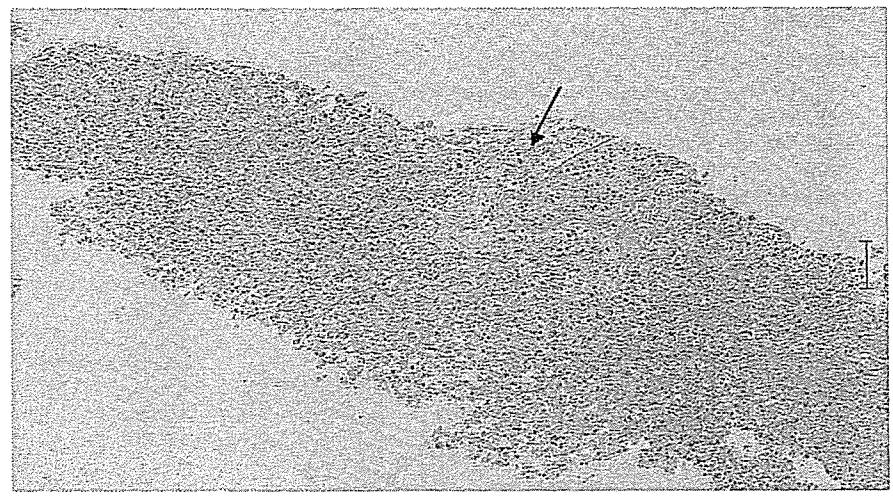

Fig. 2. Fibro-edematous thickening of the intima of a central vein (arrow) (needle biopsy on day 8, H&E, ×100).

was VOD associated with moderate acute cellular rejection.

Discussion

One review article mentions that about 1.9% of patients with cadaveric liver transplantation suffer from VOD at various times post-transplant (4). In another older report, 43% of the patients after liver transplantation had hepatic venular stenosis with immunosuppression using azathioprine, even though part of those episodes were transient (6). The cause and pathophysiology of VOD after liver transplantation is not resolved. Azathioprine has been cited as one of the causative agents for VOD after renal transplantation or liver transplantation (3, 6). However, this drug cannot be the only one cause because not all the VOD was reversible after discontinuation of the drug (4). In the present case, azathioprine was not used. Weigel et al. reported that azathioprine may contribute to the endothelial injury (7). Sebagh et al. suspected that VOD results from an immunological phenomenon (4). In this case, acute cellular rejection triggered by the attenuation of immunosuppression may have precipitated VOD. In the present case, needle biopsy specimen showed pathological findings compatible VOD, associated with acute cellular rejection. Clinically, the transaminase level increased with the attenuation or discontinuation of immunosuppression. This suggests the possibility of a contribution of immunological factors.

In LDLT, it is quite rare to see early graft failure except in cases of incompatible ABO-blood type matching, thrombosis of reconstructed vessels, or marked mismatch of the graft size. Acute cellular rejection can occur within a week after LRLT, but usually is treated with enhanced maintenance immunosuppression or steroid pulse therapy except the cases of incompatible matching. In the present case, the patient had no pre-formed reactive antibody. Therefore, hyperacute rejection is not likely. Most of the reported cases of VOD were diagnosed by liver biopsy and there were few cases of VOD diagnosed clinically. At the early phase after transplantation, it is difficult to diagnose VOD without pathological findings. In the present case, the first diagnostic clue was inflammation, increase of ascites and abnormal coagulation function. These were nonspecific, but the diagnosis of VOD should be considered with such symptoms even after LDLT.

In reported cases of VOD after liver transplantation, the age of the recipients ranged from 5 to 63 yr. Onset of VOD ranged from 11 to 3972 days after transplantation (4). So far, our case was the youngest recipient with the early onset of VOD. The survival rate of the VOD after liver transplantation has been reported to be very poor (4). Re-transplantation was the only way to save the patient in our case. If the diagnosis of VOD was possible before completion of the graft failure, anticoagulation therapy might be one treatment option, although the effectiveness is not approved (8).

In conclusion, VOD should be included as one of the causes of the early graft failure after LDLT. It can be complicated by acute rejection, and early suspicion may be effective for the successful treatment.

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CASE REPORT

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Rescue for rare complications of the hepatic artery in living donor liver transplantation using grafts of autologous inferior mesenteric artery

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Abstract This report describes two rescued cases with rare complications of the hepatic artery in living-donor liver transplantation (LDLT). In both cases a segment of the autologous inferior mesenteric artery (IMA) was successfully used as an arterial graft for re-vascularization under microsurgery. The first case was that of a pseudoaneurysm of the hepatic artery, which caused massive gastrointestinal bleeding. The hepatic arteries of the pre- and post-aneurysm were divided, and the arterial graft from the recipient's IMA was interposed for reconstruction. The second case was that of an intimal dissection of the recipient's hepatic artery. Because the dissection extended to the root of the common hepatic artery, the autologous IMA was interposed between the donor's hepatic artery and the proximal stump of the recipient's splenic artery. Reconstruction using the arterial graft of the autologous IMA is feasible for re-vascularization of the hepatic artery in liver transplantation.

Keywords Living-donor liver transplantation · Hepatic artery · Pseudoaneurysm · Intimal dissection · Microsurgery · Autologous IMA

Introduction

As hepatic artery thrombosis (HAT) in the early stage after liver transplantation is frequently fatal, urgent treatment such as re-transplantation or re-vascularization is essential. Especially in countries where brain-dead donors are scarce, graft salvage and patient survival depend on early recognition and correction of HAT. Therefore, early postoperative and repeated Doppler ultrasound surveillance is, clearly, important. Angiography is recommended where necessary. Surgical thrombectomy or reconstruction of the artery should be considered immediately in the case of HAT, especially in the early postoperative period. In such cases, vascular grafts are sometimes required. In previous reports, the autologous radial artery, sigmoid artery or saphenous vein, as well as the donor's iliac artery, were used as graft vessels [1, 2, 3]. There is no report, however, regarding the autologous inferior mesenteric artery (IMA).

Though pseudoaneurysm or intimal dissection of the recipient's artery is rare among hepatic arterial complications following liver transplantation, both conditions are as serious, if not more so, than thrombosis. In this report, we present two cases of rare complications of the hepatic artery, in which the autologous IMA was successfully used as an interposed graft for hepatic re-vascularization in living-donor liver transplantation (LDLT).

Case report

Case 1

A 49-year-old man underwent LDLT for familial amyloid polyneuropathy (FAP), with the right lobe graft taken from his daughter. The recipient's right hepatic artery (3.5 mm in outer diameter) was sutured with the

donor's right hepatic artery (3.0 mm in diameter) in an end-to-end fashion by microsurgery. The interrupted suture was performed with 8-0 Prolene. There were no complications during operation, and postoperative Doppler ultrasound showed good arterial flow. The patient recovered without any complications such as abdominal bleeding or bile leakage in the early period after transplantation.

Nineteen days after transplantation, he developed acute rejection and steroid pulse therapy was performed. On postoperative day 31, he suddenly went into shock due to massive gastrointestinal bleeding. An urgent endoscopic examination revealed a duodenal ulcer with active arterial bleeding. The ulcer was 1.8 cm in diameter and located in the posterior wall of the duodenal bulb. Endoscopic hemostasis was not successful. An emergency laparotomy was therefore performed. Oversuturing the ulcer through duodenotomy controlled the bleeding. However, re-bleeding occurred 10 days later. On this occasion, emergency angiography was performed and revealed massive bleeding from the pseudoaneurysm located near the anastomotic site of the hepatic artery. Coil embolization of the aneurysm was performed through the celiac artery, and bleeding was successfully controlled.

Though temporary hemostasis was attained, intestinal bleeding occurred again 11 days later, and coil embolization was performed again (Fig. 1). Once more, hemostasis was achieved, but re-bleeding was a serious concern. Surgical repair of the aneurysm was quickly decided upon and performed on day 56, 4 days after the third episode of gastrointestinal bleeding. Severe adhesion between the hepatic artery and the duodenal wall was observed. It was suggested that the pseudoaneurysm existed inside the granulation in the adhesion. Both the proximal and distal ends of the aneurysm were dissected as long as possible. The artery was transected on both sides without touching the pseudoaneurysm itself. The proximal stump was fragile and considered too inadequate for the new anastomotic site. Therefore, an arterial graft, 3 cm in length, which was taken from the recipient's IMA, was interposed between the graft side hepatic artery and the recipient's gastroduodenal artery (Fig. 2). A segment of IMA was dissected and resected from the root to the first branching. Arterial suturing was performed again by microsurgery. Since this repair was carried out, adequate flow in the hepatic artery has been maintained, and no further gastrointestinal bleeding has occurred. The patient continues to do well 2 years after the operation.

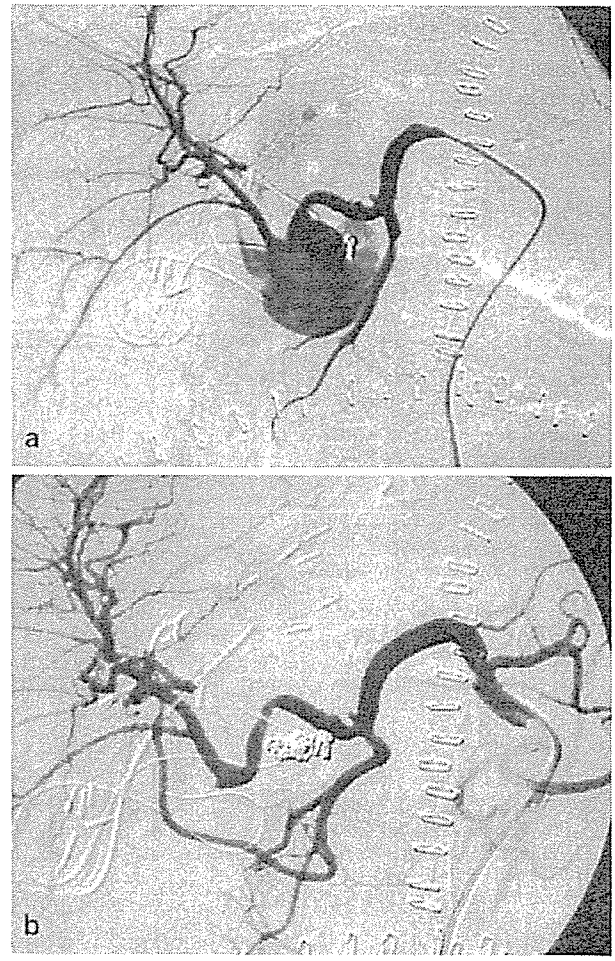

Fig. 1 Case 1: a celiac angiography on postoperative day 52. Bleeding from the pseudoaneurysm to the duodenal cavity is shown. b After coil embolization of the pseudoaneurysm

Case 2

A 30-year-old woman suffering from autoimmune hepatitis underwent LDLT with the left hepatic lobe from her father. Splenectomy was performed at the transplant because the spleen was infected. The donor's left hepatic artery (3.0 mm in diameter) and the recipient's right hepatic artery (3.5 mm in diameter) were sutured in an end-to-end fashion. Though the intima of the stump of the recipient's right hepatic artery seemed to be partially peeled away from the wall, anastomosis was performed as usual after confirmation of a good outflow from the recipient side. However, the hepatic arterial signal suddenly disappeared on Doppler ultrasound 6 days after the transplantation. Emergency laparotomy was performed for reconstruction. Liver function tests were almost stable at the time, despite the inadequate flow in the hepatic artery. Intraoperative Doppler study demonstrated an arterial flow, but it was very unstable and depended on the position of the artery.

We cut the recipient's side of the anastomosis and found intermittent and poor flow from the stump.