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Heterozygous protein C deficiency and dysfibrinogenemia acquired by liver transplantation

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Abstract Orthotopic liver transplantation is now a successful treatment for end-stage liver diseases. Since most components of the coagulation system are synthesized by liver parenchymal cells, there is always a risk of genetic defects of hemostasis being transmitting by liver transplantation. Some coagulation factor defects, such as protein C deficiency, do not induce abnormalities in routine coagulation tests and, thus, go undetected before organ procurement. We report the first case, to our knowledge, of the transmission of heterozygous protein C deficiency, an autosomal recessive genetic defect, associated with dysfibrinogenemia, an autosomal dominant trait, by liver transplantation. Both the recipient and the donor presented with severe thrombotic complications. This case shows that potentially morbid genetic defects can be transmitted by organ transplantation, and it emphasizes the difficulty associated with organ procurement criteria, particularly for liver transplantation, in which routine blood tests appear insufficient for determining whether or not organs can or should be procured from a given donor.

Key words Protein C deficiency, liver transplantation · Liver transplantation, protein C deficiency · Liver transplantation, dysfibrinogenemia · Dysfibrinogenemia, liver transplatation

Introduction

Orthotopic liver transplantation (OLT) is the best treatment for a large group of end-stage liver diseases [15]. However, because most components of the hemostasis system are synthesized by liver parenchymal cells, there is always a risk of acquiring morbid genetic defects of hemostasis through liver transplantation.

Hematologic evaluation before liver procurement usually consists of whole blood count, prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen level. Some head injuries can induce coagulation abnormalities such as disseminated intravascular coagulation (DIC) [11, 12]. Such a trauma induces hemostatic activation by releasing tissue thromboplastins from the injured tissue, resulting in thrombin generation [12]. This can make the interpretation of coagulation tests before liver procurement quite difficult. Clarkson and colleagues reported a factor XI deficiency acquired through liver transplantation. The donor, who died of intracerebral bleeding, had an isolated, prolonged aPTT [2]. Moreover, some defects of coagulation factors, such as protein C deficiency, do not alter routine hemostatic tests, making their detection before organ procurement highly unlikely.

We report the first observation, to our knowledge, of two genetic abnormalities – heterozygous protein C deficiency and dysfibrinogenemia – transmitted by OLT.

Table 1 Laboratory data after liver transplantation

Days post-OLT	Date	Bilirubin (μmol/l)	ASAT (UI/l)	Fibrinogen (g/l)	Ethanol test	D-dimers (μg/ml)	Factor V (%)	PT (%)	aPTT (s)	Protein C (%)	Events	Treatment
−1	28/06/93	16	51	2.64	−	0	100	100	33	58		
0	29/06/93	67	958	1.99	+	8	58	58	43			Frozen plasma
1	30/06/93	54	560	1.86	+	8	53	68	41			
2	1/07/93	38	573	1.34	+	8	100	74	35			
3	2/07/93	56	256	0.92	+	8		76	34			Fibrinogen
4	3/07/93	54	106	1.40	+	16	100	73	28			
5	4/07/93	58	52	1.27	+	2	100	69	35			
6	5/07/93	68	42	0.38	+	4	100	70	30			Fibrinogen
7	6/07/93	60	34	1.90	+	8	100	65	34			
8	7/07/93	58	23	1.10	+	8		81	30			
9	8/07/93	53	30	1.15	+	4	100	74	30			
10	9/07/93	33	34	0.97	+	8	100	77	33			
11	10/07/93	50	16	0.92	+	2	100	69	31			
12	11/07/93	41	22	1.22	+	2		81	34			
13	12/07/93	32	25	1.12	−		100					
14	13/07/93	21	25	1.30								
15	14/07/93	24	23				100	68	40			
16	15/07/93	18	22	0.81	−	4						
20	19/07/93	25	22	1.37	+	8		85	32			
24	23/07/93	17	39	1.58	+	16	100	71	40			
27	26/07/93	20	57		+							
31	30/07/93	26	164	0.93	+	16	100	74	30			
35	3/08/93	42	177	1.08								Fibrinogen
36	4/08/93	39	183	1.77								
37	5/08/93	51	157	1.16								Fibrinogen
38	6/08/93	57	129	2.03							Pulmonary embolism	Enoxaparin
39	7/08/93	51	147	1.80								
41	9/08/93	44	213	1.42								
44	12/08/93	66	307	1.7	−	0		90	33			
48	16/08/93	105	297	1.01	−	0	97	84	33			
56	24/08/93	184	112	1.32	+	0.5	97	65	41			
65	2/09/93	115	89	0.23	−	0		70	33			Fibrinogen
73	10/09/93	50	45	0.92	+	2	100	80	32			
87	30/09/93	30	137	0.4	+	2	100	88	30			
100	13/10/93	27	158	0.78	−		100	73	31	29	Venous thrombosis	Enoxaparin
145	24/11/93	19	43	1.31	−			90	36	41		Enoxaparin

Normal values: Bilirubin 3–18 μmol/l, ASAT < 45 UI/l, Fibrinogen 2.5–4 g/l, aPTT 31 s, Factor V and PT 70 %–100 %, Protein C 70 %–120 %)

Case report

In April 1991, a 57-year-old Caucasian man with no significant medical history was hospitalized for a cholecystectomy. During this hospitalization, a hepatitis C virus-related cirrhosis was found. His total bilirubin level was normal (16 μmol/l, normal 3–18 μmol/l), aminotransferases were 1.5 times normal, and PT was 90 % (normal range 70 %–100 %). In December 1992, magnetic resonance imaging (MRI) showed a hepatic nodule of 2 cm corresponding to a hepatocarcinoma. The patient was registered in the hepatic transplantation program in June 1993; at that time, total bilirubin, alkaline phosphatases, and γ-glytamol transferase (γ GT) were normal, aminotransferases were 1.5 times normal, PT was 100 %, aPTT was 33 s ratio 1, fibrinogen was 2.6 g/l (normal range 2.5–4 g/l), factor V was 100 %, factors VII + X were 68 %, factor

Table 2 Coagulation tests in the donor and in the recipient (*Fa* fibrinogen activity, *ND* not done)

Date	Donor				Recipient			
	aPTT (s)	PT (%)	Fa (g/l)	protein C (%)	aPTT (s)	PT (%)	Fa (g/l)	protein C (%)
09/92			1	30	ND	ND	ND	ND
06/93	30	35	0.48	ND	33	100	2.6	58
OLT								
13/10/93					31	73	0.78	29
24/11/93					36	90	1.31	41

II was 72 % (normal range 70 %–100 %), and D-dimers were below 0.5 μg/ml. The activity of antithrombin III, protein C, and protein S was measured using automated chromogenic substrate assays. The results were: antithrombin III 80 % (normal range 70 %–120 %), protein C 58 % (normal range 70 %–120 %), and protein S 90 % (normal range 70 %–130 %).

On 27 June 1993, in the emergency room, a 50-year-old Caucasian woman was declared brain-dead after a spontaneous intracranial hemorrhage. She had been treated during the preceding year with fluindione for a venous thrombosis of the right leg. Upon her admission to the hospital, the laboratory data were: total bilirubin, alkaline phosphatases, γ GT, and aminotransferases normal, aPTT 30 s ratio 1, PT 35 %, and international normalized ratio (INR) 2. As a result of the fluindione therapy, she had a low level of fibrinogen (0.48 g/l), a weakly positive ethanol test, and D-dimers of 0.5 μg/ml. She received two units of fresh frozen plasma and two fibrinogen transfusions, after which fibrinogen was 1.8 g/l, while the ethanol test and D-dimers were negative. The liver was procured and ABO-compatible, HLA-unmatched OLT was performed on 29 June 1993. Immunosuppression consisted of cyclosporin, azathioprine (100 mg/day), and methylprednisolone. Then, 1 month later, cyclosporin and prednisolone (0.3 mg/kg per day) were given for maintenance therapy. There was no hepatic rejection. Postoperative data are summarized in Table 1. By day 27 post-OLT, cholestasis and cytolysis had appeared due to a recurrence of hepatitis C virus.

In November 1993, liver tests showed a return to normal values. However, problems appeared in coagulation testing. A few hours after transplantation (day 0, Table 1), fibrinogen was 1.99 g/l, PT was 58 %, aPTT was 43 s ratio 1.4, and factor V was 58 %. Three days after transplantation, with no plasma transfusions having been performed since the operation, fibrinogen was 0.92 g/l, PT 76 %, aPTT 34 s ratio 1.1, factor V 100 %, and D-dimers 8 μg/ml. The patient received a fibrinogen transfusion, but 3 days later the fibrinogen level once again dropped to 0.38 g/l without signs of DIC or important fibrinolysis. From that point on, fibrinogen remained decreased.

In August 1993, at which time the patient was under preventive treatment of thrombosis by low-molecular weight heparin (enoxaparine), he presented with a pulmonary embolism with a thrombosis of the inferior vena cava. Treatment consisted of augmentation of enoxaparine.

In October 1993, 3 months post-transplantation, in spite of the maintenance of prophylactic treatment with enoxaparine, the patient presented with another venous thrombosis of the right leg. Because the recipient's fibrinogen level was under 1.5 g/l and also because of the two deep venous thromboses he presented, other coagulation parameters were tested. Fibrinogen antigen was 2.8 g/l (nephelometric measure, normal range 2.5–4.5 g/l) but fibrinogen activity was 1 g/l (normal range 2.5–4 g/l), indicating dysfibrinogenemia; antithrombin III activity was 97 %, protein S activity 84 %, but protein C activity was only 29 %.

Protein C deficiency was confirmed in November 1993 (41 %). Because these two abnormalities did not exist before transplantation, transmission by OLT was suspected, and the donor's coagulation tests were studied retrospectively. They showed both a dysfibrinogenemia and a protein C deficiency. Not only was the fibrinogen level (fibrinogen activity) measured in the emergency room before transfusion low, as stated earlier (0.48 g/l), but it was measured at 1 g/l in September 1992, during a hospitalization for deep venous thrombosis. Protein C activity was found to be 30 % before anticoagulant treatment at that time (Table 2).

Treatment with fluindione was started in the recipient to raise the INR to three. Fifteen months later – 18 months after OLT – no thrombosis has reoccurred.

Discussion

Most components of clotting and fibrinolytic systems are synthesized by liver parenchymal cells; exceptions are von Willebrand factor, tissue-plasminogen activator (t-PA), and urokinase-type plasminogen activator [8]. Moreover, factors II, VII, IX, and X, and proteins S and C require vitamin K for their functions [8]. This explains the high frequency of transient hemostatic disorders after liver transplantation.

When detected before OLT, these disorders reflect the severity of liver damage. In liver diseases, the initial defect is usually the diminution of the vitamin K-dependent factors [8]. Factor V levels are correlated to the intensity of hepatic parenchymal cell damage [8]. Hyperfibrinolysis, DIC, thrombocytopenia, thrombocytopathy, and abnormal fibrinogen have also been described [4, 5, 8]. In this case report, the recipient had a post hepatitis C cirrhosis stage A, according to Child-Pugh's classification, with moderate coagulation abnormality (coagulation tests were normal except for protein C activity, which was slightly low at 58 %).

There is little in the literature concerning the recovery of hemostatic functions after OLT. In two series [14, 16], the course of coagulation tests after transplantation has been described as follows:

1. A rapid normalization of PT and aPTT is seen. The mean time elapsed before PT returns to normal is 3.7 ± 2.8 days after surgery [14] and, in most patients, aPTT is normal on the 1st postoperative day [14, 16]. The normalization of PT and aPTT reflects the normalization of the clotting factors. All of them, except factor VIII, which is increased, reach normal activity on day 3 or 4 [16].
2. Fibrinogen is normal on the 2nd postoperative day [16].
3. Coagulation inhibitors show a different pattern. Activity of antithrombin III requires from 10 to 14 days to a reach normal level ; protein C and protein S return to normal levels 3–5 days after OLT [14]. Thus, there is an imbalance between hemostatic and thrombotic mecha-

nisms, and the presence of elevated thrombin/antithrombin complexes favors activated coagulation and a hypercoagulable state [14, 16].

In this case report, after OLT, hypofibrinogenemia was recorded several times, despite normalization of the clotting factors (Table 1). This hypofibrinogenemia occurred without DIC, hyperfibrinolysis, or other major hepatic dysfunction.

The liver is the major site of fibrinogen synthesis, so fibrinogen levels were checked in the donor's records. In the emergency room, the donor's fibrinogen (i.e., fibrinogen activity) was decreased to 0.48 g/l. This disorder may be caused by the presence of tissue thromboplastin or activated coagulation factors after brain injury [11]. Neither the ethanol test nor D-dimer levels were determined for this patient at the time of admission to emergency care. The hypofibrinogenemia was assumed to have been due to the intracranial hemorrhage, and so we proceeded with liver procurement. However, retrospectively, we can conclude that the patient presented with a congenital dysfibrinogenemia since the fibrinogen antigen level determined by nephelometric methods was 4.46 g/l while fibrinogen activity was decreased. This dysfibrinogenemia was also noted when she was hospitalized in August 1990 and in September 1992 for deep venous thrombosis. Congenital dysfibrinogenemia has an autosomal dominant transmission and is uncommon [4]. Usually, the presence of abnormal fibrinogen is clinically silent, but it sometimes leads to hemorrhage or thrombosis [12]. Only 1 % of unexplained venous thrombosis is related to congenital dysfibrinogenemia [9, 13], so when the recipient was hospitalized the second time for a deep venous thrombosis, we looked for a cause other than hypofibrinogenemia for these thromboses. A decreased protein C activity (29 % and 41 %) was then discovered.

The liver is also the major site of protein C synthesis, and homozygous protein C deficiency can be corrected by hepatic transplantation [1]. Because the normalization of protein C activity practically always occurs early after transplantation (mean 3.7 ± 2.5 days), the persistence of low levels of protein C in the recipient 3 and 4 months after transplantation, with no apparent reason, suggests the transmission of a genetic heterozygous protein C deficiency by OLT. The retrospective study of the donor's coagulation parameters showed, in September 1992, in addition to dysfibrinogenemia, a protein C deficiency (protein C activity was 30 %). This disorder is either transmitted as an autosomal recessive trait or as an autosomal dominant trait with variable penetrance [9]. Only levels under 55 % in adults in stable condition, without anticoagulant treatment or liver disease, are highly predictive of heterozygosity [3]. The incidence of heterozygosity varies from 1 in 200 to 1 in 300 adults [3].

The recipient, as well as the donor, presented with recurrent thrombosis. The role of heterozygous protein C deficiency in thrombosis is still uncertain. Miletich and colleagues [10] measured levels of protein C antigen in 5422 blood donors. They found 79 heterozygous patients, but none of them had a personal or family history of thrombosis. They concluded that heterozygous protein C deficiency, in the absence of other abnormalities, is not in itself an important risk factor for thrombosis [10]. Harenberg and colleagues [16] also suggested that acquired protein C deficiency in liver disease is not related to thromboembolism. However, according to other studies, only 25 % of the patients with heterozygous protein C deficiency are asymptomatic, and the prevalence of protein C deficiency among patients younger than 40–45 years of age with unexplained venous thrombosis is 5 %–8 % [9]. In OLT in children, the protein C level remains low longer than in adults. This explains perhaps in part the higher incidence of hepatic arterial or portal thrombosis in child liver recipients [7].

In our case, the protein C deficiency was associated with a dysfibrinogenemia in the donor and then, after OLT, in the recipient. The association of these two abnormalities probably explains the thrombotic events in the donor and also in the recipient after OLT because they occurred in spite of preventive treatment with enoxaparine in the recipient.

Despite a shortage of available livers, we must be very careful in evaluating organ function before procurement. Bilirubin, hepatic enzymes and coagulation tests (aPTT, PT, and fibrinogen) must be performed to evaluate liver functions and carefully considered before organ procurement. If coagulation abnormalities are found, other parameters, such as an ethanol test and D dimer levels, are useful. However, some congenital hemostasis abnormalities, such as protein C deficiency, do not lead to perturbations in routine coagulation tests. To our knowledge, we report the first case that associates two genetic abnormalities – heterozygous protein C deficiency and dysfibrinogenemia. This case report emphasizes the importance of the donor's medical history, but also the difficulty in establishing organ procurement criteria, particularly in liver transplantation.

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