NDT **PLUS**
Nephrology Dialysis Transplantation

Letters

Acute renal allograft thrombosis in 'seronegative' antiphospholipid syndrome

Sir,
We present a 32-year-old Asian female with ESRD secondary to lupus nephritis who received a deceased donor kidney transplant but lost the graft 5 days later. Her medical history was significant for a pregnancy complicated by hypertension and intra-uterine growth retardation with absent end diastolic flow. This necessitated emergency caesarian section at 28 weeks of gestation. Pathology of the placenta revealed vascular thrombosis and decidual necrosis.

At this hospitalization, the patient received a four-antigen match deceased donor kidney without intra-operative complications. The allograft functioned promptly after transplantation. However, on post-operative Day 5, she acutely decompensated with hypotension and diminished level of consciousness. Surgical exploration revealed a pale nonviable kidney necessitating a nephrectomy.

Histologic examination revealed that one of the hilar vessels had a luminal thrombus associated with haemorrhage (Figure 1A). Thrombi or endothelial swelling was also noted in afferent arterioles and glomerular capillaries (Figure 1B). Interstitial inflammation, tubulitis and arteritis were absent. Immunofluorescence was negative for immunoglobulins and C3 in the glomeruli and C4d in peritubular capillaries, ruling out recurrent lupus and humoral rejection. At the time of transplant, tests for lupus anticoagulant and anti-cardiolipin antibody were negative on two separate occasions. However, her serum did show increased thrombotic activity in a new test performed in our laboratories, which is a proposed surrogate test in patients at risk for thrombosis (Figure 1C). A diagnosis of graft failure due to thrombotic microangiopathy and seronegative anti-phospholipid antibody syndrome was rendered.

Thrombosis is the major cause of graft loss in the first year of survival following kidney transplantation. The
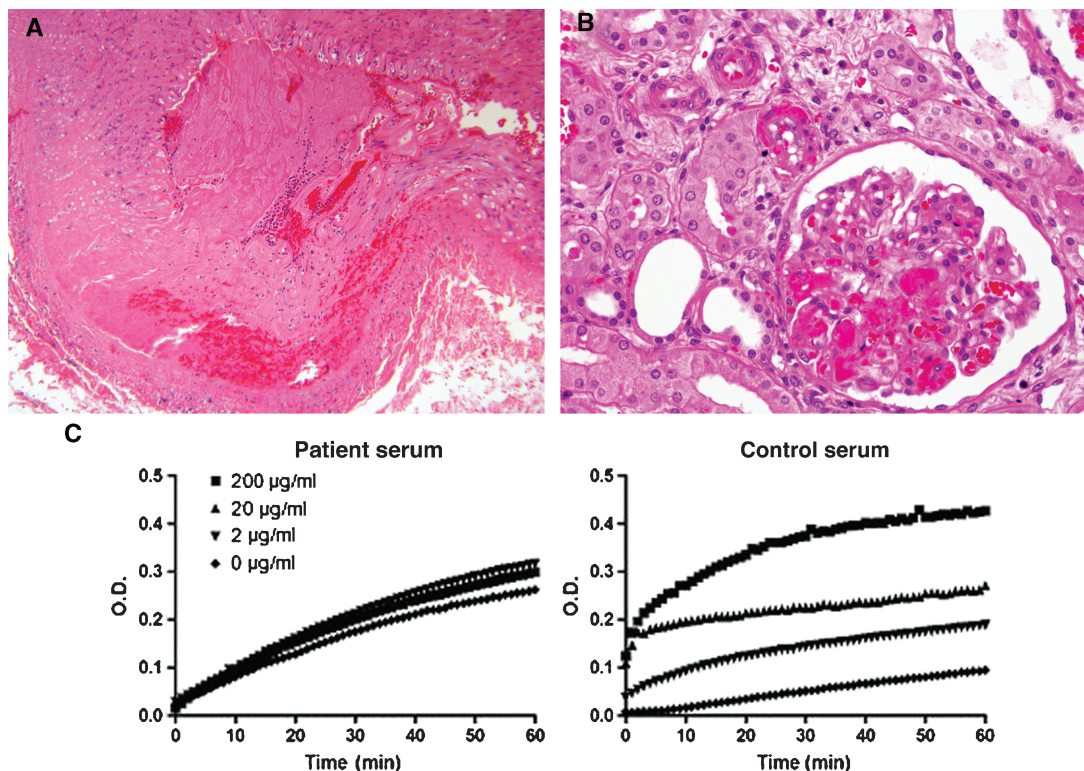

Fig. 1. (**A**) Explant nephrectomy performed on Day 5. There is extravasation of red cells and fibrin in the muscular wall and thrombosis of the renal artery (H&E ×100). (**B**) Explant nephrectomy. Glomerular capillary thrombi and thickening and endothelial swelling in adjacent arteriole (H&E ×200). (**C**) Kinetics of kallikrein activation by negatively charged molecules. Patient serum (30 µl) or control human serum (30 µl) was incubated with 20 µl of oversulfated glycosaminoglycans (OS-GAG) at a final concentration of 0, 2, 20 and 200 µg/ml at 37°C for 5 min. Kallikrein amidolytic activity was assessed by addition of 150 µl of 50 mM N-Benzoyl-Pro-Phe-Arg-*p*-nitroanilide hydrochloride chromogenic substrate. O.D. readings were taken every minute for 60 min. The auto-activated kallikrein activities (without OS-GAG induction) in patient serum suggest the presence of a negatively charged contact system inducer in the patient's blood.

APLS is a hypercoagulable state that predisposes patients to arterial and/or venous thromboses that can result in graft failure [1]. A 2006 consensus conference defined patients with definite APLS as having at least one laboratory criterion (e.g. persistent, elevated titres of APL) and one clinical criterion (e.g. deep vein thrombosis, pregnancy complications) [2]. However, APLS encompasses a wide range of clinical symptoms [3]. Notably, a subset of patients manifest thrombotic complications without detectable antiphospholipid titres. These patients are classified as having seronegative antiphospholipid syndrome (SNAPS) [2]. This patient's history of pregnancy complications was subtle, and the absence of LAC and ACA antibodies did not raise the possibility of APLS at the time of transplantation. We therefore propose the diagnosis of SNAPS in this patient. SNAPS increases the risk of graft thrombosis and necessitates anti-coagulation before and after the transplant operation [4]. Therefore, a second transplant operation in this patient would be considered high risk and would require peri-operative anti-coagulation [5]. This case highlights the difficulty of diagnosing 'seronegative' APLS within the current diagnostic guidelines. It also identifies a need for new diagnostic tests to identify at-risk patients.

Conflict of interest statement. None declared.

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Severe thiamine deficiency complicated by weight loss protects against renal ischaemia-reperfusion injury in rats

Sir,

Injury due to reperfusion after prior ischaemia (IRI) is an important cause of delayed graft function after renal transplantation [1]. Studies in dogs and rats suggest that thiamine is protective against IRI in heart and brain [2–4]. It has been argued that many organs, including kidneys are deficient in thiamine at the moment of transplantation [4]. We aimed to investigate the effect of severe tissue thiamine deficiency on ischaemia-reperfusion injury in rat kidneys.

Male inbred Lewis rats ($\pm$ 270 g) (Harlan, Zeist, The Netherlands) were fed with a thiamine-deficient diet (Arie Blok, Woerden, The Netherlands). The diet only contained trace amounts of thiamine (0.16 μg/kg, equalling $\sim$0.04% of the thiamine content of regular chow). Control animals were orally supplemented with 400 μg thiamine/day in a 2.5% sucrose solution, whereas the thiamine-deficient animals only received the same volume of the sucrose solution. After 4 weeks, ischaemia-reperfusion procedures were performed. Anaesthesia was induced by 5% isoflurane and maintained on 3% isoflurane. The rats were placed on a homothermic table to maintain core body temperature at 37°C and the left kidney was subjected to 45 min of ischaemia, followed by reperfusion. Nephrectomy of the contralateral right kidney was performed during ischaemia of the left kidney. Kidney tissue samples were snap-frozen and stored at $-80°$C in 4% formalin. Plasma and red blood cells were also stored at $-80°$C. Tissue transketolase activity was measured according to the kinetic method of Chamberlain *et al.* [5]. Thiamine, thiamine monophosphate and thiamine pyrophosphate were determined by HPLC with fluorimetric detection [6]. All experimental procedures were approved by the Committee for Animal Experiments of the University of Groningen and performed according to the principles of laboratory animal care.

In the third week of the experiment, growth of the thiamine-deficient rats was significantly slower than that in the control rats (12.1 $\pm$ 6.3 g versus 21.0 $\pm$ 4.7 g, respectively, $P = 0.003$). In the fourth week, the thiamine-deficient rats lost weight, whereas the control rats gained weight (-9.5 ± 8.8 g versus 15.4 $\pm$ 5.2 g, respectively, $P < 0.001$), resulting in a significant difference in body weight before ischaemia reperfusion (326 $\pm$ 13.8 g versus 355 $\pm$ 22.8 g respectively, $P = 0.006$). Induction of thiamine deficiency resulted in significant decreases in renal biochemical and functional thiamine status at the moment of ischaemia reperfusion (Table 1). There was no difference in baseline plasma creatinine concentrations prior to ischaemia reperfusion between thiamine-deficient and control rats (16.7 $\pm$ 1.8 μmol/L versus 16.9 $\pm$ 2.4 μmol/L respectively, $P = 0.88$). At the first day after

Table 1. Transketolase activity and thiamine and thiamine metabolites

	Control ($n = 11$)	Thiamine deficient ($n = 8$)	P-value
TK activity	13.9 (2.4)	7.7 (1.5)	<0.001
TPP	81.2 (13.2)	15.7 (6.5)	<0.001
TMP	26.2 (4.9)	0.4 (0.4)	<0.001
THM	90.2 (15.2)	2.5 (0.8)	<0.001

TK activity is expressed as mU/mg protein. TPP, TMP and THM are expressed as pmol/mg protein.