Additional anti-HLA antibody detection by using PRA-STAT

Sir,

The diagnostic test PRA-STAT (SangStat) is an ELISA kit for the detection of HLA-specific IgG antibodies, which occur after sensitizing events such as pregnancy, blood transfusion, and organ transplantation. The ELISA offers some direct technical advantages over the conventional complement-dependent cytotoxicity (CDC) method: it does not require any cell preparation and the cell panel from which HLA molecules are derived to coat the ELISA plates does not vary from lot to lot [1]. Moreover, it gives additional results on antibodies that cannot be detected by standard CDC [2–4]. Indeed the ELISA method detects all types of IgG antibodies, including non-complement-fixing IgG2 and IgG4, while IgM auto- and alloantibodies are not identified.

Although recent reports have been made on the clinical applications of this test for the patients awaiting organ transplantation or experiencing graft rejection [5–9], there is a need to further document the benefits by prospective studies. The following observation illustrates one particular approach.

In 1995 a 41-year-old patient had been treated by haemodialysis since 2 August for end-stage renal failure due to chronic glomerulonephritis. As he was severely anemic, and as routine preparation for kidney transplantation (he did not have any cytotoxic antibodies detected by CDC and PRA-STAT), he received two bags of phenotyped non-filtered blood from two different donors. Two weeks later, the patient was screened for the presence of anti-HLA antibodies: he was found negative by CDC (carried out at 25°C, then 37°C on 22 different cell samples) but when the same serum was tested by PRA-STAT, it was shown to contain significant amounts of specific antibodies towards HLA-A10, HLA-B27 and HLA-B62. As a consequence the HLA pheno-type of the two blood donors was determined. Donor 1 was found to be A3 A34 (split of A10) B62 B70 and donor 2 was found A26 (split of A10) A29 B35 B56. The antigen HLA-B27 was not found, which could imply either that the anti-B27 reaction of the tested serum is due to some other activation mechanism or to a broad cross-reactivity.

Since only CDC results were available, we continued to give an additional blood transfusion from a third donor. Two weeks later, the serum of the patient was screened for the presence of anti-HLA anti-bodies and was found again to be negative by CDC. In contrast it showed a significantly positive result on PRA-STAT; this time with a specificity always towards HLA-B27 and HLA-B62 but also towards HLA-B57. Therefore the HLA phenotype of the third donor was determined and found to be A2 A24 B18 B57.

The agreement between the anti-HLA antibodies detected by PRA-STAT and the HLA phenotypes of the three donors was in sharp contrast with the negative results obtained by CDC. For this reason the sera were tested independently by flow cytometry on a selected cell panel (A. Cesbron and J. D. Bignon, CRTS, Nantes, France). They were shown to contain a clear anti-B26 reactivity; the specificities towards HLA-B27, HLA-B57 and HLA-B26 were more weakly present.

These data, with the concordance between PRA-STAT and flow cytometry, suggest that additional antibodies detected by PRA-STAT and not by CDC might belong to a category that does not fix complement efficiently, as antibodies belonging to the IgG2 and IgG4 isotypes.

The clinical relevance of the additional antibodies detected by PRA-STAT is to be further documented by prospective studies. At the moment, neither CDC nor PRA-STAT gives the maximum of information on the HLA immune status of patients. Therefore, if these antibodies detected by ELISA but not by CDC are relevant for blood transfusions and organ transplantation, it would be advisable to use both ELISA and CDC in screening policy to obtain more information. Additional costs of this complementary technique would be weighed against the significant cost of unwarranted blood transfusions and the potential post-transplant complications like acute rejection and graft loss, which require expensive drugs, longer hospitalization, and eventually result in an early return to dialysis. The use of additional diagnostic techniques may lead to significant savings in the global hospital budget and, as such, justifies the initial extra cost on the laboratory budget.

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Sir,  
High pretransplant haematocrit (Hct) is a recognized risk factor for delayed graft function (DGF) and primary non-function (PNF). In a retrospective study, Schmidt et al., reported that a Hct of greater than 30% or an increasing pretransplant Hct, significantly increased the risk of delayed graft function [1]. Prolonged cold ischaemia time is also a well-known risk factor contributing to DGF and PNF, and influencing ultimate transplant survival. These two risk factors in combination may lead to an even poorer outcome.

We report a patient with pretransplant polycythaemia in whom we used intraoperative phlebotomy to induce haemodilution, since prolonged ischaemia time precluded this manoeuvre preoperatively.

A 50-year-old male, was admitted for a 0–1–1 mismatched cadaveric renal transplant from a 45-year-old donor who had died of intracerebral haemorrhage. The aetiology of the renal failure was diabetic nephropathy and the patient had been on CAPD for the previous 2 years. He had been a heavy smoker for 30 years. Four months prior to the transplant call-up he was found to have symptomatic normochromic normocytic anaemia with low ferritin level. This was appropriately treated with an i.v. iron infusion. His haemoglobin (Hb) increased after this from 12.6 to 18 g/dl. At this stage he was called for the cadaveric renal transplant.

Since the cold ischaemia time of this kidney was 41 h at this time, it did not permit preoperative phlebotomy and haemodilution. We therefore proceeded with the transplant operation.

On removal of the vascular clamps the graft remained dusky and patchily perfused. We therefore venaected 2 litres of blood from the external iliac vein and replaced it with Haemacol and saline to maintain haemodynamic stability. This improved perfusion of the kidney significantly. The recipient was commenced on cyclosporin, azathioprine, and prednisolone postoperatively as per our unit protocol.

A postoperative radioisotope perfusion scan showed a well-perfused graft without any significant function. Subsequently the patient underwent a protracted period of DGF requiring dialysis. The Hb dropped from 18 g/l preoperatively to 8 g/l postoperatively. Transplant biopsy on day 5 showed acute tubular necrosis with foci of pyelonephritis which was treated appropriately. The day 10 biopsy revealed continuing acute tubular necrosis and moderate cellular rejection which was treated with i.v. methylprednisolone over 3 days.

The patient’s graft function started to recover spontaneously from day 18, with serum creatinine improving to 200 μmol/l by day 30. After this there was a further episode of rejection and the cyclosporin was replaced by FK506. His serum creatinine stabilized at around 200 μmol/l. At 3 months the Hb remained stable and the patient remained clinically fit and well. His serum erythropoietin levels were normal before transplant and during the period of delayed graft function but rose significantly on establishment of successful graft functions.

It has been suggested that low Hct is beneficial for graft perfusion and haemodilution can prevent normothermic renal ischaemia [2–4]. However, in a retrospective study, Linde et al. did not find any significant difference in onset of graft function, graft survival or serum creatinine levels at 1 year in rHuEpo-treated recipients [5].

The effect of pretransplant Hct on graft function is thus controversial, although it is generally felt that high levels have a deleterious effect. Rising Hct levels in the 3 months preceding transplant also predisposes to DGF [1]. Medullary congestion with erythrocytes may contribute to reperfusion injury by contributing to incomplete return of medullary blood flow [2,6]. Congestion and stasis can be prevented by haemodilution. The degree of erythrocyte trapping has been correlated with the duration of cold ischaemia [3].

Haemodilution has been reported to reduce medullary erythrocyte trapping in rat kidneys [2, 3]. The deleterious effects of high haematocrit and long cold ischaemic times can be additive, as was demonstrated by this patient. It has been shown that intraoperative albumin administration favourably affects the outcome of cadaver renal transplant [7].

We treated this polycythaemic patient with intraoperative phlebotomy and haemodilution. This, to our knowledge, has not been reported previously and can be successfully done if long cold ischaemia time precludes preoperative phlebotomy and haemodilution. This patient achieved good graft function despite the long cold ischaemia time, high preoperative Hct, and absence of medullary stasis. In patients with high Hct, intraoperative phlebotomy and haemodilution can therefore be successfully used, as illustrated in our patient.