Original Article

Prolonged aPTT after kidney transplantation due to transient lupus anticoagulants

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Abstract

Background. After kidney transplantation, a renal biopsy may be needed to elucidate the reasons for lack of graft function. If the activated partial thromboplastin time (aPTT) is prolonged, the biopsy will often be postponed, as increased risk of bleeding must be expected. However, aPTT prolongation is not always due to lack of coagulation factors, but can be due to the presence of lupus anticoagulants (LAs). Clinical observations in our department indicated that a large proportion of recently kidney-transplanted patients developed prolonged aPTT values without clinical complications.

Methods. A prospective study of patients receiving a kidney transplant in 2004 was conducted to investigate the frequency and cause of prolongation of the aPTT.

Results. Twenty-seven patients were included in the study; none had prolonged aPTT or LAs before the transplantation. In the post-transplantation period, 19 patients (70.4%) had a significantly prolonged aPTT. Further investigation showed that for all 19 patients, prolongation was due to acquired antibodies: 13 had developed LAs and six had developed unspecific antibodies. The acquired antibodies were transient and did not affect clinical outcome.

Conclusions. This is the first study investigating prolonged aPTT in the post-transplantation period. All patients with prolonged aPTT had acquired transient antibodies, i.e. LA or ‘LA-like’. If a renal biopsy was requested, 70.4% of the transplanted patients would presumably have their biopsy postponed due to prolonged aPTT, but as LAs do not increase the risk of bleeding, such a delay would be unnecessary. Immediate LA investigation is therefore recommended if a recently transplanted patient requiring surgical procedures has a prolonged aPTT.

Keywords: antiphospholipid antibodies; coagulation; kidney transplantation; renal biopsy

Introduction

When allograft function fails in the early phase after kidney transplantation, renal biopsy is often performed. Before such a procedure, the coagulation status is monitored [i.e. measurement of platelets, prothrombin time and activated partial thromboplastin time (aPTT)] and, if the aPTT is prolonged, the biopsy will often be postponed, until coagulation parameters are normalized. In the days after kidney transplantation, we observed several patients with a prolonged aPTT without any evident explanation. A small retrospective study in our department (2003; unpublished) showed that prolongation of the aPTT after transplantation was a frequent problem. Of 33 consecutive kidney-transplanted patients with a normal aPTT before transplantation, 24% developed a prolonged aPTT within the first 24 h and 61% within the initial 5 days. A few months after transplantation, only one patient had a persisting prolonged aPTT. A prolonged aPTT can be due to lack of coagulation factors, but can also be caused by the presence of lupus anticoagulants (LAs), i.e. antiphospholipid antibodies interfering with the analysis. As development of a prolonged aPTT in these patients was not correlated with clinical outcome such as bleeding or graft malfunction, it could indeed be due to acquired LAs. If the aPTT prolongation was due to the presence of LAs, it would not contraindicate a graft biopsy, as LAs have no anticoagulation effect in vivo.

Antiphospholipid antibodies are a very heterogeneous group of acquired antibodies directed against proteins with affinity for phospholipid surfaces, mainly β2 glycoprotein I (β2GPI) and prothrombin, but also other phospholipid-binding proteins such as protein C,
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(protein S, annexin, etc. [1,2]. Antiphospholipid antibodies are detected by prolongation of phospholipid-dependent coagulation tests as LAs or by solid-phase immunoassays quantitating various more or less specific antibodies, e.g. anticardiolipin antibodies (ACAs), β2GPI antibodies or antiprothrombin antibodies. In most patients, the LA activity is due to a mixture of different antibodies [1,2]. LA exerts an in vitro anticoagulant effect by interference with phospholipid surfaces upon which coagulation depends, and the aPTT is thereby prolonged. In vivo, however, LA is considered a risk factor for venous and arterial thrombosis, as it promotes hypercoagulability by interfering with proteins on phospholipid surfaces [1,2]. LAs may be shorter living antibodies, e.g. due to infection [3,4], which incidentally are found because of prolongation of the aPTT, but these antibodies are usually a transient phenomenon and therefore without clinical importance. Critically ill patients may also develop LAs with no correlation with thromboembolic events or bleeding [5]. Persistent LAs, however, can arise due to autoimmune diseases, malignancy [6], or without any known reason, and these may result in a thrombogenic state called phospholipid antibody syndrome (secondary or primary).

A prospective study of consecutive patients receiving a kidney transplant was performed to investigate the frequency and cause of prolongation of the aPTT. A high frequency of acquired LA would indicate the need for routine performance of the LA test in such situations to accelerate surgical procedures.

Subjects and methods

Thirty-one consecutive patients, who received a kidney transplant in the period April 2004-June 2005, were included in the study. Four received high-dose heparin in the post-transplantation period and were excluded. The remaining 27 patients, 11 females and 16 males, were included in the study.

Immunosuppressive treatment

Treatment with tacrolimus or cyclosporin was initiated perorally immediately before transplantation: cyclosporin as Sandimmun Neoral® (Novartis) 15 mg/kg/day with dose adjustment to obtain a target blood level of 1500 nmol/l, and tacrolimus as Prograf® (Fujisawa) 0.25 mg/kg/day with dose adjustment to a target blood level of 10–20 ng/ml. All patients received mycophenolate mofetil (Cellcept®/C223 (Novartis) 20 mg intravenously (i.v.) 1.5 g of Cellcept daily. Basiliximab was given as Simulect® (Novartis) 20 mg intravenously (i.v.) before transplantation, followed by a second dose of 20 mg 4 days after transplantation. Thymoglobulin was given i.v. as Thymoglobulin® (Sangstat) 1.5 mg/kg at the day of transplantation and repeated once daily to a total of 5 days. No glucocorticoids were used. The different combinations of medication are shown in Table 3.

Anticoagulant regimen

All patients were given Enoxaparin (Clexane®, Aventis Pharma) 20 mg/day during the first 5 days after transplantation. Patients given heparin during or after transplantation were excluded.

Collection of data

Clinical data regarding dialysis status (including dialysis age), remaining diuresis, number of transplantations and smoking habits were collected. Changes in P-creatinine were recorded, along with transplantation medication, number of renal biopsies performed and duration of the transplantation admission period. Finally, clinical outcome was noted in terms of rejection status, the latest P-creatinine measurement and admissions related to thromboembolic events after the transplantation.

Blood sampling

Blood samples were drawn the day before transplantation, the day after transplantation and on several occasions in the following weeks until any acquired LAs had disappeared. All blood sampling was performed prior to daily Clexane administration. Blood was collected in plastic tubes containing 110 mM sodium citrate and centrifuged for 15 min at 2500 g followed by filtration (Acrodisc 0.2 μm) to remove the remaining platelets. For determination of ACAs and β2GPI antibodies, blood was collected in plastic tubes containing EDTA, and plasma was separated from cells within 20 min of collection. In all cases, the plasma was dispensed in aliquots tested immediately or otherwise frozen at −80°C.

Laboratory methods

Coagulation assays were performed on a STA-R coagulometer (Diagnostica Stago, Asnieres, France). The aPTT was measured with STA aPTT reagent containing cephalin as the source of phospholipids and silica as a particulate activator (Diagnostica Stago).

LA determinations were performed according to the recommendations from the Scientific and Standardization Committee (Subcommittee for Standardization of LA) [7], which are based on the following principles:

1. Prolongation of a phospholipid-dependent clotting assay. Here aPTT was used.
2. Evidence of inhibition demonstrated by mixing studies with normal plasma. The presence of LAs is indicated if normalization of the aPTT does not occur after mixing patient plasma and normal plasma 1:1. An in-house pool from five healthy donors centrifuged and filtrated as the blood samples was used as normal plasma.
3. A confirmatory test to demonstrate phospholipid-dependent inhibition. We used the platelet neutralization procedure (PNP) as described by Triplett et al. [8]: platelets past their use-by date from the blood bank were washed with physiological saline and freeze–thaw lysed to produce a platelet suspension. The aPTT was determined on a 1:1 mixture of patient’s plasma and the platelet suspension, and a 1:1 mixture of patient plasma and saline.
It was considered positive if the aPTT in the mixture with platelets was shortened and was > 5 s shorter than the mixture with saline [8].

4. Performance of clotting factor analysis if antibodies against factors are suspected. This was not performed since no patients had any signs of bleeding tendency.

To exclude heparin as the cause of a prolonged aPTT, thrombin time with and without addition of protamine sulfate (Novo Nordic, Denmark) was measured using Test Thrombin Reagent (Dade Behring, Marburg, Germany).

Diluted Russell viper venom time (dRVVT) was measured with the LA Screen assay (Dade Behring, Marburg, Germany), containing Russell’s viper venom, phospholipids, calcium and an antiheparin agent, capable of neutralizing heparin concentrations up to 1.0 U/ml in the plasma sample. The principles of the test are the same as outlined above and the presence of LAs was confirmed with the LA Confirm assay (Dade Behring, Marburg, Germany) with reagents as described above, but containing a higher phospholipid concentration in order to neutralize LAs. The ratio between the LA Screen assay (dRVVT) and the LA Confirm assay was considered positive when it was > 1.5. Controls included in the assay were analysed in each run.

Measurement of ACAs and β2GPI IgG and IgM antibodies was performed by means of an enzyme-linked immunosorbent assay (ELISA) (Euro-Diagnostica, Arnhem, The Netherlands). The cut-off value was 10 U/ml for both classes of immunoglobulin.

Statistical analysis
Data are shown as means±SD. Relevant continuous variables were compared by using Student’s t-test. Non-parametric tests were used on discrete variables with non-normal distribution.

Ethics
The study was performed according to the principles of the Declaration of Helsinki.

Results
Figure 1 shows representative time courses of aPTT from patients developing LAs (Figure 1A) and from those without (Figure 1B). A wide variety is noted in the patients developing LAs, but with all but one having normalized within the first week.

All patients had a normal aPTT and no LAs before the kidney transplantation. After the transplantation, 19 patients, i.e. 70.4%, had a statistically significant prolonged aPTT (*P* < 0.0001) (see Table 1). Prothrombin time was slightly shortened after transplantation, equivalent to the change in aPTT, but did not differ between the groups. Platelet counts were normal for both groups.

Further investigations revealed that aPTT prolongation was due to acquired LAs in 13 patients (48.2% of all patients), while six (22.2% of all patients) had ‘unspecific antibodies’, i.e. factor deficiency was excluded by mixing studies (no normalization by a 1:1 mixing with normal plasma), but all the criteria for LAs were not fulfilled, i.e. no clearly positive PNP. Thus, during the post-transplantation period, a total of 70.4% of the patients acquired antibodies interfering with the aPTT assay. Within the first week, LAs disappeared in all patients but one, who after 2 months still had persisting LAs, which had no effect on clinical outcome. Thrombin time for the patients with a prolonged aPTT was in all cases within the normal reference range, and comparative analysis with addition of protamine sulfate showed no indication of heparin or heparin analogues as the cause of prolonged aPTT. Out of 19 patients with a prolonged aPTT, 10 had a dRVVT (ratio > 1.5): seven of those had a positive LA, while three had ‘unspecific antibodies’. None of the patients with a normal aPTT had a positive dRVVT. No ACAs were detected. One patient had developed a relatively low titre of β2GPI antibodies (35 U/ml).

As shown in Table 2, pre-transplantation data for the two groups did not differ significantly in any of the issues investigated (age, gender, dialysis mode, dialysis time, diuresis before transplantation and number of previous transplantations). However, although not statistically significant, there were a higher percentage of smokers in the group of patients with prolonged aPTT.

Post-transplantation data for the two groups also revealed no significant differences (Table 3). There was a tendency towards a higher percentage of patients with prolonged aPTT having a renal biopsy. On the other
hand, outcome as far as improvement of P-creatinine was concerned showed a favourable tendency in the group of patients with a prolonged aPTT. One patient without aPTT prolongation rejected the kidney transplant compared with no patients from the other group (not shown).

The immunosuppressive medication regime used was slightly skewed: a higher proportion of patients with a prolonged aPTT received the most powerful immunosuppressive compounds, tacrolimus and thymoglobulin (Table 3). The number of patients, however, was far too small to make certain statements in this respect.

### Discussion

The aim of the study was to clarify the reasons for a prolonged aPTT after kidney transplantation, because a prolonged aPTT has clinical impact, e.g. postponement of biopsies or other surgical procedures. A high incidence of LAs in patients after a kidney transplantation was found: out of 27 patients without coagulopathy or treatment with any antithrombotic drugs, 70.4% developed a prolonged aPTT in the post-transplantation period, all due to transient antibody production. As a prolonged aPTT due to the presence of LAs does not increase the risk of bleeding,
postponement of surgical procedures in the 70.4% of the patients was unnecessary, and could have been avoided, if the LA test was performed immediately.

Different aPTT reagents have different sensitivity to LAs [3,7,9]. Our routine method is rather sensitive to LAs, but reagents which are more sensitive and other reagents which are less sensitive to LAs exist. Therefore, the size of the problem of LAs, i.e. prolongation of aPTT in transplanted patients, depends on the reagents used. Furthermore, different aPTT assays may also depend more or less on the type of antibody, i.e. whether they are directed against β2GPI or prothrombin or some other epitope [9]. The choice of reagent depends on whether the purpose is to detect LAs, e.g. as part of a thrombophilia screen, or mainly to detect lack of coagulation factors. In the diagnosis of LAs, it is recommended to use at least two phospholipid-dependent assays, e.g. aPTT and dRVVT, following the principles described in Subjects and methods. However, it should be stressed that testing for LAs is poorly standardized including the mixing studies and confirmatory tests, and is a matter of on-going work for improving the analyses and control [1,9,10].

Only 52.6% of the 19 patients with a prolonged aPTT due to antibody production had an LA ratio >1.5. This is not surprising, since various tests for detection of LAs have a rather wide variety of sensitivity and specificity for LAs [9,10]. In this study, the LA ratio was more specific, i.e. it did not detect as many of the transient antibodies as the aPTT test, but it could not be used to exclude the presence of these antibodies, since it was also negative in many of the patients with prolonged aPTT. In this respect, measurements of antibodies against anticardiolipin and β2GPI were more specific in this study, as only one patient had a positive test; generally, however, these tests are not considered to be more specific. In contrast, LA tests are better risk markers for thrombophilia than ACAs and β2GPI antibodies in the case of persistent LAs [11], but not regarding transient LAs as in the present study. Antibodies against prothrombin were not quantified in this study, and these antibodies are generally found to be less well correlated to thrombosis than anti-β2GPI antibodies [2]. As mentioned, prothrombin and β2GPI are believed to be the major protein targets, but several other epitopes have been suggested, and the activity of LAs may often be caused by a mixture of different antibodies [1,2]. Thus, the type of antibodies in the transplanted patients cannot be determined from this study.

None of the patients developed thromboembolic events, which is in accordance with the benign nature of these transient phospholipid antibodies as also seen, for example, during infections. Patients with antiphospholipid antibodies are known to have an increased risk of transplant failure [12,13], and screening for thrombophilia has been recommended prior to kidney transplantation [14]. It is also recommended that such patients undergoing kidney transplantation are anticoagulated with heparin, even for patients without a prior history of thromboembolic events [15]. In this study, however, LA was not present before the transplantation. As the presence of LA did not affect kidney transplantation outcome, it does not seem to represent a risk factor for transplant failure or thrombosis in this context. Additionally, none of the patients had signs of bleeding. Bleeding is very rare in patients with LAs and is almost always dependent on the presence of certain antibodies against prothrombin or severe thrombocytopenia [16,17] which were absent in our patients.

A possible explanation for the development of LAs in these patients could be the implantation of antigenic foreign material (the kidney transplant), causing an activation of the immune system, although this is mainly suppressed by the immunosuppressive treatment. A plausible additional cause could therefore be the prednisolone-free transplantation regime conducted in our department. Treatment with prednisolone suppresses the immune system in itself, and additional immunosuppression would therefore be expected if prednisolone had been a part of the medication regime. It should, however, be emphasized that the presence of LAs had no clinical impact and therefore should not be considered a problem in the context of the prednisolone-free transplantation regime.

In conclusion, patients with a prolonged aPTT should be investigated with the diagnostic panel suggested by the International Society on Thrombosis and Haemostasis [5], in order to rule out other reasons for the prolonged aPTT. If LA is diagnosed, specific treatment is only needed in relation to specific clinical conditions. Furthermore, the prolonged aPTT due to the presence of LA does not justify postponement of surgical procedures necessary in the post-transplantation period, and valuable time will be saved.

Conflict of interest statement. None declared.

References


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