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ORIGINAL ARTICLE

Soluble CD30 and ELISA-detected human leukocyte antigen antibodies for the prediction of acute rejection in pediatric renal transplant recipients

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biopsy-proven acute rejection, human leukocyte antigen antibodies, pediatric renal transplantation, soluble CD30, steroid-free immunosuppression.

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Conflicts of Interest
The authors have declared no conflicts of interest.

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Summary

Biomarker-based post-transplant immune monitoring for the prediction of impending graft rejection requires validation in specific patient populations. Serum of 28 pediatric renal transplant recipients within the framework of a well-controlled prospective randomized trial was analyzed pre- and post-transplant for soluble CD30 (sCD30), a biomarker reflecting mainly T-cell reactivity, and anti-human leukocyte antigen (anti-HLA) antibody reactivity, a biomarker for B-cell activation. A sCD30 concentration ≥ 40.3 U/ml on day 14 was able to discriminate between patients with or without biopsy-proven acute rejection (BPAR) with a sensitivity of 100% and a specificity of 76%. Six of seven patients (86%) with BPAR showed a sCD30 above this cut-off, whereas only 3/21 patients (14%) without BPAR had a sCD30 above this cut-off ($P = 0.004$). For pre- and post-transplant anti-HLA class II reactivities by enzyme-linked immunosorbent assay, a cut-off value of 140 optical density was able to discriminate rejecters from nonrejecters with a sensitivity of 86% or 71% and a specificity of 81% or 90%, respectively. Withdrawal of steroids was associated with a approximately twofold higher serum sCD30 compared to controls, but did not affect anti-HLA reactivities. An increased post-transplant sCD30 serum concentration and positive pre- and post-transplant anti-HLA class II reactivities are informative biomarkers for impending BPAR in pediatric renal transplant recipients. (TWIST, Clinical Trial No: FG-506-02-43)

Introduction

Despite the availability of potent modern immunosuppressive drugs, acute allograft rejection remains an important complication after renal transplantation because it may lead to early graft loss and, perhaps more importantly, may predispose to chronic T-cell- and antibody-mediated rejection, which are the major causes for late graft loss both in pediatric and adult renal transplant recipients [1,2]. Currently, the diagnosis of acute graft rejection is based on serial monitoring of graft function by serum creatinine determinations and confirmation of suspected rejection by graft biopsy; these signals become

apparent relatively late during the rejection cascade, when significant graft damage by inflammation may have already occurred. It would therefore be advantageous to diagnose impending graft rejection at an earlier stage by use of noninvasive biomarkers, which reflect the degree of immune activation as the net result of intensity of immunosuppressive therapy and immunoreactivity of an individual patient. Such biomarker-assisted individualization of immunosuppressive therapy to avoid under- or overimmunosuppression has the potential to improve outcome in renal transplant recipients [3].

A promising tool for post-transplant immune monitoring is the determination of the serum content of the T-cell activation marker CD30. The CD30 molecule, a member of the tumor necrosis factor/nerve growth factor receptor superfamily, is a relatively large 120-kD glycoprotein that was originally identified as a cell surface antigen on Hodgkin's and Reed Sternberg cells [4]. After activation of $CD30^+$ T cells, a soluble form of CD30, soluble CD30 (sCD30), is released into the bloodstream. CD30 is a co-stimulatory molecule that plays an important role in the generation of memory T-cell responses and regulation of the balance between Th1- and Th2-type immune responses [5]. Previous studies in adult renal transplant recipients have shown that post-transplant sCD30 is a good predictor of impending graft rejection [5,6] and an indicator of an increased risk of subsequent graft loss [7]. Because sCD30 is a biomarker that mainly reflects T-cell reactivity, it might be advantageous to combine it with a biomarker for B cell activation, for example the human leukocyte antigen (HLA) antibody status [7].

Before biomarkers are introduced into routine clinical diagnosis, they require validation in specific patient populations. Clinically relevant developmental patterns for the function of many leukocyte subpopulations, including $CD4^+$ T lymphocytes have been described [8], suggesting that data obtained in adults cannot be necessarily extrapolated to the pediatric patient population. In addition, the utility of specific biomarkers in patients receiving novel immunosuppressive regimens such as early steroid withdrawal under interleukin 2 receptor antibody coverage requires investigation. The aims of the present study were therefore (i) to validate the use of sCD30 and enzyme-linked immunosorbent assay (ELISA)-detected HLA antibodies for the prediction of acute rejection in pediatric renal transplant recipients in the early period post-transplant and (ii) to investigate the effect of early steroid withdrawal on T- and B-cell reactivity. These analyses were performed within the framework of a well-controlled prospective randomized trial, the TWIST study [9].

Materials and methods

Study design and patient population

This was an exploratory immunologic substudy in the framework of a large, randomized, open-label, comparative multicenter trial to evaluate the impact of early steroid withdrawal on growth and other steroid-related metabolic complications during the first 6 months post-transplant in pediatric renal allograft recipients (TWIST, Clinical Trial No: FG-506-02-43). The results of the main trial have been published previously [9]. Inclusion and exclusion criteria were the same as in the main study and have been published previously [9]. The logistics of serum sampling for analyses in a central laboratory in the framework of a multicenter trial in pediatric renal transplantation is complicated; therefore only 6 of 15 study centers participating in the TWIST study recruited patients for this immunologic substudy. Review and approval of the study protocol was obtained from the institutional review board at each center, and the study was conducted according to the Declaration of Helsinki. Written informed consent was obtained from parents or legal guardians and assent from the older patients if appropriate. Study monitoring and database analyses followed Good Clinical Practice guidelines.

Immunosuppressive regimen

Tacrolimus and mycophenolate mofetil (MMF) was administered to both groups as previously reported [9]. The dosage of tacrolimus and MMF and the exposure to tacrolimus were comparable between the steroid withdrawal and control group (Table 1). Patients in the steroid withdrawal group received the first dose of daclizumab 1.0 mg/kg within 24 h of reperfusion. The second and final dose of 1.0 mg/kg was given on day 14. Both the dose and the duration of corticosteroid administration differed between the groups. The steroid withdrawal group received corticosteroids as a bolus dose of 300–600 mg/m^2 i.v. on day 0. The dose on day 1 was 60 mg/m^2 reduced to 40 mg/m^2 on day 2, 30 mg/m^2 on day 3, and 20 mg/m^2 on day 4 and then discontinued on day 5. The corticosteroid bolus dose and the day 1 dose were the same for the control group. Corticosteroid dose was reduced to 40 mg/m^2 starting on days 2–7, and then reduced in decrements of 10 mg/m^2 per week to <10 mg/m^2 on days 43–183 at the discretion of the investigator.

Immunologic analyses

Serum for the analysis of immunologic parameters was collected prior to renal transplantation and on day 7, 14, and 60 post-transplant. Serum sCD30 content was determined using the commercially available ELISA kit of Bender MedSystems (Vienna, Austria). As indicated by the manufacturer, the intra-assay variance of this assay is 10% and the interassay variance is 20%. Anti-HLA class I and class II reactivities were detected using the solid-phase ELISA of Biotest (Dreieich, Germany). The presence of donor-specific anti-HLA antibodies (DSA) in the patient's serum was

Table 1. Patient characteristics.

	Entire study group	Steroid withdrawal	Control
Number of patients	28	15	13
Recipient age (years)	12.1 ± 4.20	12.1 ± 3.95	12.0 ± 4.61
Female gender, *n* (%)	9 (32)	5 (33)	4 (31)
Re-transplant, *n* (%)	2 (7)	1 (6)	1 (7)
HLA-DR mismatches, *n* (%)			
0 MM	5 (18)	2 (13)	3 (23)
1 MM	14 (50)	9 (60)	5 (38)
2 MM	9 (32)	4 (27)	5 (38)
Tacrolimus daily dose (mg/kg)*	0.33 ± 0.14	0.32 ± 0.14	0.34 ± 0.13
Tacrolimus trough level (ng/ml)*	9.7 ± 4.0	8.7 ± 2.9	10.9 ± 4.6
MMF daily dose (mg/m^2)*	1118 ± 239	1095 ± 292	1149 ± 148
Biopsy-proven acute rejections, *n* (%)	7 (25)	5 (33)	2 (15)
Donor age (years)	34.2 ± 14.5	31.4 ± 15.0	36.2 ± 14.5
Donor type			
Living-related, *n* (%)	7 (25)	4 (26)	3 (23)
Deceased donor, *n* (%)	21 (75)	11 (74)	10 (77)
Delayed graft function, *n* (%)	4 (14)	1 (7)	3 (23)

Data are mean ± SD. MMF, mycophenolate mofetil.

*Immunosuppressive drugs and respective exposure represent values from day 7 post-transplant.

investigated using the highly sensitive Luminex single antigen methodology, which utilizes plastic beads coated with recombinant HLA molecules (LABScreen Single Antigens, One Lambda Inc., Canoga Park, CA, USA). Only A, B, and DR MM were considered for DSA. Because no clinically validated cut-off for the Luminex assay is recommended by the provider company, mean fluorescence intensity (MFI) of $\geq$500 was used to define the cut-off for antibody positivity, based on recent data from the CTS serum study that a cut-off $\geq$500 MFI gives meaningful results, if *de novo* antibodies are analyzed [10].

Other laboratory evaluations

The estimated glomerular filtration rate (eGFR) of renal allografts was assessed by the creatinine clearance calculated according to Schwartz *et al.* [11]. Tacrolimus blood trough concentrations were collected for central analysis using the HPLC–tandem mass spectrometry [12].

Statistical analysis

Results are expressed as mean ± SD or stated otherwise. Any differences between the control and the steroid withdrawal group were evaluated using the Student's *t*-test or, if normality failed, using the Mann–Whitney *U* rank-sum test. The one-way repeated measurements analysis of variance was used to detect any significant changes in laboratory data over time within each study group. A repeated measures analysis of variance with the baseline value included as explanatory variable was performed. The associations of sCD30 or HLA class I and class II antibodies with biopsy-proven acute rejection (BPAR) were explored using the Fisher's exact test. Receiver operating characteristics (ROC) plots of sensitivity versus 1-specificity were generated to determine whether a particular biomarker could discriminate patients with a BPAR from those who experienced no rejection. The areas under the ROC curves, sensitivity, specificity, and the corresponding 95% confidence intervals (CI) were calculated [13]. As cut-off, the value with the highest Youden's Index was chosen. In case of multiple values with same sensitivity and specificity the mean was chosen. Bootstrapping was performed to validate the cut-off with 1000 replicates and $n = 28$. Pearson correlation coefficient between post-transplant sCD30 and eGFR was calculated. A *P*-value of <0.05 was considered as statistically significant. Statistical calculations were performed using the IBM SPSS Statistics (PASW version 18.0, SPSS Inc., Chicago, IL, USA) and SAS package (version 9.2; SAS Institute, Cary, NC, USA). Graphs were produced using Sigmaplot (SYSTAT Software Inc, San Jose, CA, USA).

Results

The clinical characteristics are summarized in Table 1. Fifteen patients were randomized to the steroid withdrawal group and 13 patients to the control group. Panel-reactive antibodies pretransplant tested in the complement-dependent cytotoxicity (CDC) assay were below 25% in all patients.

During the 6-month study period, seven of 28 patients (25%) experienced a BPAR, classified according to Banff '97 [14] and Banff '05 criteria [15] either as borderline changes ($n = 6$) or acute T-cell-mediated rejection type IA

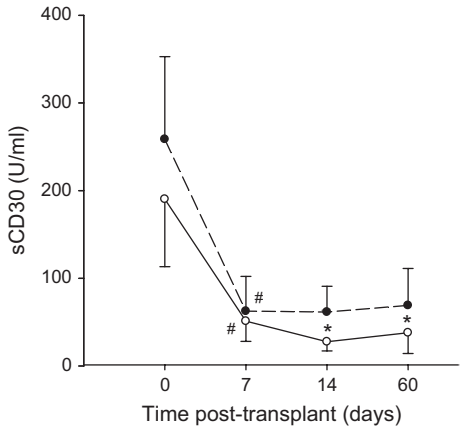

Figure 1 Time course of serum sCD30 concentrations pre- and post-transplant and the effect of early steroid withdrawal (closed circles, dashed line versus controls on maintenance steroids, open circles, straight line). Data are given as mean ± SD. $^{\#}P = 0.001$ versus baseline (day 0); $^{*}P = 0.003$ steroid withdrawal versus control; $^{\S}P = 0.002$ steroid withdrawal versus control.

($n = 1$); staining for C4d was negative in these biopsies. The median time period post-transplant until occurrence of BPAR was 57 days (range, 15–68 days). There were no repeated BPAR in a single patient. Each BPAR responded well to methylprednisolone pulse therapy; there was no graft loss within the 2-year follow-up period.

Soluble CD30

The time course of serum sCD30 concentrations pre and post-transplant and the effect of early steroid withdrawal versus maintenance steroids (controls) are depicted in Fig. 1. Mean pretransplant sCD30 concentration in the steroid withdrawal group (260 ± 102 U/ml) was not significantly different from that in controls (198 ± 68.4 U/ml; $P = 0.072$). In both groups, there was a sharp decline in serum sCD30 by 75% of baseline (steroid withdrawal, 68.8 ± 39.5 U/ml; control, 51.0 ± 23.1 U/ml; $P = 0.167$), most likely because of the effect of immunosuppressive therapy. After day 7 post-transplant, the mean serum sCD30 content in controls further declined, whereas the corresponding values in patients off steroids were significantly higher than in controls both on days 14 and 60 post-transplant (Fig. 1). The absolute sCD30 values were: day 14 post-transplant, steroid withdrawal, 62.9 ± 29.3 U/ml; control, 28.3 ± 10.3 U/ml ($P = 0.003$); day 60, steroid withdrawal, 79.5 ± 41.6 U/ml; control, 37.8 ± 22.7 U/ml ($P = 0.002$). A repeated measures analysis of variance with the baseline value included as explanatory variable was also performed. We found a significant difference between the steroid withdrawal and the control group ($P = 0.013$). This difference in post-transplant serum sCD30 concentrations preceded a numerically higher incidence of BPAR in patients after steroid withdrawal ($n = 5$) than in controls ($n = 2$).

To define a cut-off value for sCD30 that discriminates between patients with or without subsequent BPAR, ROC curves were calculated (Table 2). While sCD30 concentrations pretransplant and on day 7 and day 60 post-transplant were not discriminative, a sCD30 concentration $\geq$40.3 U/ml on day 14 post-transplant was able to discriminate between patients with or without subsequent BPAR with a sensitivity of 100% (95% CI, 59.0–100.0) and a specificity of 76% (95% CI, 52.8–91.8). The bootstrap-validated estimate of the area under the ROC curve AUC for sCD30 on day 14 was 0.653. Bootstrapping resulted in a mean cut-off for sCD30 on day 14 of 41.1 U/ml (range, 35.8–89.3), which is comparable to the cut-off obtained using ROC curve analysis. The positive predictive value for

Table 2. Areas under the ROC curves for pre- and post-transplant sCD30 and ELISA-detected anti-HLA class I or class II antibody reactivities to discriminate between patients with or without biopsy-proven acute rejection.

	Parameter	Area under the ROC curve	95% Confidence interval	*P*-value
Pretransplant	sCD30	0.629	0.345 to 0. 913	0.372
	HLA class I	0.616	0.324 to 0.907	0.436
	HLA class II	0.776	0.504 to >0.99	0.047
Post-transplant day 7	sCD30	0.511	0.257 to 0.764	0.934
	HLA class I	0.614	0.253 to 0.976	0.536
	HLA class II	0.771	0.482 to >0.99	0.066
Post-transplant day 14	sCD30	0.786	0.611 to 0.961	0.001
	HLA class I	0.611	0.247 to 0.974	0.550
	HLA class II	0.775	0.485 to >0.99	0.063
Post-transplant day 60*	sCD30	0.608	0.219 to 0.998	0.586
	HLA class I	0.667	0.060 to >0.99	0.590
	HLA class II	0.650	0.012 to >0.99	0.645

sCD30, soluble CD30; HLA, human leukocyte antigen.
*Only three patients experienced a BPAR after day 60 (the other four with previous BPAR were excluded from this analysis).

the prediction of BPAR was 71%, the corresponding negative predictive value 91%. Six of seven patients (86%) with BPAR showed a sCD30 above this cut-off, whereas only 3 of 21 patients (14%) without BPAR had a sCD30 above this cut-off ($P = 0.004$).The mean sCD30 concentration on day 14 in rejecters was 58.6 ± 21.5 U/ml, as compared to 50.0 ± 39.6 U/ml in nonrejecters ($P = 0.041$). On day 60 post-transplant, the mean sCD30 serum concentration was not different in patients with subsequent BPAR (49.0 ± 8.6 U/ml; $n = 3$) or without BPAR (63.6 ± 44.3 U/ml; $n = 21$). An earlier study reported a correlation between higher sCD30 concentrations and increased serum creatinine concentrations [16]. In the present study, there was no correlation between the post-transplant serum sCD30 concentration and eGFR ($r^2 = 0.017$, $P = 0.952$). This finding is consistent with our previous observation in a large cohort of adult renal transplant recipients [7].

Anti-HLA antibody reactivity

We analyzed the relationship between anti-HLA class I and class II antibody reactivities pre- and post-transplant in rejecters versus nonrejecters. Pretransplant, five of seven patients (71%) with BPAR had positive anti-HLA antibody reactivities, class I and class II in two patients, and class II in three patients, whereas only four of 21 (19%) patients without BPAR showed pretransplant positive anti-HLA antibody reactivity (class I and II, $n = 0$; class I, $n = 1$; class II; $n = 3$; $P = 0.009$). Post-transplant, sera obtained on day 7 and/or day 14, i.e., prior to BPAR, were considered for analysis. Five of seven patients (71%) with BPAR showed positive anti-HLA reactivities (class I and II, $n = 4$; class II, $n = 1$) compared to only two positive (class II) patients of 21 nonrejecters (10%; $P = 0.004$). In the five rejecters, the positive anti-HLA reactivities pretransplant remained elevated or further increased on day 7 and day 14 post-transplant, whereas the pretransplant positive anti-HLA reactivities in three of four nonrejecters either turned negative ($n = 3$) or decreased from 414 to 150 optical density (OD) ($n = 1$) during the first 14 days post-transplant. Four of seven patients (57%) with BPAR showed post-transplant anti-HLA ELISA-double positivity compared with 0 patients without BPAR ($P = 0.002$).

All pre- and post-transplant sera were also tested using the Luminex assay for DSA. Pretransplant, only two of 28 patients (7%) had DSA against class I HLA-antigens, and no patient had DSA against class II HLA-antigens. One patient with positive pretransplant DSA developed BPAR on day 56 post-transplant. Post-transplant, four patients developed *de novo* DSA (class I, $n = 3$; class II, $n = 1$) on day 7 (mean MFI of 1240 ± 405 (SD)), on day 60, MFI decreased to a mean of 415 ± 218. None of these patients developed a BPAR. Hence, the determination of DSA pre- or post-transplant was not informative for the prediction of BPAR.

To define cut-off values for anti-HLA class I or II antibody reactivities that discriminate between patients with or without BPAR, ROC curves were calculated (Table 2). For pretransplant anti-HLA class II reactivity, a cut-off value of 140 OD was able to discriminate between patients with or without subsequent BPAR with a sensitivity of 85.7% (95% CI, 42.1–99.6) and a specificity of 81.0% (95% CI, 58.1–94.6). Anti-HLA class I reactivity was not discriminative (Table 2). Post-transplant, a cut-off value of 140 OD for anti-HLA class II antibodies on day 14 was discriminative between rejecters and nonrejecters (Table 2) with a sensitivity of 71.4% (95% CI, 29.0–96.3) and a specificity of 90.5% (95% CI, 69.6–98.8). The positive predictive value for the prediction of BPAR was 71%, the corresponding negative predictive value 95%. The determination of anti-HLA class II antibodies on day 7 post-transplant or the determination of anti-HLA class I antibodies did not significantly discriminate between patients with or without BPAR (Table 2). Regarding the combined analysis of anti-HLA and sCD30, four of seven patients with BPAR (57%) had a sCD30 concentration ≥40.3 U/ml and positive

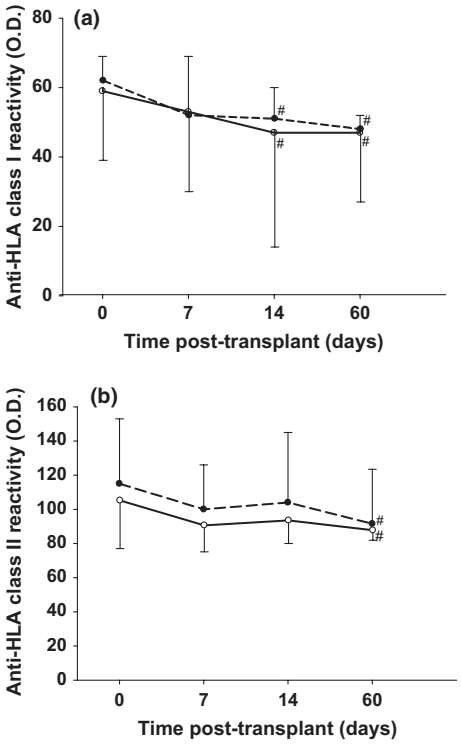

Figure 2 Time course of anti-HLA class I (a) and class II (b) reactivities pre- and post-transplant and the effect of early steroid withdrawal (closed circles, dashed line versus controls on maintenance steroids, open circles, straight line). Data are given as mean ±SD. $^{\#}P < 0.05$ versus baseline (day 0).

anti-HLA reactivity on day 14 compared with two of 21 patients (29%) without BPAR ($P = 0.024$).

Next, we analyzed the effect of early steroid withdrawal on the course of anti-HLA class I and II reactivities during the first 60 days post-transplant (Fig. 2). Anti-HLA values at day 60 of those patients that had already experienced a BPAR prior to day 60 were eliminated from this analysis. The mean pretransplant anti-HLA class I (steroid withdrawal, 66 OD; control 59 OD) and class II reactivities (steroid withdrawal, 115 OD; control 105 OD) were not significantly different. In controls, anti-HLA class I reactivities declined significantly by 26–74% of baseline ($P = 0.038$) (Fig. 2a). In patients undergoing steroid withdrawal, there was less pronounced decline in anti-HLA class I reactivities, which did not reach statistical significance ($P = 0.085$). Anti-HLA class II antibody reactivities also declined post-transplant to 78% of baseline in controls and to 82% in steroid withdrawal patients (Fig. 2b). At none of the time points studied was there a significant difference in anti-HLA class I or II reactivities between patients off steroids and controls.

Discussion

This is the first prospective study in pediatric renal transplant recipients in which the immunological biomarkers sCD30 and ELISA-detected anti-HLA antibodies for the prediction of BPAR were analyzed. We observed that a sCD30 serum concentration of >40.3 U/ml on day 14 post-transplant is able to differentiate between subsequent rejecters and nonrejecters with a sensitivity of 76% and a specificity of 86%. These data are consistent with previous observations in adult renal transplant recipients [6,7,17,18]. The small differences with respect to the informative time point for sCD30 sampling between the study of Pelzl *et al.* and our study are likely attributable to differences in the immunosuppressive regimen (antilymphocyte induction therapy, type of calcineurin inhibitor), the associated time point of BPAR occurrence (median 9 days post-transplant in the study of Pelzl *et al.* [6] versus 57 days in our study) and differences in the patient cohort (adult versus pediatric). Several studies have demonstrated that post-transplant measurement of the serum T-cell activation marker sCD30 allows prediction of subsequent T-cell-mediated acute rejection episodes and subsequent graft loss in kidney transplant recipients [7]. Regarding the predictive cut-off value for sCD30, we have recently observed in a prospective multicenter study in 2322 adult deceased-donor kidney recipients that patients with a high sCD30 of ≥ 40 U/ml on day 30 post-transplant showed a subsequent graft survival rate after 3 years of 78.3 ± 4.1%, significantly lower than the 90.3 ± 1.0% rate in recipients with a low sCD30 on day 30 of <40 U/ml [7]. It is interesting that the independently performed present study revealed almost the same cut-off value for post-transplant sCD30 for impending BPAR in the ROC curve analysis. On the other hand, we reported previously that the pretransplant serum content of sCD30 > 100 U/ml is associated with chronic graft loss [19]. Hence, it should be noted that the cut-off values of sCD30 is the pre- and post-transplant setting are different and that it is the post-transplant sCD30 serum concentration which makes the difference, after the immunosuppressive therapy exerted its effect on the recipient's immune system. sCD30 is an attractive biological marker because it is, as a relatively large molecule, resistant to repeated thawing cycles and temperature differences and easily determined using commercial ELISA [5]. Whether sCD30-based prospective adjustment of immunosuppressive therapy can prevent BPAR and irreversible graft damage and thereby improve long-term graft outcome awaits evaluation in randomized controlled trials.

We report here for the first time the value of sCD30 as a biomarker for the prediction of BPAR in the setting of early steroid withdrawal. We observed a twofold difference in post-transplant serum sCD30 concentrations in patients off steroids versus controls, which was associated with a numerically higher incidence of subsequent BPAR in patients off steroids, most likely because of the lacking immunosuppressive effect of steroids on T cells. Hence, sCD30 appears to be a promising biomarker for the early detection of impending BPAR also in the setting of early steroid withdrawal.

We observed that positive anti-HLA reactivities by ELISA both pre- and post-transplant allow prediction of subsequent presumed T-cell-mediated BPAR. It is noteworthy that especially anti-HLA ELISA-double positivity, i.e., reactivities both against class I and class II antigens, was predictive of subsequent BPAR. We interpret positive anti-HLA reactivity as detected in ELISA prior to BPAR as an immunologic signature of increased immunoreactivity. Previous studies had only analyzed the predictive value of HLA antibody positivity using ELISA on renal graft survival [20,21]. The precise mechanism of how nondonor-directed HLA antibodies are associated with the later development of T-cell-mediated BPAR remains to be elucidated. It is known that when a specific HLA antibody is generated in response to humoral epitopes during a stimulation episode, there is simultaneous stimulation of T cells responding to cellular epitopes on the same HLA molecule [22–24]. Hence, although nondonor-directed HLA antibodies may not contribute directly to the rejection episode, they may act as surrogate markers of T-cell activation.

Regarding the predictive cut-off value for post-transplant anti-HLA reactivities using ELISA, we observed in a previous study that 19 patients with high sCD30 of ≥ 40 U/ml and high HLA class I or II antibody reactivity of ≥ 250 OD

on post-transplant day 30 showed a 3-year graft survival rate of only 65.2 ± 13.5%, in contrast to a 90.9 ± 1.1% rate in 835 patients with low sCD30 and low HLA antibody reactivity on day 30 ($P < 0.001$) [7]. The predictive value of post-transplant anti-HLA reactivities alone, i.e., independent of sCD30, was not investigated in our previous study. The cut-off value for post-transplant anti-HLA reactivities in the latter study (250 OD) is higher than the cut-off value derived from the present study (140 OD). This observation could be owing to differences in the study endpoint (BPAR in the present study versus graft survival in our previous analysis [7]) and/or to differences in the study population (pediatric versus adult).

We observed in serial measurements of anti-HLA reactivities, a 26% decline in class I antibodies and a 20% decline in class II antibodies during the first 60 days post-transplant. This moderate decline in anti-HLA reactivities compared to the pronounced decline (75%) in the T-cell activation marker sCD30 indicates that the immunosuppressive medication used in this study was primarily directed against T cells. Early steroid withdrawal was not associated with an increase or, compared to control, less pronounced decline in anti-HLA reactivities in our study. This finding is consistent with the observation that early steroid withdrawal is not associated with an increased rate of acute antibody-mediated rejection in the early post-transplant period [25,26].

The strength of our study is that it was based on a prospective multicenter randomized trial where the outcomes were clearly defined and rigorously ascertained, and the data were carefully collected in a standardized fashion. In addition, and in contrast to some of the previous studies on this topic, all acute rejections were biopsy-proven and classified according to Banff criteria. The limitations are the relatively small number of patients investigated, but this is an inherent problem for all studies in the pediatric renal transplant population. The number of cases included is small, and therefore the cut-off values calculated for post-transplant sCD30 and anti-HLA antibody reactivities should be considered preliminary. In addition, they require validation in an independent study.

In conclusion, this study suggests that post-transplant sCD30 is a useful biomarker for impending T-cell-mediated BPAR in pediatric renal transplant recipients and informative for increased T-cell reactivity also in the setting of early steroid withdrawal. Positive anti-HLA class II reactivities as measured in ELISA pre- and post-transplant, especially anti-HLA ELISA-double positivity, also predict subsequent T-cell-mediated BPAR. These data allow a more precise estimation of the individual immunologic risk in pediatric renal transplant recipients in the early period post-transplant, which will hopefully improve patient outcome by individualization of immunosuppressive therapy.

Authorship

HB, CS, and BT: participated in literature search, study design, grant application, data collection, analysis and interpretation, and preparation of the manuscript. RF, BH, and GO: participated in literature search, study design, data collection, analysis and interpretation, and preparation of the manuscript. JO, KV, RG, SF, DM, AS, and ML: participated in sample and data collection, data analysis, and interpretation of results.

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