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ORIGINAL ARTICLE

Assessment of pretransplant inflammation in pediatric renal allograft recipients

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Summary

Pretransplant (Tx) inflammation is linked to adverse outcomes in adult Tx recipients but no such data exist for children. Our study evaluated the predictive value of three pre-Tx inflammatory markers: serum C-reactive protein (CRP), Neopterin (Neo) and interleukin (IL) 12, in determining outcome. Pre-Tx serum on 51 children (mean age 11 years) transplanted between 1985 and 2000 was analyzed. Data on other variables were abstracted from patient records. Primary end-points were graft survival and acute rejection (AR). Kaplan-Meier and log-rank statistics compared endpoints in patients at different quartiles for each marker. Cox regression analysis was used to determine the independent effect of the markers on the end-points. The mean CRP, Neo, and IL-12 were 1.3 mg/l, 1.78 ng/ml, and 123 pg/ml, respectively. The mean CRP, Neo, and IL-12 were not different between the patients with and without AR or graft loss ($P > 0.4$ for all). Neither rejection-free survival nor graft survival was affected by CRP, Neo, or IL-12 quartiles. Cox-regression analysis demonstrated no predictive value of any marker on outcome. Unlike adults, a single pre-Tx determination of inflammatory markers was not predictive of AR or graft loss in children, indicating that the pathogenesis of AR may be different in children.

Introduction

There has been recent interest in the potential value of pre-Tx inflammatory markers as predictors of acute rejection (AR) and graft loss in adult renal-Tx patients. The most widely studied marker of inflammation is C-reactive protein (CRP), an acute phase reactant protein synthesized by the liver in response to interleukin 6 (IL-6), tumor necrosis factor-α and IL-1b. High levels of CRP, therefore, are reflective of a pro-inflammatory state. Moreover, CRP also has pro-inflammatory effects of its own such as complement activation and stimulation of natural killer cell activity. Although the half-life of CRP is only 4–6 h, a high concordance in levels over extended periods of time (>6 months) has been demonstrated [1]. In addition, CRP is easily measured in the serum, and its level rises over a 1000-fold with inflammation, all of which make it a good marker of chronic inflammation.

There is a substantial existing body of literature on the predictive value of serum CRP measurements, even at a single point in time, in adults. The CRP levels are elevated in adults with chronic kidney disease (CKD), even in the early stages, with levels being even higher in dialysis patients [2]. This has been attributed to the chronic inflammation in uremic patients due to the accumulation of 'uremic toxins,' from infection of vascular accesses and from the dialysis procedure itself. High serum levels of CRP are predictive of future cardiac disease and mortality in dialysis patients, patients with coronary artery disease and even in healthy individuals [3]. PreTx serum CRP also predicts AR [4], chronic allograft nephropathy (CAN) [5] and even death in adult renal Tx patients [6].

Serial monitoring of serum CRP has also been shown to be of value in the post-Tx period as an early marker of some complications such as bacterial and viral infections and AR, in both adults and children [7,8]. The CRP has been shown to rise on postoperative days 2 and 3 as a response to stress and then fall by days 7–10. A lack in drop of the serum CRP by days 7–10 or a subsequent rise are both markers of impeding complications, although it has limited predictive value in differentiating between infections and AR. To date, there are no data on the predictive value of a pre-Tx CRP in predicting adverse outcomes in pediatric Tx recipients.

Other inflammatory markers such as Neopterin (Neo), which is derived from γ-interferon stimulated monocytes, and IL-12, which stimulates the development of the Th1 subset of CD4 positive T cells, have been less well studied in the Tx population. Serum Neo levels are elevated in adult dialysis patients, and fall after successful renal Tx [9]. A persistently elevated serum Neo after Tx is believed to be a sensitive marker of AR, although its specificity for AR is very low since elevations in Neo can also be seen in the setting of infectious processes [9–11]. A recent study evaluated the predictive value of pre-Tx IL-12 elevations in adult renal Tx recipients and found that it was an independent predictor of AR [12]. This is understandable since IL-12 is the most important cytokine promoting polarization of CD4 positive T cells towards a pro-inflammatory Th1 phenotype, eventually leading to activation of CD8 T cells and also natural killer cells, all of which act as effectors of graft damage [13].

There are very few studies that have looked at any of the aforementioned inflammatory markers in children with CKD and none, to our knowledge, that have evaluated the predictive value of these markers, measured in the pre-Tx period, in determining post-Tx outcomes. Clearly children have much more to gain from preventing AR, CAN and cardiovascular morbidity. Identifying high-risk children pre-Tx would allow optimal tailoring of therapy to improve graft outcome, and perhaps even reduce cardiac disease in the long-term.

The objective of our study was to measure three inflammatory markers (CRP, Neo, and IL-12) in the pre-Tx period in children with CKD who subsequently received a renal Tx and to determine if a single pre-Tx serum CRP, Neo, or IL-12 predicted AR or long-term graft loss in this cohort.

Patients and methods

Stored pre-Tx sera from all pediatric renal Tx recipients cared for at the University of California, Davis Medical Center, from October 1985–2000 were retrieved. An aliquot from the serum collected from patients for their final crossmatch was used for measurement of the inflammatory markers: serum CRP, Neo, and IL-12 levels all of which were measured using a commercially available ELISA kit (BioSource International Inc., Camarillo, CA, USA). Normal values for these markers were: <5 mg/l for CRP, <2.48 ng/ml for Neo and <175 pg/ml for IL-12. Specimens had been kept frozen at −70 °C after collection until the time of the assays of the inflammatory markers. All assays were run in duplicate. Demographic data and data pertaining to the cause of CKD, need for and duration of dialysis, and events around the time of Tx [such as ischemia times, organ donor source, Human Leukocyte Antigen (HLA) match, and delayed graft function (DGF)] were abstracted from the patient medical records by a single investigator (LB). Medical records from follow-up visits were reviewed for determining the occurrence of AR, and to evaluate long-term graft survival. The primary outcomes were rejection-free graft survival and overall graft survival. The diagnosis of AR was based on the renal pathologist's interpretation of renal biopsy findings. Early rejection was defined as the occurrence of AR within the first 3 months after Tx. Graft loss was defined as return to long-term dialysis or a re-Tx. Immunosuppressive protocols were quite diverse. Until 1995 maintenance immunosuppression consisted of corticosteroids, cyclosporine/tacrolimus and azathioprine. After August 1995, mycophenolate mofetil replaced azathioprine. Induction therapy consisted of OKT3, antithymocyte globulin, daclizumab or none.

Statistical analyses

The Student's *t*-test and the Fisher's exact test were used to investigate univariate associations between outcomes and the continuous and categorical variables, respectively. Kaplan-Meier survival curves with the log-rank test were used to evaluate differences in rejection-free and overall graft survivals between patients at different quartiles of CRP, Neo, and IL-12. The Cox proportional multivariable analysis was used to evaluate the independent effect of each of the markers on the primary outcomes. The covariates used included the need for dialysis, number of HLA mismatches, cold ischemia time (CIT), DGF, and organ donor source.

All analyses were carried out using the SPSS software (Chicago, IL, USA), Version 11.5.0 for Windows. Statistical significance was set at $P < 0.05$.

Results

Demographic data

Between October 1985–2000, 61 Tx were performed in 56 pediatric recipients at our center. Of these 61 Tx, 7 were

Table 1. Pre-Tx demographic data of the 51 patients.

Gender (% female)	41%
Cause of CKD	
Reflux/dysplasia	19 (37%)
Glomerulonephritis (GN)	19 (37%)
Obstructive uropathy	9 (18%)
Other	4 (8%)
Mean (SD) age at Tx (years)	11.2 (4.8)
Median age	12.5
Mean (SD) duration of dialysis (years)	2.0 (1.5)
Median duration	1.6
Number of patients receiving dialysis	36 (71%)
Peritoneal dialysis	22
Hemodialysis	11
Both	4
Donor source (% Cadaveric)	50%
Mean (SD) CIT (h)	15.5 (11)
Median time	16
Mean (SD) number of HLA mismatches	3.1 (1.6)
Median HLA mismatches	3
Mean (SD) number of HLA DR mismatches	1 (0.6)
Median HLA DR mismatches	1
Mean (SD) final PRA	8.5 (19.5%)
Median PRA	0%
DGF (%)	2%

Pre-Tx, pretransplant; CKD, chronic kidney disease; CIT, cold ischemia time; HLA, human leukocyte antigen; PRA, panel reactive antibody; DGF, delayed graft function.

lost in the immediate post-Tx period from technical complications and were excluded from the study. In an additional two patients, medical records could not be retrieved, leaving a total of 52 Tx in 51 patients for our study. Basic demographic data on the patients is presented in Table 1. The mean/standard deviation (SD) follow-up time for the study group was 4.9 (3.4) years. At the time of last follow up, 15 (29%) grafts had been lost and 26 (50%) had experienced at least one episode of clinical AR.

Inflammatory markers

The mean (SD) and median values of the three inflammatory markers were as follows: CRP 1.3 (2.2) mg/l and 0.5 mg/l, Neo 1.8 (0.9) ng/ml and 1.5 ng/ml, and IL-12 123 (67) pg/ml and 112 pg/ml, respectively. The percentage of patients who had elevated values for these markers were 7.7% for CRP, 19% for Neo, and 21% for IL-12. There was a very strong correlation between all three inflammatory markers, the strongest correlation being between Neo and IL-12 ($r = 0.85$, Pearson correlation statistic). There was no correlation between any of the markers and either dialysis vintage ($r = 0.02$ for CRP, $P = 0.9$) or age at Tx ($r = 0.14$ for CRP, $P = 0.3$).

Univariate analyses

There were no statistically significant differences in the mean levels of CRP, Neo and IL-12 between patients who did and did not experience graft loss, AR, steroid-resistant AR, and those whose original disease was glomerular versus nonglomerular (Table 2). As seen in Table 2, children who were receiving dialysis pre-Tx had a significantly higher mean CRP and Neo level, compared to those who were not receiving dialysis. In fact, all four children who had an elevated CRP, 9 of the 10 children (90%) who had an elevated Neo, and 10 of the 11 children (91%) with an elevated IL-12 level, were receiving some form of dialysis pre-Tx. There was no significant difference in the mean value of any indi-

Table 2. Univariate analyses of the inflammatory markers and outcome variables.

	Mean (SD), CRP (mg/l)	*P* value	Mean (SD), Neo (ng/ml)	*P* value	Mean (SD), IL-12 (pg/ml)	*P* value
Etiology of CKD						
GN	1.9 (2.9)		1.9 (1.0)		137.8 (63)	
Others	1.0 (1.7)	0.20	1.7 (0.9)	0.64	114.5 (69.3)	0.2
AR	1.4 (2.3)		1.9 (1)		131.6 (71)	
No AR	1.3 (2.3)	0.95	1.7 (0.8)	0.57	114.4 (63.8)	0.36
Steroid-resistant AR	1.0 (2.1)		1.6 (0.9)		115.8 (58.9)	
All others	1.5 (2.3)	0.52	1.9 (1.0)	0.29	125.7 (70.8)	0.64
Steroid-resistant AR	1.0 (2.1)		1.6 (0.9)		115.8 (58.9)	
Steroid-responsive AR	1.8 (2.5)	0.40	2.2 (1.2)	0.65	150.2 (81.6)	0.23
Graft Loss	0.9 (2.0)		1.6 (0.8)		126 (50.1)	
No graft loss	1.5 (2.4)	0.43	1.9 (1.0)	0.26	121.8 (73.8)	0.84
Dialysis yes	1.6 (2.6)		1.9 (1.0)		131 (72)	
Dialysis no	0.6 (0.8)	0.046	1.5 (0.6)	0.048	103 (52)	0.13
Hemodialysis	1.6 (2.3)		1.9 (1.1)		124 (98)	
Peritoneal dialysis	1.5 (2.4)	0.9	1.9 (1.0)	0.9	132 (55)	0.8

CRP, C-reactive protein; Neo, neopterin; IL, interleukin; CKD, chronic kidney disease; GN, glomerulonephritis; AR, acute rejection.

vidual inflammatory marker among patients receiving different dialysis modalities.

Kaplan-Meier and log-rank tests

Kaplan-Meier survival curves for rejection-free and overall graft survival were not statistically different for children at different quartiles of CRP, Neo, or IL-12 even when grouped quartiles were used (e.g. first quartile versus all others, fourth quartile versus all others and first two quartiles versus last two quartiles; data not shown).

Multivariate analysis

On multivariable analyses, none of the inflammatory markers had a predictive role in determining graft loss or AR (neither early nor late rejection).

Discussion

Unlike in adult renal Tx recipients, our study did not find a predictive value of any the pre-Tx inflammatory markers CRP, Neo, and IL-12 for AR or graft loss in the pediatric population. This could indicate that the pathogenesis of AR and CAN are different in children and in adults, or at least that factors other than subclinical inflammation are more important in determining graft outcomes in children. On one level this is intuitive when one explores the 'source' of inflammation in healthy adults and also adult CKD patients. While this question has yet to be answered definitively, it has been postulated that in otherwise healthy adults, factors such as chronic smoking, gastritis, periodontitis, bronchitis, obesity or even oxidative stress from aging itself lead to an inflammatory response manifesting as an elevated CRP, which then can *cause* adverse events such as atherosclerosis. Another school of thought is that inflammation within the atherosclerotic plaque per se is the source of the CRP. Since neither atherosclerosis nor the aforementioned extra-vascular factors (such as chronic bronchitis) are as common or advanced in most children, it is understandable that children would be expected to have a lower inflammatory burden [14]. Even when one looks at the CKD population, the factors postulated to promote a pro-inflammatory state, such as occult vascular access infections, use of bio-incompatible dialyzers and the hemodialysis procedure itself [2], may be expected to affect children less, since most children are on dialysis for a much shorter period of time, in part due to the pediatric preference given on them on the cadaveric donor organ wait list (the pre-Tx median duration of dialysis in our study cohort was only 1.6 years). This hypothesis is supported by the observation in our study that the majority of the children had normal levels of pre-Tx CRP, IL-12 and Neo, which is in striking contrast to the study in adult renal Tx recipients, in which the mean and median CRP levels were 14.5 and 9.0 mg/l, respectively [4] compared to 1.3 and 0.5 mg/l, respectively, in our study population. That dialysis is undoubtedly a pro-inflammatory state is also supported by our observation that the majority (91–100%) of the children with abnormally elevated values of CRP, Neo, and IL-12 were receiving dialysis pre-Tx. Moreover, although not in our study, others investigators have shown that serum CRP levels in children are positively correlated with how long children had been on dialysis [15]. Unlike Goldstein *et al.* [15], only 11% of our patients who were receiving dialysis pre-Tx had an elevated CRP, compared to 77% of their population. This difference may be explained by two factors. First, the children studied by Goldstein *et al.* were on dialysis for a considerably longer time than ours (median duration of dialysis 2.3 years). And second, there may likely have been differences in the dialysis prescription and dialyzer selection, both of which could affect the CRP level.

Levels and the biological activity of many of the inflammatory markers in the body are known to be under significant genetic control. The same has been shown in the case of CRP, IL-12, and Neo [16–18]. Polymorphisms in the genes controlling these inflammatory markers were not studied by us and could certainly have confounded our study results.

Due to the small number of subjects, our study was also likely very underpowered to detect significant differences in the primary outcomes between patients in the four separate quartiles of each of the inflammatory markers. However, even when children were analyzed after being grouped together (e.g. first quartile versus fourth quartile, first two quartiles versus last two quartiles), no differences were seen in outcomes. What is more important is that no *trends* were seen for differences in the outcomes either.

Our data underscore the importance of focusing on other factors that have been associated in the past with an increased risk of rejection and CAN in children such as DGF, HLA-DR mismatching, absence of induction immunosuppression, and calcineurin-inhibitor toxicity. Another area that needs investigation is the reason why adolescents have poorer graft survival compared to all other age groups. This has been attributed in the past to medication noncompliance [19,20], and may have been a contributor to the high incidence of graft loss in our population, most of whom were adolescents. Nonadherence to medication regimes is such a pervasive problem in pediatric and adolescent recipients, that its effect is likely to overwhelm other factors, such as occult inflammation, which may also contribute to graft loss.

In conclusion, while our study does have some limitations, those being its retrospective nature, small sample size and the lack of uniform immunosuppression in the

subjects (due to the long study duration) our pilot data do raise the issue that alloantigen independent factors (such as chronic inflammation) that are a significant determinant of the serum CRP, Neo, and IL-12 level may play a much smaller role in the pathogenesis of graft rejection in children compared to adults. Children and especially adolescents are at high risk of AR and graft loss related largely to nonadherence to medical regimens, the effect of which might supersede any impact of an underlying inflammatory state. While the stability of serum specimens frozen for such long periods of time also needs to be questioned, similarly collected, stored and processed specimens from adult renal Tx recipients at our center have been studied by us and consistently shown to have a predictive value in identifying adults at risk of rejection and graft loss [4,12,21]. Clearly, larger prospective studies are needed to elucidate the predictive value of chronic inflammation in outcomes after pediatric renal Tx. Serial monitoring of inflammatory markers in larger numbers of children in the post-Tx period and analysis of gene polymorphisms may lead to a better understanding of the role of inflammation in graft function and survival in children.

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