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Soluble CD30 does not predict late acute rejection or safe tapering of immunosuppression in renal transplantation

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ABSTRACT

Background: Previous reports revealed the potential value of the soluble CD30 level (sCD30) as biomarker for the risk of acute rejection and graft failure after renal transplantation, here we examined its use for the prediction of safe tapering of calcineurin inhibitors as well as late acute rejection.
Methods: In a cohort of renal transplant patients receiving triple immunosuppressive therapy we examined whether sCD30 can be used as a marker for safe (rejection-free) discontinuation of tacrolimus at six months after transplantation (TDS cohort: 24 rejectors and 44 non-rejecting controls). Also, in a second cohort of patients (n = 22, rejectors n = 11 and non-rejectors n = 11), participating in a clinical trial of rituximab as induction therapy after renal transplantation (RITS cohort), we examined whether sCD30 could predict the occurrence of late (>3 months post-transplant) acute rejection episodes. sCD30 was measured by ELISA in serum taken before and at several time points after transplantation.
Results: Overall, in the TDS cohort sCD30 decreased after transplantation. No difference in sCD30 was observed between rejectors and non-rejecting controls at any of the time points measured. In addition, in the RITS cohort, sCD30 measured at three months after transplantation were not indicative for the occurrence of late acute rejection.
Conclusion: In two prospectively followed cohorts of renal transplant patients we found no association between sCD30 and the occurrence of either late acute rejection or acute rejection after reduction of immunosuppression.

1. Introduction

Kidney transplantation is the only cure for end-stage renal failure. With the advent of current immunosuppressive therapy both short-term and long-term graft survival have greatly improved, although chronic deterioration of graft function remains a major clinical problem. A major drawback of this type of therapy is that the drugs are non-specific and have serious side effects, like a greater risk of infection, increased susceptibility to malignancies and faster development of cardiovascular disease [1,2]. Moreover, calcineurin inhibitors, like tacrolimus, are also nephrotoxic and this feature may contribute to long-term graft loss [2,3]. Therefore, an important goal is to safely reduce the use of immunosuppressive drugs; i.e. lowering the number of drugs or their dose without increasing the risk of rejection. The main problem of this approach is that as yet there are no validated biomarkers to predict safe tapering. Recently, the concentration of soluble CD30 (sCD30) was suggested by Susal *et al.* as a reliable biomarker for the prediction of kidney graft outcome [4]. Other groups found a similar association between high sCD30 levels and graft loss [5–9]. But as regards the prediction of acute rejection, the data are conflicting [10–12].

The CD30 molecule was originally identified on the surface of Reed-Sternberg cells in Hodgkin lymphoma. It is a member of the tumour necrosis factor/nerve growth factor super family and is a relatively large glycoprotein of 120 kDa [13]. CD30 is not expressed on resting immune cells but on diverse activated cells like T- and B-lymphocytes, and dendritic cells. (14) Recent studies have shown that CD30 has an important role in the generation of memory T-cell responses and the regulation of the balance between Th1-/Th2-responses, as it acts as a co-stimulatory molecule [14]. Another important finding is that even under cyclosporine treatment, CD30+ lymphocytes can still be induced by alloantigens. The absolute number of CD30+ cells is decreased by cyclosporine, but T cell activation still occurs [15]. Therefore it can be envisaged that

Abbreviations: Aza, Azathioprine; AUC, Area under curve; HD, Haemodialysis; MMF, Mycophenolate mofetil; PD, Peritoneal dialysis; PRA, Panel reactive antibody; ROC, Receiver operating characteristic; RITS, Rituximab Induction Therapy Study; sCD30, Soluble CD30; TDS, Tacrolimus Discontinuation Study.

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CD30+ cells have a role in graft rejection even under immunosuppressive treatment. Upon activation of T cells, CD30 can be released as a soluble molecule in the bloodstream. Due to its size, sCD30 is not filtrated by the kidney and its concentration is not influenced by renal failure, although the route of metabolization of sCD30 is still unknown.

Here, we investigated if sCD30 could predict the occurrence of acute rejection in renal transplantation patients in whom immunosuppression was reduced. In the same patient cohort, we previously showed that the ratio between memory T cells and Tregs, and the changes in distribution of naïve, effector and memory T cells enabled to identify patients in whom immunosuppression could safely be reduced at six months after transplantation [16]. In the current study, we furthermore examined in another patient cohort whether sCD30 could predict late acute rejection episodes (beyond 3 months after transplantation) using a case-control design. To our knowledge, this is the first study addressing the role of sCD30 in the prediction of acute rejection after tapering of immunosuppression and late acute rejection.

2. Materials and methods

2.1. Patient cohorts and immunosuppressive treatment

2.1.1. Tacrolimus Discontinuation Study (TDS)

The patients included in this study received a renal allograft at the Radboud university medical center between January 2000 and December 2004. Standard triple immunosuppressive therapy consisted of tacrolimus in combination with mycophenolate mofetil and prednisolone. Patients received 100 mg of prednisolone intravenously during the first 3 days after transplantation and subsequently an oral dose of 15–25 mg/day (depending on body weight), which was tapered to a maintenance dose of 0.1 mg/kg/day. Tacrolimus was started at day 1 or 2 after transplantation at 0.15 mg/kg/day (administered twice daily orally) and the dose was subsequently adjusted to achieve target whole-blood concentrations of 15–20 ng/milliliter during days 0–14, 10–15 ng/milliliter during weeks 3–6, and 5–10 ng/milliliter from week 7 onwards. Mycophenolate mofetil (MMF) was administered at 1000 mg twice daily with a dose reduction to 750 mg twice daily at 2 weeks after transplantation, unless the patient weighed more than 90 kg. Induction therapy with polyclonal or monoclonal antibodies was not used.

At 4 months after transplantation, immunological low risk patients were selected for reduction of their immunosuppression (including withdrawal of tacrolimus to prevent long term nephrotoxicity). They had to meet the following inclusion criteria: stable graft function, at least 1 HLA-B and 1 HLA-DR match between donor and recipient, panel reactive antibodies (PRA) ≤85% before transplantation, and Caucasian race. Patients who received a kidney from a HLA-identical living donor, patients with two or more previously failed grafts, and patients who had experienced a steroid-resistant acute rejection episode after their current transplantation were excluded for immunological reasons. In addition, patients with severe osteoporosis and patients with bone marrow suppression were excluded because they received an alternative treatment regimen.

First, MMF was substituted for azathioprine (Aza, 3 mg/kilogram daily). The dose of Aza was adjusted in case of leukocytopenia or elevated liver enzymes. In case patients did not tolerate a minimum Aza dose of 2 mg/kilogram/day, MMF was reintroduced (750 mg twice daily). Two months later, at six months after transplantation, the tacrolimus dose was gradually reduced to zero over a period of 4 weeks. Meanwhile, the prednisolone dose was increased to 0.15 mg/kilogram/day. The resulting maintenance immunosuppressive therapy after conversion consisted of azathioprine (at least 2 mg/kilogram/day; otherwise MMF 750 mg twice daily) and prednisolone (0.15 mg/kilogram/day). Patients were evaluated for acute rejection episodes during the first 6 months after withdrawal of tacrolimus. All acute rejection episodes were histological confirmed.

Table 1
Tacrolimus Discontinuation Study (TDS) patient characteristics.

Parameter	Rejectors	Non-rejectors	*p*-value
Number of patients	24	44	
Female (%)	8 (33%)	16 (36%)	1.00
Patient age (years, mean ± SD)	45.4 ± 15.5	41.6 ± 14.2	0.804
Donor age (years, mean ± SD)	49.4 ± 13.5	47.0 ± 12.4	0.595
Cold ischemic time (hours, mean ± SD)	11.0 ± 9.74	12.0 ± 8.81	0.637
Retransplantation (%)	0 (0%)	7 (16%)	0.004
Living donor (%)	12 (50%)	18 (41%)	0.610
HLA A/B/DR mismatches (mean ± SD)	2.3 ± 0.8	1.9 ± 1.2	0.095
PRA >5% (%)	3 (13%)	15 (34%)	0.083
Dialysis before transplantation			
None (%)	2 (8%)	6 (14%)	0.703
HD (%)	15 (63%)	22 (50%)	0.445
PD (%)	7 (29%)	11 (25%)	0.771
HD and PD sequentially (%)	0 (%)	5 (11%)	0.153
eGFR six months post transplantation (ml/min/1.73 m^2; median, (range))	70 (38–126)	76.0 (37–147)	0.741
eGFR ten months post transplantation (ml/min/1.73 m^2; median, (range))	65 (26–125)	81 (42–145)	0.039

HLA, human leukocyte antigen; PRA, panel reactive antibody; PD, peritoneal dialysis; HD, haemodialysis; eGFR, estimated glomular filtration rate (calculated with MDRD4-formula).

2.1.2. Rituximab Induction Therapy Study (RITS)

Patients included in this cohort participated in a randomized clinical trial assessing the efficacy and safety of rituximab induction therapy when added to standard triple immunosuppressive therapy in renal transplantation. Participants received a renal allograft in the Radboud university medical center between December 2007 and June 2012. Inclusion criteria were: Age above 18 years and for female patients the absence of pregnancy and willingness not to become pregnant within 12 months after transplantation. Patients with haemolytic uremic syndrome as original disease, recurrence of focal segmental glomerosclerosis in a previous graft, more than two previously failed

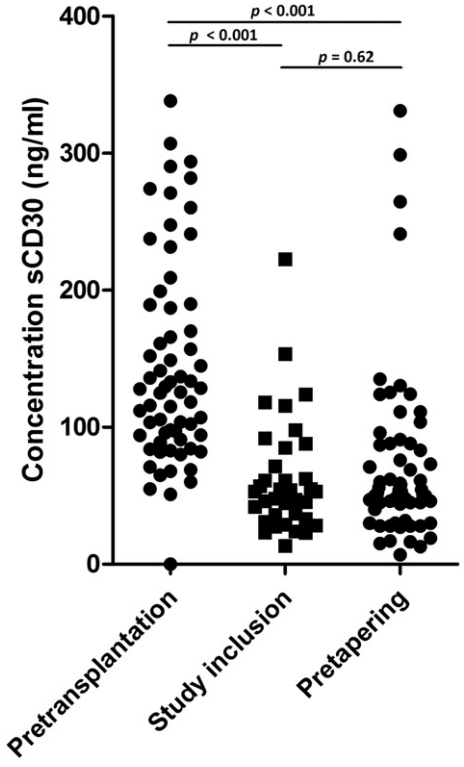

Fig. 1. Scatter plot of soluble CD30 concentration pre transplantation, at 4 months after transplantation (inclusion of study), and pre tapering of immunosuppression (6 months after transplantation) in the Tacrolimus Discontinuation Study cohort. Every dot represents the result from a single patient, the median is given.