Post-transplant soluble CD30 levels are associated with early subclinical rejection in kidney transplantation

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ABSTRACT

Several studies have shown association of high pre- or post-transplant levels of soluble CD30 (sCD30) with acute rejection and poor late kidney transplant outcome. Our goal was to investigate whether sCD30 levels at month-3 post-transplant are associated with subclinical rejection, presence of CD30$^+$ cells within the graft, and expression of immune response genes in peripheral blood mononuclear cells. The study comprised 118 adult first kidney graft recipients, transplanted at a single center, receiving tacrolimus in low concentration. All were submitted to a protocol biopsy at month-3. Subclinical rejection was identified in 10 biopsies and sCD30 levels ≥ 61.88 ng/mL (P = 0.004), younger recipient age (P = 0.030) and non-Caucasian ethnicity (P = 0.011) were independently associated with this outcome. Rare CD30$^+$ cells were present in only two biopsies. There was a correlation between sCD30 levels and CD30 gene expression in peripheral blood mononuclear cells (r = 0.385, P = 0.043). These results show that high sCD30 levels are independent predictors of graft dysfunction and may contribute to patient selection protocols by indicating those who could benefit from a more thorough evaluation.

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1. Introduction

A major area of interest in the kidney transplantation field is the identification of early risk assessment markers capable of predicting the occurrence of rejection, graft loss or dysfunction. Ideally, these markers should be non-invasive, measured in blood or urine, and useful even to identify subclinical inflammation processes.

Several studies have shown that high pre- or post-transplant serum levels of soluble CD30 (sCD30) are associated with poorer graft survival and increased acute and chronic rejection rates [1–10]. We have previously shown, in a study with 511 recipients with a functioning graft for at least three years, that sCD30 levels were independently associated

with graft loss and with interstitial lesions in biopsies for cause [11]. However, sCD30 levels were not found to be useful as markers of subclinical acute rejection [10].

CD30 is a type I transmembrane receptor of the tumor necrosis factor receptor family (TNFRS8), with an apparent molecular weight of 120 kDa. It was initially described on the surface of the malignant cells of Hodgkin's lymphoma and anaplastic large cell lymphoma [12,13]. CD30 is detected on activated T and B cells as well as memory and regulatory T cells [14–18]. The biological function of CD30 in mature T lymphocytes (activated or memory) is still discussed, but there is evidence that CD30 participates in signal transduction, that leads to a fast NF-κB activation [19].

sCD30 is a 85 kDa protein that results from the cleavage, by the metalloproteinases ADAM17 [20] and ADAM10 [21], of the extracellular domain of CD30 molecule on activated lymphocytes. Therefore, the amount of sCD30 levels may be indicative of the state of activation of the immune system [22].

2. Objective

The aim of this study was to evaluate the relationship between sCD30 levels and subclinical rejection at three months after kidney transplantation, in patients receiving an immunosuppressive regimen

with reduced tacrolimus (TAC) exposure, prednisone and mycophenolate [23]. We also investigated whether sCD30 levels were associated with the presence of CD30$^+$ cells within the graft inflammatory infiltrate, and with gene expression of *CD30*, *CD30 ligand (CD153)*, *ADAM10*, and *ADAM17* in peripheral blood mononuclear cells (PBMC).

3. Materials and methods

3.1. Patients

The included 118 kidney transplant recipients were recruited from a prospective study in low-immunologic risk kidney transplant recipients (first transplants, panel reactive antibodies (PRA) less than 30%) receiving reduced tacrolimus exposure, prednisone and mycophenolate, randomized at three months to be converted or not to sirolimus [23]. The present investigation was conducted at three months after transplantation, just before the conversion. All transplants were performed at the Hospital do Rim e Hipertensão/Federal University of São Paulo. The study was approved by the institutional medical ethics committee and informed consent was obtained from each patient prior to enrollment in the study. The patients were enrolled between October 2008 and May 2010. All were older than 18 years, were recipients of primary kidney grafts from ABO compatible non-HLA identical living-related or ABO identical deceased donors and were transplanted with a negative T and B complement dependent cytotoxicity crossmatch. All subjects had a peak HLA class I calculated PRA lower than 30%, according to inclusion criteria of the study [23], and none had preformed HLA-A,-B,-DR donor-specific antibodies (DSA) with normalized mean fluorescence intensity (MFI) ≥1500, as detected by Luminex Single Antigen assay (One Lambda, Canoga Park, USA), according to our previously described practice [24]. Immunosuppression consisted in TAC (0.1 to 0.2 mg/kg/day), adjusted to maintain trough blood concentrations from 8 to 10 ng/mL during the first month and from 5 to 7 ng/mL thereafter, mycophenolate sodium (MPS, 1440 mg/day) and prednisone (0.5 mg/kg/day). Induction therapy with basiliximab was used in 88.7% of the recipients of kidneys from deceased donors.

A protocol biopsy was performed at month-3 post-transplant and a blood sample was collected at the time of the biopsy. A pre-transplant serum sample of each of the recipients was obtained for reference.

3.2. Histopathology evaluation of allograft biopsies

Protocol biopsies performed at month-3 after transplantation were obtained as an out-patient procedure using a 16-gauge biopsy needle guided by ultrasound, and were graded according to Banff'07 classification [25]. All biopsies were reviewed by an independent pathologist, who was blinded to clinical and serological data. Infiltrate type, localization and the total inflammation (ti) score were reviewed according to the characteristics described by Mengel et al. [26,27]. C4d deposition was evaluated by immunofluorescence (Quidel, San Diego, USA) and also scored according to Banff'07 [25]. All clinically indicated biopsies, performed during the follow-up period, were graded by the local pathologist.

3.3. Immunohistochemistry

Protocol biopsies with subclinical rejection and/or ti score affecting ≥ 10% of the renal parenchyma that had paraffin embedded tissue available were sent to a specialized laboratory for immunohistochemistry performance (Laboratório Bacchi—http://www.conspat.com.br/conspat/) for identification of CD30 (BerH2 mouse, Dako, Glostrup, Denmark) and CD3 (LN10 mouse, Novocastra, Nussloch, Germany) positive cells. Tonsils were used as positive control staining for CD3 and CD30.

The sections were analyzed by the detection system EnVision FLEX (Dako). Briefly, antigen retrieval was performed by immersing the sections in sodium citrate buffer (pH 6.1) or Tris-EDTA (pH 9.0), according to the staining, in a microwave for 20 min. The sections were depleted of endogenous peroxidase activity by adding a peroxidase blocker. After overnight incubation with polyclonal antibodies, the samples were incubated with the secondary antibodies. This was followed by a 10 minute incubation with 3,3′-diaminobenzidine (EnVision FLEX Substrate Working Solution). The sections were washed and counterstained with Meyer hematoxylin. The positivity of the immunohistochemistry stain was examined without knowledge of clinical information, since the support laboratory did not receive any clinical data regarding these biopsies.

3.4. Measurement of sCD30 levels

Pre-transplant and three month post-transplant sCD30 serum levels were measured in duplicate by ELISA (Affymetrix eBioscience, San Diego, USA), according to the manufacturer's instructions. The detection limit was 0.3 ng/mL and the intra-assay and inter-assay coefficients of variation were less than 10% and 20%, respectively.

3.5. Anti-HLA antibody determination

Sera were screened for the presence of anti-HLA class I and II antibodies by LabScreen Mixed assay (One Lambda) in Luminex platform. Positive samples were tested by LabScreen Single Antigen assay (One Lambda). The criterion for antibody positivity in post-transplant sera was MFI ≥ 300.

3.6. Gene expression

RNA from PBMC was extracted with *mirVana™ miRNA Isolation kit* (Ambion, CA, USA), according to the manufacturer's instructions. Total RNA (200 ng) was reverse transcribed with RevertAid H Minus MMuLV kit (Fermentas, Hanover, MD, USA), reaction Buffer for M-MuLV, dNTPs, Oligo-dT primer (Integrated DNA Technologies, Coralville, IA, USA) and RiboLock™ Ribonuclease Inhibitor (Thermo Fisher Scientific, Pittsburgh, PA, USA). *CD30*, *CD153*, *ADAM10*, *ADAM17* and *PPIA* (used as the reference gene) gene expression quantification was performed in duplicate and determined by ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Thermo Fisher Scientific), using primers and probes from Applied Biosystems (CD30-Hs01114488_m1, CD153-Hs00174286_m1, ADAM10-Hs00153853_m1, ADAM17-Hs01041915_m1 and PPIA-Hs99999904_m1). Duplicates with a variation coefficient greater than 25% were excluded from the analyses. The $2^{-\Delta\Delta Ct}$ method [28] was used to calculate changes in gene expression, normalized to the reference gene and relative to the expression of these genes in 10 healthy donors' samples. Data were $\log_2$ transformed. PBMCs for RNA extraction were obtained only from the last 29 patients included.

3.7. Statistical analysis

Categorical and continuous variables were compared by Fisher's exact and Mann–Whitney tests, respectively. Spearman correlation was used to investigate association between sCD30 levels, estimated glomerular filtration rate (eGFR) and gene expression. A receiver operator characteristic (ROC) curve was used to determine the cutoff value for sCD30 levels as predictors for subclinical rejection. Multivariate logistic binary regression analysis (forward conditional method) was performed to investigate the independency of factors associated with subclinical rejection. Pearson correlation was used to investigate the association between gene expression of *CD30*, *CD153*, *ADAM10* and *ADAM17*. Statistical analyses were performed using PASW v18 for Windows. A two-tailed P < 0.05 indicated statistical significance.