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Soluble CD30 in patients with antibody-mediated rejection of the kidney allograft

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Abstract

The aim of our retrospective study was to evaluate the clinical significance of measurement of the soluble CD30 (sCD30) molecule for the prediction of antibody-mediated (humoral) rejection (HR). Sixty-two kidney transplant recipients (thirty-one C4d-positive and thirty-one C4d-negative patients) were included into the study. Soluble CD30 levels were evaluated before transplantation and during periods of graft function deterioration. The median concentrations of the sCD30 molecule were identical in C4d-positive and C4d-negative patients before and after transplantation (65.5 vs. 65.0 and 28.2 vs. 36.0 U/ml, respectively). C4d+ patients who developed DSA de novo had a tendency to have higher sCD30 levels before transplantation (80.7±53.6 U/ml, $n=8$) compared with C4d-negative patients (65.0±33.4 U/ml, $n=15$). Soluble CD30 levels were evaluated as positive and negative (≥100 U/ml and <100 U/ml respectively) and the sensitivity, specificity and accuracy of sCD30 estimation with regard to finding C4d deposits in peritubular capillaries were determined. The sensitivity of sCD30+ testing was generally below 40%, while the specificity of the test, i.e. the likelihood that if sCD30 testing is negative, C4d deposits would be absent, was 82%. C4d+ patients who developed DSA de novo were evaluated separately; the specificity of sCD30 testing for the incidence of HR in this cohort was 86%.
Conclusion: We could not confirm in our study that high sCD30 levels (≥100 U/ml) might be predictive for the incidence of HR. Negative sCD30 values might be however helpful for identifying patients with a low risk for development of DSA and antibody-mediated rejection.

Keywords: Kidney transplantation; Soluble CD30; Antibody-mediated rejection; C4d

1. Introduction

Antibody-mediated or humoral rejection (HR) is a deleterious immunological complication associated with (de novo) production of antibodies to mismatched donor antigens (donor-specific antibodies, DSA) and a poor prognosis of the kidney allograft [1–3]. The diagnosis of HR is verified by the detection of DSA and the finding of diffuse C4d deposits in graft biopsies. Various factors, however, may complicate the clinical recognition of HR. C4d deposits in the graft peritubular capillaries are a sign of complement activation due to previous antibody binding to graft antigens [4]. Nevertheless, focal C4d staining may be found during viral or other pathological conditions that trigger the classical complement cascade [5,6]. Alternatively, DSA may be sporadically difficult to detect in the periphery (by crossmatching with donor cells/or other techniques) due to antibody extraction by binding to antigens of the graft. Besides, the standard complement-dependent (CDC) and flow-cytometry (FCXM) crossmatch tests are generally performed with donor lymphocytes, while the antibody response may be also directed

Abbreviations: CDC, complement-dependent cytotoxicity; DSA, donor-specific antibodies; FCXM, flow-cytometry crossmatch; HR, humoral rejection; PRA, panel reactive antibodies; sCD30, soluble CD30.

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to endothelial (or other) antigens not expressed on lymphocytes [7,8]. At present, only a few commercial tests for detection of non-HLA antibodies are available.

Recently, there has been growing evidence in the literature that elevated concentrations of the soluble CD30 (sCD30) molecule in kidney recipients' sera are a risk factor for acute kidney allograft rejection and impaired graft survival [9–13]. It was also reported by two independent groups that high sCD30 levels are helpful in predicting vascular rejection [14–16]. On the other hand, no correlation was found in a similar study between sCD30 and antibody production [17]. Therefore, our objective was to test the hypothesis that measurement of sCD30 levels might be helpful for the clinical prediction and diagnosis of HR. Sixty-two kidney transplant patients (thirty-one C4d-positive and thirty-one C4d-negative control patients) were included into the study. Kidney biopsies were carried out during periods of graft function deterioration, sCD30 measurements, the CDC and FCXM tests were performed before transplantation and at time points related to the performance of kidney graft biopsies.

2. Materials and methods

2.1. Patients and immunosuppressive protocols

Patients included into the study were transplanted in our center in the period between November 1998 and December 2004; fifty-three patients received cadaveric kidneys and nine patients obtained grafts from living donors. Informed consent was obtained from all patients. The post-transplant diagnoses of patients, who were divided into C4d-positive and C4d-negative groups are shown in Fig. 1, the demographic characteristics and grades of rejection are represented in Table 1. Once the diagnosis of humoral rejection had been confirmed, patients were treated by plasmapheresis, OKT3/or ATG antibody and/or methylprednisone. Patients before second/or third transplantation received induction therapy of ATG 6 mg/kg within 6 days along with FK506, mycophenolate mofetil and methylprednisone. Repeated HLA mismatches (split and broad) are acceptable according to the Czech organ allocation criteria.

2.2. Measurement of soluble CD30

The quantitative detection of sCD30 in patients' sera was performed according to the manufacturers' instructions by enzyme-linked immunosorbent assay (ELISA) obtained from Biotest (Dreieich, Germany), using a QuickStep ELISA/ELFA Processor (Biotest, Dreieich, Germany). The absorbance of microwells was measured at 450 nm. The concentrations of sCD30 were determined by comparing the optical density (OD) of sample wells with the OD of wells with standard dilutions of sCD30 (standard curve). Due to the small amount of sera, sCD30 determination could not be performed with all pre-/or post-transplant samples from each patient.

2.3. C4d detection

Biopsies and C4d staining were performed in all C4d+ and C4d− patients. The median time point of performing biopsies in C4d+ patients was 10 days after transplantation (min — 5, max — 33). Graft biopsies in C4d-negative patients were carried out due to graft function deterioration (median day 8, min — 1, max — 730). Kidney graft pathology was classified using the definitions given by the updated Banff classification [18,19]. Immunofluorescence was applied for C4d detection in all biopsies starting from 2001; the remaining cases were stained by immunohistochemistry. Focal C4d positivity was considered as negative.

1. Immunofluorescence: Frozen sections were incubated with 5% normal swine serum and then with mouse anti-C4d monoclonal antibody (Quidel Corp. San Diego, CA). Slides were washed and incubated with anti-mouse IgG-FITC conjugate (Sewapharma, Czech Rep.) diluted in PBS. After washing, sections were mounted under a coverslip using glycerin solution.
2. Immunohistochemistry: Immunohistochemical staining of paraffin-embedded tissues was performed using anti-C4d polyclonal antibody (Biomedica, Austria). After deparaffinization and rehydration, the slides were treated in a pressure-cooker (10 mmol/L citrate buffer, pH 6.0). Endogenous biotin was blocked using a Biotin blocking system and non-specific background staining was blocked by Protein Block (DakoCytomation, Denmark). Detection of C4d antibody was performed using a horseradish peroxidase-labeled biotin–streptavidin system (LSAB Plus Kit, DakoCytomation, Denmark). Sections were then incubated with 3.3-diaminobenzidine, counterstained with Harris's hematoxylin and mounted in Entellan (Merck, Germany).

2.4. HLA typing and detection of donor-specific antibodies (DSA)

Patients and kidney donors were HLA typed for class I antigens routinely by serology; in cases of ambiguities, DNA methods were applied; class II antigen typing was performed by DNA techniques only. The standard CDC and FCXM tests were performed before and after transplantation at time points related to the performance of kidney graft biopsies.

1. Complement-dependent cytotoxicity (CDC) test and measurement of panel reactive antibodies (PRA): The complement-dependent cytotoxicity (NIH) test was performed according to the standard procedures [20]. The CDC crossmatch test was considered positive when cell kill was higher than 10%. PRA were tested by CDC on a panel of fifty healthy blood donors.
2. Flow-cytometry crossmatch (FCXM): Donor spleen lymphocytes (or peripheral blood lymphocytes, in cases when transplantation from a living donor was performed) were incubated with patient sera/or negative control (male AB) serum. Patient IgG bound to donor cells was detected by FITC-labeled goat anti-human IgG (heavy chain) antibody (Jackson ImmunoRes. Lab., West Grove, PA, USA). CD3+ and CD19+ cells were stained by PE-conjugated anti-CD3 and PerCP-labeled anti-CD19 monoclonal antibodies,

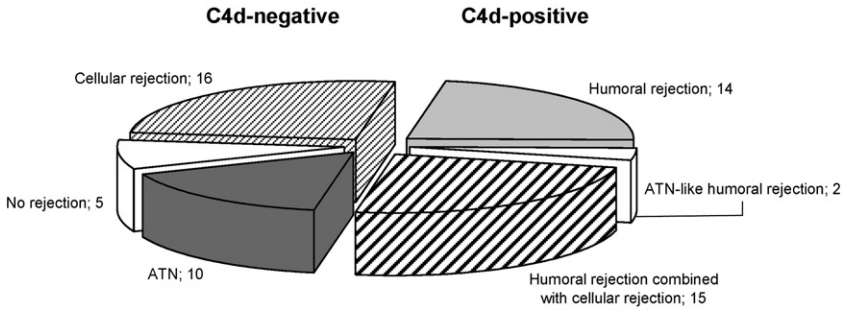

Fig. 1. Post-transplantation clinical diagnoses of C4d-negative and C4d-positive patients.