High variation of individual soluble serum CD30 levels of pre-transplantation patients: sCD30 a feasible marker for prediction of kidney allograft rejection?

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Abstract

Background. Previous studies have suggested that the pre-transplant levels of the soluble CD30 molecule (sCD30) represent a non-invasive tool which can be used as a biomarker for the prediction of kidney allograft rejections.

Methods. In order to evaluate the feasibility of sCD30 for pre-transplantation monitoring the sera of potential kidney recipients (n = 652) were collected four times in a 3 months interval. Serum from healthy blood donors (n = 203) served as controls. The sCD30 concentrations of all samples were determined using a commercially available ELISA. This strategy allowed the detection of possible variations of individual sCD30 levels over time.

Results. Heterogeneous sCD30 concentrations were found in the samples obtained from individual putative kidney transplant recipients when quarterly measured over 1 year. Total 95% of serum samples obtained from healthy controls exhibited sCD30 values <30 U/ml, whereas most recipients displayed higher serum levels (>30 U/ml). Total 524 patients (80.4%) constantly exhibited serum concentrations of <100 U/ml during the period investigated, whereas 109 patients (16.7%) showed variations by exceeding the proposed ‘cut off’ of 100 U/ml for one to three times. The frequency of samples exhibiting sCD30 values >100 U/ml was significantly lower than that previously reported.

Conclusions. The high degree of variation does not allow the stratification of patients into high and low immunological risk groups based on a single sCD30 value > 100 U/ml. Due to the heterogeneity of sCD30 levels during time course and the high values of SD, its implementation as a pre-transplant marker cannot be justified to generate special provisions for the organ allocation to patients with single sCD30 values >100 U/ml.

Keywords: graft survival; haemodialysis; kidney transplantation; soluble CD30

Introduction

The identification of pre- and post-transplant parameters of recipients bearing an increased risk of allograft rejection is an important prerequisite for the successful implementation of individually tailored immunosuppression of transplant patients. Although the treatment of transplant patients with immunosuppressive therapies has significantly increased the graft survival, a high dose immunosuppression should be avoided at least for low-risk recipients due to the massive side effects such as cancer development, infection and toxicity. The determination of panel reactive antibodies (PRA), which at present are exclusively used as indicators for an increased risk of graft rejection, is currently being critically discussed. For this reason novel markers are urgently needed for proper monitoring of pre- and post-transplant risks.

The CD30 molecule, a 120 kDa transmembrane molecule and member of the tumour necrosis factor receptor (TNF-R) superfamily, was originally identified as a surface antigen on the Hodgkin Reed Sternberg (HRS) cells [1–3]. Increased serum levels of the soluble 85 kDa CD30 protein (sCD30) in Hodgkin disease (HD) patients were further correlated with their clinical outcomes [3]. In most healthy individuals sCD30 serum concentrations are only detectable at low levels. CD30, which is expressed on CD4+ and CD8+ T lymphocytes, B cells, natural killer (NK) cells and some cells of non-lymphoid origin, appears rather to be expressed on Th2 cytokine-producing
T lymphocytes than on Th1 cells. In addition to HD, increased serum sCD30 levels have been frequently associated with diseases exhibiting Th2 dominated-immune responses including viral infections, lupus erythematosus and atopic dermatitis. The expressions of CD30 and CD30L as well as the potential role of CD30/CD30L interactions in humoral immunity have been extensively reviewed by Kennedy et al. [4].

Recently, there has been some evidence that high pre-transplant serum levels of sCD30 indicate the risk of an impaired graft outcome of kidney transplants [5–8]. Thus, up-regulated sCD30 levels were shown to be indicative for an increased risk of transplant loss emphasizing their clinical relevance and the implementation of sCD30 as a predictive biomarker for allograft rejection upon transplantation of different solid organs. In all studies published so far sCD30 levels as a prediction marker have only been determined once per patient prior to transplantation, whereas the pre-transplantation monitoring of sCD30 levels in putative kidney recipients over time has not yet been performed. We here postulate that an additional monitoring of individual changes of sCD30 levels in organ recipients over time is important for the validation and the clinical impact of this serum marker. Since individual sCD30 levels showed a significant heterogeneity over time, in particular with respect to the ‘cut off’ level of 100 U/ml which is currently used for distinguishing between high and low risk patients [5–8], we propose a quarterly monitoring of individual sCD30 values.

Patients and methods

The study was initiated at 737 patients representing the complete waiting lists of the four kidney and pancreas/kidney transplantation centres located in the ‘Deutsche Stiftung für Organtransplantation (DSO) Region East’ and completed with 652 recipients. This reduced patient number was caused by grafting, through the deregistrations of patients for unknown reasons by the transplant centres and by their deaths during the study time. The patient samples were quarterly collected for 1 year. Serum samples from 203 healthy blood donors, kindly provided by the Blood Donation Service of the Martin Luther University in Halle (Germany), served as controls.

For the analysis of sCD30 levels the commercially available sCD30 ELISA (Biotest AG, Dreieich, Germany) was used. Briefly, this ELISA is based on the sandwich-technique using two different anti-sCD30 monoclonal antibodies (mAbs) for capturing and detecting the antigen. The kit includes the immobilized capture Ab and antigen for the standard curve pre-dropped in duplicate by the manufacturer which comprises the range between 1.6 and 100 U/ml. The day-to-day coefficient of variation was 3.9% for high (50 U/ml) and 11.4% for low (3.2 U/ml) sCD30 concentrations, thus demonstrating the assay to be reliable and reproducible. To exclude an influence of the different lot numbers, standard curves for each lot were performed which showed no significant differences. Serum samples yielding optical density (OD) values higher than 2.0 were repeated at a higher dilution to reach OD values covered by the standard curve. All measurements were at least performed in duplicate at different days to exclude errors due to the individual handling of one experiment and to demonstrate the reproducibility of the values. The data calculations were performed using the WINSTAT program.

Results

Serum samples from putative recipient patients were quarterly monitored over a period of 1 year. As shown in Figure 1, most of the recipients exhibit higher sCD30 serum levels (30–90 U/ml) than healthy blood donors (0–30 U/ml) which is in accordance with nearly all previous studies investigating this aspect [e.g. 9–10]. The results of the mean values (MV) at the different time points suggest no seasonal dependency of sCD30 concentrations, and the high standard deviations (SD) of the MV (quarter 1: 52.25 ± 48.21 U/ml; quarter 2: 67.25 ± 39.27 U/ml; quarter 3: 58.47 ± 35.76 U/ml; quarter 4: 68.19 ± 42.29 U/ml) are in accordance with most previous studies. Although a ‘cut off’ level of 100 U/ml had been proposed as a risk predictor [6–8], the frequency of sCD30 serum levels >100 U/ml was considerably lower in our study when compared with the results published by Suesal and co-workers [6] (Figure 2). These lower levels were not the result of a degradation process of serum sCD30 since 5-fold freezing and thawing of six serum samples with sCD30 concentrations ranging from 10 to 110 U/ml and their consecutive storing for at least 2 h at room temperature or at −20°C, respectively, did not lead to any alterations in the sCD30 serum values (data not shown).

The novel approach of the present study was the monitoring of the patient distribution with sCD30 serum levels higher or lower than 100 U/ml
during the quarterly measurements. Based on the individual sCD30 levels during the time course, 524 patients (80.4%) exhibited constant serum concentrations of <100 U/ml during the period investigated, whereas 19 of 652 recipients (2.9%) showed sCD30 values higher than the proposed ‘cut off’ level of 100 U/ml in all four measurements (Figure 3A). In contrast, 109 patients (16.7%) demonstrated variations by exceeding 100 U/ml sCD30 one to three times. Randomly chosen representative results of patients exhibiting sCD30 levels higher than 100 U/ml only once (Figure 3B) and patients exceeding this value for two or three times are shown in Figure 3C, respectively. The implementation of 30 U/ml as threshold value to distinguish between healthy persons and haemodialysis patients described previously [9,10] was confirmed by our results (Figure 1). From the 652 putative recipients only 11 patients (1.7%) showed values lower than 30 U/ml in all four serum samples, whereas 158 patients (24.2%) demonstrated sCD30 serum levels lower than 30 U/ml for one to three times. It is noteworthy that only two of 652 recipients investigated (0.3%) exhibited an extraordinary fluctuation of the sCD30 serum levels during the study ranging from levels lower than 30 U/ml to levels significantly higher than the proposed ‘cut off’ level of 100 U/ml (data not shown).

Discussion

It has recently been suggested that the serum sCD30 level may be a potential marker for the prediction of acute allograft rejection of kidneys. The determination of sCD30 serum concentrations might therefore offer a promising non-invasive tool to recognize patients with an increased risk for developing an acute graft rejection. Based on published results pre-transplant sCD30 serum levels higher than 100 U/ml have been classified as a risk factor for the survival of kidney allografts [5–8,10]. In our present study we show (i) that the frequency of kidney pre-transplant patients exhibiting sCD30 levels which exceed the proposed ‘cut off’ of 100 U/ml is lower than that previously reported [6] and (ii) that there exists a high degree of time-dependent variations in the individual sCD30 levels.
temporarily exceeding the proposed upper 100 U/ml-threshold level (16.7% of the patients) as well as temporarily falling below the lower 30 U/ml-threshold level (24.2% of the patients) distinguishing between haemodialysis patients and healthy individuals. To the best of our knowledge, so far in all studies published pre-transplant sCD30 levels have only been determined once per individual. Thus, there exists no information regarding possible time-dependent variations of individual sCD30 levels during the course of disease. This aspect is of particular importance since in Middle Europe the median waiting time for the reception of a kidney graft is about 4 years, although many kidney recipients exhibit waiting times from 5 to 10 years. During this period the dialysis patients can develop additional diseases based on the continued intoxication due to the lack of proper kidney functions [11–14]. However, whether the high incidence of variation might be associated with clinical data of patients including intoxication, infections or other physiological factors has still to be investigated. The unexpectedly high individual time-dependent variations favour the concept that mainly individual immunological events, rather than the genetic disposition of a patient, as discussed previously, [6] may lead to the observed individual increases and decreases of serum sCD30. Although a certain statistical impact for the implementation of serum sCD30 as pre-transplant rejection marker had been suggested, the predictive value was highly narrowed by Rajakariar et al. [15]. These authors described an increased sCD30 level only during a rejection episode of the vascular type, whereas the dominating tubulointerstitial type showed decreased sCD30 levels even lower than those of healthy volunteers. Two other studies [16,17] in which no significant differences between the pre-transplant serum sCD30 levels of rejecting and non-rejecting patients were observable demonstrated the lack of the predictive pre-transplant value of this molecule. These two studies together with others [16–19] rather point to an importance of sCD30 post-transplant monitoring for the prediction of allograft rejection/survival. Indeed, a substantial difference in the post-transplant sCD30 levels between rejecting and non-rejecting patients has been described in the early post-transplantation period [16–19]. Patients with rejections had significantly higher sCD30 values at post-transplant days 5 [17,19] to days 14 or 15 [10,16,18] but with different threshold values proposed by the authors which allow the distinction between rejecting and non-rejecting kidney recipients. Thus, an integration of the individualized evaluation of post-transplant sCD30 serum level as one biomarker, together with accompanying diseases which affect the immunological reactivity post-transplantation, may be a feasible approach for the non-invasive post-transplant prediction of acute kidney allograft rejection.

In conclusion, our data do not support the transplantation of only HLA well-matched organs to recipients displaying pre-transplant sCD30 levels higher than 100 U/ml only once as proposed previously [6–8], but rather argue for a quarterly monitoring of sCD30 concentration prior to transplantation.

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Conflict of interest statement. None declared.

References


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