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Post-transplant hypocomplementemia: A novel marker of cardiovascular risk in kidney transplant recipients?☆

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ABSTRACT

Background and aims: Cardiovascular disease (CVD) is a leading cause of mortality after kidney transplantation (KT). The potential role of the complement system in the pathogenesis of post-transplant CVD remains unexplored.
Methods: Serum complement (C3 and C4) levels were measured at baseline and post-transplant months 1 and 6 in 447 kT recipients. The study outcome was post-transplant atherothrombotic event (PAE), a composite of acute coronary syndrome, critical peripheral arterial disease, stroke and/or transient ischemic attack.
Results: After a median follow-up of 4.2 years, 48 PAEs occurred in 43 patients (cumulative incidence: 9.6%; incidence rate: 2.6 events per 100 transplant-years). No differences were found in C3 and C4 levels at baseline or month 1 between patients with or without PAE. However, C3 levels at month 6 were significantly lower in patients developing PAE beyond that point (i.e., late PAE) (96.9 ± 22.3 *vs.* 109.6 ± 24.0 mg/dL; $p = 0.013$). The presence of C3 hypocomplementemia at month 6 was associated with a lower PAE-free survival ($p = 0.002$). After adjusting for conventional CVD risk factors and acute graft rejection, C3 hypocomplementemia at month 6 remained as an independent risk factor for late PAE in all the exploratory models (minimum hazard ratio: 3.24; $p = 0.011$). With respect to a model exclusively based on clinical variables, the inclusion of C3 levels at month 6 improved predictive capacity (areas under ROC curves: 0.788 and 0.812, respectively).
Conclusions: Post-transplant monitoring of serum C3 levels might be useful to identify KT recipients at increased risk of CVD.

1. Introduction

Cardiovascular disease (CVD) is a leading cause of mortality after kidney transplantation (KT). Accurate estimation of post-transplant risk may help to identify those recipients in which the benefit expected from cardiovascular risk-modifying drugs (i.e., antiplatelet or lipid-lowering therapies) outweighs potential adverse effects and concerns for drug-to-drug interactions. However, CVD risk prediction in the setting of KT is not precise. Classical approaches, such as the Framingham risk score, have been demonstrated to underestimate the occurrence of events in this population [1,2]. This could be partially explained by the underweighting of traditional risk factors, such as diabetes-attributable risk. In addition, novel risk factors not captured in the classical risk prediction equations (i.e., homocysteine) may contribute to the higher than

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expected incidence of CVD events after KT [3], as may the impact of decline in graft function, cytomegalovirus (CMV) infection or maintenance immunosuppression [4–6].

The complement system exerts a number of immune, inflammatory and metabolic functions that have been linked to the development of CVD at different levels [7–10]. Overall, the complement system seems to play a dual role in the pathogenesis of atherosclerosis since it is involved in the removal of debris but also in promoting local inflammation through the amplification of the inflammatory response and the recruitment of immune cells [11]. Complement components can be locally produced in the atherosclerotic plaque or reach it through the bloodstream after being released from the liver or other organs or from migratory cells [12–14]. Moreover, understanding the relationship between the complement system and the atherogenic process is further complicated by the fact that different effects on the CVD risk may depend upon the complement pathway involved or the stage and type of event considered [7,15].

Most published data have been focused on the association between C3 and the occurrence of CVD. The complement factor C3 is a soluble protein that initiates the cascade of activation in the alternative pathway following spontaneous hydrolysis induced by direct interaction with pathogens or "altered-self" cells (such as cancer cells). It is also the factor in which the three main activations pathways converge to initiate the terminal membrane attack complex (C5b-C9) [9]. Higher serum and plasma C3 levels have been related with coronary heart disease and stroke in healthy population [16–18] and in patients with established CVD [19,20]. To a lesser extent, plasma levels of the C4 component have been also positively associated with the risk of CVD in the general population [17], whereas mannose-binding lectin concentrations seem to exert an inverse correlation among hemodialysis patients [21].

There is a growing interest in the role of the complement system in solid organ transplantation due to its participation in the molecular mechanisms involved in the ischemia-reperfusion injury and graft rejection [13]. However, no previous studies have addressed the involvement of the complement system in the pathogenesis of post-transplant CVD. The present research was designed to gain novel insight into the potential value of C3 and C4 serum levels as surrogate markers of CVD risk in a large cohort of KT recipients.

2. Materials and methods

2.1. Study population and setting

The present prospective cohort study with complementary retrospective data collection was conducted at the University Hospital "12 de Octubre" (Madrid, Spain). Between November 2008 and March 2013, consecutive adult (≥18 years) patients receiving KT at our institution underwent an immune monitoring based on serial measurements of serum complement levels (C3 and C4), among other immunological parameters [22,23]. We excluded patients with documented pre-transplant primary immunodeficiency or human immunodeficiency virus infection, as well as those who died or suffered from graft loss within the first week after transplantation. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and had been approved by the local Clinical Research Ethics Committee. Written informed consent was obtained from each patient included in the study.

2.2. Study design and immune status assessment

The study outcome was the occurrence of any CVD event during the post-transplant period (see definition below for post-transplant atherothrombotic event [PAE]). A number of pre-transplant, perioperative and post-transplant variables were prospectively recorded. In addition, specific data on conventional CVD risk factors, use of cardiovascular risk-modifying drugs (antiplatelet, lipid-lowering, beta-blocker and anticoagulant therapies), and CVD events was retrospectively gathered by using a standardized data collection form. To ensure adequate case capture, electronic medical records of primary care physicians were also screened through the Madrid Electronic Health Record (HORUS) system, which integrates comprehensive patient information from the entire regional healthcare system. Case adjudication was performed by a panel of three nephrologists with extensive experience in CVD that were blinded to complement levels. Estimated glomerular filtration rate (eGFR) was performed using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [24]. To characterize trajectories in graft function throughout different periods, variations in eGFR (ΔeGFR) between time points (i.e., T_1 and T_2) were calculated (ΔeGFR = [eGFR at T_2 − eGFR at T_1/eGFR at T_1] x 100).

Serum samples were collected just before transplantation (baseline) and at post-transplant months 1 and 6 (±2 weeks). Serum C3 and C4 levels were measured by nephelometry (Image 800, Beckman Coulter, Villepinte, France). Normal ranges, as established by our laboratory, were between 83.0 and 171.0 mg/dL for C3, and between 14.0 and 38.0 mg/dL for C4. Patients were enrolled at the time of transplantation and followed-up for at least one year (unless death or graft loss occurred earlier). We divided the post-transplant period in three intervals: early (first month), intermediate (months 1–6) and late (>6 months).

2.3. Immunosuppression and prophylaxis regimens

Descriptions of the induction therapies, maintenance immunosuppressive regimens, and post-transplant prophylaxis are available as Supplementary Data.

2.4. Study definitions

Hypocomplementemia was defined as a decrease in serum complement levels below the lower normal range given by our laboratory (as detailed above). Post-transplant atherothrombotic event was a composite of acute coronary syndrome (myocardial infarction, unstable angina, or need for percutaneous coronary intervention or coronary artery bypass graft surgery), critical peripheral arterial disease (ischemic pain at rest, ulcer or gangrene in one or both legs attributed to proven peripheral arterial disease), stroke, transient ischemic attack and/or need for carotid revascularization procedure. Cerebrovascular events of presumable cardioembolic origin were excluded. Late PAE was that occurring beyond the sixth post-transplant month. Definitions used for myocardial infarction, stroke and transient ischemic attack were based on commonly accepted criteria [25,26].

2.5. Statistical analysis

Quantitative data were shown as the mean ± standard deviation (SD) or the median with interquartile ranges (IQRs). Qualitative variables were expressed as absolute and relative frequencies with 95% confidence intervals (CIs). All variables were tested for normality of distribution by means of the Kolmogorov-Smirnov test. Categorical variables were compared using the X^2 test, Fisher's exact test or McNemar test for repeated measures, as appropriate. Student's *t*-test or Mann-Whitney *U* test were applied for continuous variables. The linear associations between normally distributed variables were assessed by Pearson's correlation coefficients. Event-free survival curves were plotted by the Kaplan-