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Alloantibodies and allograft arteriosclerosis: Accelerated adversity ahead?

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In this issue, Loupy and colleagues report that, among renal allograft recipients who develop anti-donor alloantibody, a subset that exhibit “severe arteriosclerosis” in the graft within the first year after transplant are at significantly increased risk to incur a major adverse cardiovascular event (MACE) or die during subsequent intermediate-term (minimum follow-up 3.4 years, median follow-up >5 years) [1]. The observations in a single-center renal transplant data set derived from a study population of over 744 patients (of 1012 transplanted between 2004 and 2009, with complete data beyond one year in 664) were largely confirmed in a second independent cohort of 321 consecutive patients (2006–2009) from another center in the same city who met study entry criteria. In order to tease out “accelerated [graft] arteriosclerosis” as an independent risk factor, 33 previously identified donor and recipient factors were taken into account in their statistical model. This careful, detailed analytic approach reflects considerable effort, which is clearly necessary and perhaps sufficient to justify confidence that the association they identify is biologically meaningful.

As expected, well-validated, mechanistically plausible risk factors for immune graft injury (retransplant status, donor specific antibodies (DSA) present on the day of transplant and/or within 1 year, and especially higher titre DSA that activate complement C1q fixation), non-immune graft characteristics (donor age, hypertension, and diabetes), and recipient cardiovascular risk profile (hypertension, diabetes, prior MACE) emerged from the univariate and multivariate analyses. The link between alloantibody elaboration and graft injury is very well established in the transplant literature [2]. Similarly, donor characteristics and prolonged graft ischemic interval are well known contributors to adverse long-term renal allograft outcomes [3]. In addition, older transplant recipients with a history of cardiovascular risk factors (especially prior MACE) are at significantly greater risk of death and MACE events relative to non-transplant patients with normal renal function and transplant recipients without cardiovascular risk factors [4]. What is provocative about the current report is the suggestion that relatively severe inflammation within the graft – which is often anti-donor antibody-associated, and presumably driven to a significant degree by DSA [5] – is apparently an independent risk factor for MACE, even after controlling for all the other known risk factors. That is, inflammation in the graft is associated with atherosclerotic lesion instability (accelerated luminal narrowing, or more plausibly increase plaque instability) elsewhere in the transplant recipient. The notion that patients who make

alloantibody exhibit a “pro-inflammatory” phenotype that may also accelerate native vessel disease is provocative. If this is true, “accelerated [graft] arteriosclerosis” may be a very useful “biomarker” to identify that subset of patients in whom novel mechanistic hypotheses regarding how this occurs can be tested, and candidate “precision” treatments to interrupt identified targets can be efficiently evaluated.

The strengths of this study include the high quality of the data collection (relatively low rate of patient drop-out due to ‘lost to follow-up’, high proportion of patients with complete data), use of a primary outcome measurement (Banff graft ‘cv’ atherosclerosis score) performed independent of information regarding graft functional status or patient clinical data, replication of the finding in an independent patient cohort, and careful attention to management of formidable statistical methodology challenges.

However dependence on qualitative histologic measurements is a relative weakness: optimally, these must be replaced with quantitative, generally reproducible assays, and applied in sufficient numbers of patients so that the resulting analysis can consider each putative parameter reflecting “graft inflammation” as a continuous independent variable. Pathology assessments, which are well known to have high variability due to both sample variation and the judgements of the pathologist, should be accomplished by an expert panel that is blinded to the clinical data of each specimen, and such that all biopsies are reviewed by the entire panel. An objective molecular analysis of specimens could perhaps supplement or even replace histologic analysis [5].

Native kidney insufficiency, renal allograft dysfunction, and end-stage, dialysis-dependent renal failure are major, well-defined risk factors for cardiovascular events and death. The spectrum of renal insufficiency can vary significantly even among recipients with highly similar serum creatinine levels at a particular time following transplant, and renal function can be highly dynamic over time in association with differences in baseline post-transplant graft function, interval clinical events, and intensity and consistency of immunosuppressive drug exposure. Thus, measurement of renal function by serum creatinine or estimated GFR may be inadequate and direct measurement of GFR by rather laborious nuclear medicine techniques may be required. Differences in renal function between “minimal arteriosclerosis” and “severe arteriosclerosis” groups, either at the time of group assignment or subsequently, could account for some or all of the apparent increase in MACE risk in the latter group, and were not taken into account in the current report.

While the relative importance of various metabolic perturbations associated with reduced functional nephron mass is difficult to parse out based on clinical studies, multiple studies have shown that renal insufficiency is associated with systemic inflammation, while other studies have linked elevation of systemic inflammatory markers with atherosclerotic plaque instability and increased MACE incidence. This report does not control for these confounding variables: it is simply stated that renal function (measured when?) correlates strongly with worse outcome. In a study of this size, the difference in the number of MACE events (21 in 91 patients in the “severe atherosclerosis with antibody” group, versus 21 in 156 in the “severe atherosclerosis without antibody” group) is probably too small to adequately power a sub-analysis. Correcting for independent contributions of many different

variables, and at what time interval after transplant each occurs (renal insufficiency, alloantibody elaboration), would require a much larger data set (perhaps tens of thousands of patients), and thus would require a registry. Graft performance at study entry and over time (GFR, including change over time, and controlling for associated alterations in immunosuppressive drug exposure) and associated metabolic perturbations (lipid profile, hypertension, and diabetes, in each instance controlling for pretransplant recipient characteristics) would have to be taken into account in future study design and data analysis.

It is worth considering what pathophysiologic mechanisms may be in play that result in the observed association. First, inflammation in the graft may result in systemic inflammation that accelerates MACE. Systemic inflammation may be a consequence of the mobile nature of the immune system and its expressed molecules. This notion could be tested by analysis of peripheral blood samples for cytokines, chemokines, growth factors, acute phase reactants, activated leukocyte subsets (T, B, DC, monocytes, NK, etc.). Second, allograft rejection is often accompanied by autoantibody development [6]. Some autoantibodies could contribute to MACE associated vascular lesions. The subset of recipients with MACE may have more or higher titred autoantibodies, a possibility easy to test in longitudinal and cross sectional studies, or may have autoantibody deposits in MACE associated lesions, which could be tested on pathology samples obtained at operation or autopsy. Third, is development of alloreactivity a consequence of a genetic or environmental predisposition to develop MACE? By this hypothesis, MACE is really the primary event, and enhanced alloreactivity is due to immune mechanisms that are engaged by inflammatory processes that drive MACE. Perhaps the massive published GWAS studies in cardiovascular medicine that have identified many candidate genes will be revealing if viewed through this lens. Lastly, the population of patients with graft inflammation and MACE may identify that subset that interacts particularly poorly with current immunosuppression, manifesting as inadequate control of alloreactivity, drug-induced metabolic syndrome, and/or direct cardiovascular effects. Genetic, cellular, and molecular studies may define individual differences in how drugs are metabolized and engage putative therapeutic and off-target pathways.

Before the transplant community accepts the authors' proposed paradigm, however, it is essential that the basic observation be independently confirmed in a significantly larger patient cohort. Only with a study population significantly larger than 1000 can methodologic weaknesses in the current report be overcome. An informative study might be accomplished through cooperation between multiple large centers where biopsies are obtained by protocol at defined intervals. Until then, we are left with a plausible but unproven clinical association, one which deserves further study to confirm the principle finding. That effort would permit exploration of scientifically intriguing and perhaps clinically actionable mechanistic connections between immune graft injury and symptomatic progression of systemic atherosclerosis.

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References

1. Loupy A, Vernerey D, Viglietti D, Aubert O, Van Huyen JP, Empana JP, Bruneval P, Glotz D, Legendre C, Jouven X, Lefaucheur C. Determinants and Outcomes of Accelerated Arteriosclerosis: Major Impact of Circulating Antibodies. *Circ Res.* 2015; 117:xxx-xxx. [in this issue].
2. Jordan SC, Vo AA. Donor-specific antibodies in allograft recipients: etiology, impact and therapeutic approaches. *Curr Opin Organ Transplant.* 2014 Dec; 19(6):591–7.10.1097/MOT.000000000000128 [PubMed: 25304815]
3. Chapal M, Le Borgne F, Legendre C, Kreis H, Mourad G, Garrigue V, Morelon E, Buron F, Rostaing L, Kamar N, Kessler M, Ladrière M, Soulillou JP, Launay K, Daguin P, Offredo L, Giral M, Foucher Y. A useful scoring system for the prediction and management of delayed graft function following kidney transplantation from cadaveric donors. *Kidney Int.* 2014 Dec; 86(6):1130–9.10.1038/ki.2014.188 [PubMed: 24897036]
4. Ojo AO. Cardiovascular complications after renal transplantation and their prevention. *Transplantation.* 2006 Sep 15; 82(5):603–11. [PubMed: 16969281]
5. Halloran PF, Reeve JP, Pereira AB, Hidalgo LG, Famulski KS. Antibody-mediated rejection, T cell-mediated rejection, and the injury-repair response: new insights from the Genome Canada studies of kidney transplant biopsies. *Kidney Int.* 2014 Feb; 85(2):258–64.10.1038/ki.2013.300 [PubMed: 23965521]
6. Halloran PF. Transplantation: Autoantibodies-epiphenomena or biological clues. *Nat Rev Nephrol.* 2013 Dec; 9(12):705–6.10.1038/nrneph.2013.221 [PubMed: 24145327]

Table 1

Risk Factors for Chronic Allograft Nephropathy: known and Possible Additional (“?”) Variables Potentially Confounding MACE Risk Assessment

Donor factors

Living vs brain-dead, heart-beating vs donation following cardiac death
 Graft ischemic time (cold, warm)
 Delayed graft function
 Best post-transplant GFR (higher better)
 Genetic disparity
 MHC antigens (HLA-A, B, C, DR)
 “minor” antigens (Y, MICA, etc)
 Donor Microbiome?

Recipient factors

Recipient MACE event history and risk factors
 Hypertension and management
 Obesity and management
 Cardiovascular fitness (MVO₂)
 Lipid risk profile and management
 Diabetes risk profile and management
 Genetic Cardiovascular GWAS:
 Lipid profile
 Family Atherosclerosis History: age of onset, rate of progression
 Autoimmune, Innate Immune, Adaptive Immune Predispositions
 Drug metabolism
 Renal failure etiology, duration, and severity
 Living donor: preemptive vs dialysis-dependent
 Renal replacement therapy (Y/N; duration, quality)
 Immune history
 Prior transplant
 Panel-reactive antibody status
 Anti-donor antibody
 Complement-fixing (C1q) vs no
 Cellular immunity
 Vaccine and infection history
 Inflammation Exposure History (response phenotype)
 Autoantibody profile
 Behavioral
 Tobacco use (cumulative exposure, primary and second-hand)
 Diet (BMI, lipid profile)
 Exercise (maximal oxygen consumption [MVO₂]; 6 minute walk)
 Compliance with immunosuppression, other medications
 Environmental? (Chemical, Dietary, Pharmacologic, “Nutraceutical”)

Recipient Microbiome?

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Table 2

Candidate Hypotheses and Putative Mechanisms Linking Anti-donor Alloantibody, Allograft Inflammation, and Accelerated Systemic Arteriosclerosis Progression

Hypothesis 1: Inflammation in graft drives inflammation in the recipient

Cytokines (eg IL-1, IL-6, IL-13)

Chemokines (eg IL-8, RANTES)

Growth factors (eg EDRF, VEGF)

Acute Phase Reactants eg C-reactive protein, TNF α)

Activated Leukocyte Subsets (eg, T, B, DC, monocytes, NK)

Hypothesis 2: Autoantibodies drive MACE

Vimentin

Collagen subtypes

Hypothesis 3: Recipient MACE risks drive alloimmunity

Genetic

Behavioral

Environmental

Hypothesis 4: Transplant drugs interact adversely with recipient genotype, MACE genes

Inadequate Control of Alloreactivity

Drug-induced Metabolic Syndrome

Drug-induced Progression of Cardiovascular Lesion