HDL: Beyond Atheroprotection

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Decades of epidemiologic studies have firmly established the inverse relationship between HDL-cholesterol (HDL-C) levels and cardiovascular events.1 However, the resulting hypothesis that raising HDL-C levels should reduce cardiovascular disease (CVD) has been called into question by the failure of recent trials designed to decrease CVD events, despite raising HDL-C levels with cholesteryl ester transfer protein inhibitors or extended release niacin. The failure of these trials to show a reduction in cardiovascular events has, in turn, stimulated interest in the concept that measures of HDL composition, HDL particle number, or HDL functions may be better predictors of cardiovascular risk than HDL-C levels. Among the antiatherogenic functions of HDL, reverse cholesterol transport, which refers to the ability of HDL to pick up cholesterol in the tissues and deliver it to the liver, has been considered the most important. Cholesterol efflux capacity (CEC) measures the ability of HDL to accept cholesterol from macrophages, which is the first step of reverse cholesterol transport. A number of recent studies has examined the ability of measures of HDL-cholesterol efflux capacity to detect radiographic prevalence of atherosclerosis and predict cardiovascular events.

In the first human study to examine CEC as a marker for atherosclerosis, Khera et al.2 showed that CEC of apoB-depleted serum (a surrogate for HDL) was inversely associated with measures of carotid and coronary atherosclerosis, independent of HDL-C levels. A case control study of subjects recruited from the cardiac catheterization laboratory also found an inverse relationship between CEC and the presence of coronary atherosclerosis.3 Surprisingly, CEC in this setting was directly associated with subsequent incident cardiovascular events. Differences in the patient populations, study design, and/or methods for assessing CEC may have been responsible for this unexpected result. Subsequently, two larger prospective trials reported that CEC was inversely associated with cardiovascular events. In contrast to the other studies, which used radiolabeled cholesterol to track efflux, Rohatgi et al.4 used fluorescence-labeled cholesterol to measure CEC in subjects from the Dallas Heart Study who were free of coronary heart disease at baseline and found an inverse association with incident atherosclerotic CVD independent of HDL-C levels. More recently, CEC was found to be inversely associated with incident coronary heart disease events in a nested case control sample from the very large prospective European Prospective Investigation into Cancer and Nutrition-Norfolk Study.5 Comparing the top and bottom tertiles of CEC in subjects with incident coronary heart disease and controls, the study found that CEC was inversely associated with incident coronary heart disease events independent of HDL-C and ApoAI levels.5 Collectively, these prospective population studies support the hypothesis that measures of HDL function serve as a marker for atherosclerosis and risk of cardiovascular events independent of HDL-C levels.

CKD is associated with low levels of HDL and increased risk of CVD. We and others have shown that functionality of HDL is altered by kidney dysfunction, including in recipients of renal transplant.6–8 Thus, the concept that impaired HDL function may contribute to the pathogenesis and risk for CVD in CKD is particularly attractive. Cardiovascular events are the main cause of death in recipients of renal transplant, with ischemic heart disease being the main culprit.9 Although renal transplantation improves long-term survival of patients with ESRD on dialysis, renal transplantation is associated with an accelerated form of atherosclerosis.9 In this issue of JASN, Annema et al.10 address the interesting possibility that CEC can predict adverse cardiovascular and renal events in recipients of renal transplants. This prospective study examined whether CEC at baseline is associated with future cardiovascular mortality, all-cause mortality, and graft failure. Baseline CEC did not predict cardiovascular mortality or all-cause mortality. However, there was a strong inverse association between graft failure and efflux capacity. Both the negative association between CEC and cardiovascular consequences as well as the positive association between CEC and graft survival are surprising and the subject of this commentary.

The lack of association of baseline CEC with cardiovascular mortality may seem to conflict with the studies showing that CEC is inversely correlated with cardiovascular events,4,5 but there is a number of differences between these studies that may contribute to the apparent difference in their results, such as (1) patient population, (2) disease process, (3) size of the cohort, (4) cardiovascular end points, and (5) method for determining CEC. The accelerated atherosclerosis in subjects with renal transplants has a complex pathogenesis involving both immunologic and nonimmunologic processes.9 Therefore, the ability of CEC and other measures of HDL function to predict mortality caused by cardiovascular...
events may differ in subjects with renal transplants because of fundamental differences in the pathogenesis of their atherosclerosis. Given that loss of graft function is associated with reduced survival after renal transplantation, one might anticipate an association with total mortality. Interestingly, however, the association between CEC and all-cause mortality reported in this study became significant after adjustment for recipient age and sex, although the association was lost with additional adjustment for ApoAI, HDL-C, and creatinine clearance. The total number of subjects studied was relatively small, and only cardiovascular mortality and total mortality were examined compared with the analyses of incident cardiovascular events in some of the studies above. Larger prospective trials with well defined cardiovascular end points would be required to further clarify the relationship between efflux capacity and other measures of HDL function with cardiovascular events and mortality after renal transplantation. The methods used to measure efflux capacity in these outcome studies have differed substantially. Some used J774 mouse macrophage cells that were not loaded with cholesterol but were labeled with H-cholesterol and stimulated with CAM to induce the cholesterol transporter ATP-binding cassette transporter A1, whereas others used a fluorescence-based assay. In the study by Annema et al., THP-1 human monocytes were loaded with acetylated LDL and 3H-cholesterol. Transfer of cholesterol between macrophages and HDL is bidirectional, with both influx of cholesterol from HDL to the macrophage and efflux of free cholesterol and phospholipids from the macrophage. Assays using cholesterol-loaded macrophages may give a better measure of net efflux capacity of HDL. Therefore, it is likely that differences in the assays yield different information regarding HDL function, and this may underlie some apparent differences in outcome results. It would be important to rigorously compare and standardize the assays of CEC used in clinical trials to make the results easier to interpret. These limitations notwithstanding, the observation that impaired CEC to HDL in recipients of renal transplants predicts graft survival is novel and very intriguing. Furthermore, the finding that the association is independent of circulating levels of ApoAI and HDL-C reinforces the concept that HDL functionality may be superior to HDL-C levels in predicting adverse events, even those beyond atherosclerosis, such as renal graft loss.

In view of the increasing appreciation of HDL’s beneficial actions, impairment in the major metric of HDL function (i.e., CEC) may reflect deficiencies in other functionalities of HDL relevant to graft survival. Indeed, HDL of adult and children recipients of renal transplants is not only defective in effecting cholesterol efflux but has suppressed antioxidative activity and reduced capacity to protect the endothelium. The latter is exemplified by decreased nitric oxide production and increased expression of vascular cell adhesion molecules. However, the correlation between HDL CEC and other potentially vasoprotective functions is variable. We found no tracking between impaired efflux capacity and heightened inflammatory response of macrophages in specific samples of uremic HDL. Similarly, HDL of recipients of heart transplants has impaired CEC and vasoprotective function, which is reflected by a blunted migratory response of endothelial progenitor cells; however, these parameters are not linked. Such findings underscore the idea that a generalized attenuation in HDL functionality may reflect distinct structural or biochemical changes in HDL particles that may or may not lead to concurrent dysfunctions in HDL. This is important, because it is currently not clear which particular functionality or panel of functionalities is physiologically relevant or whether a particular beneficial or detrimental metric of HDL function actually predicts a hard clinical end point. Thus, although it is likely that other HDL functionalities are impaired in recipients of renal transplants, the results reported by Annema et al. emphasize that HDL’s CEC strongly predicts the clinical end point of graft loss. What are the possible reasons for this association?

Annema et al. point to parallelism between atherosclerosis and the transplant vasculopathy that leads to chronic transplant dysfunction and ultimately, graft failure. Annema et al. suggest that impaired capacity of HDL to remove cholesterol from macrophages as well as other parenchymal cells, such as endothelial cells, promotes the vasculopathy, whereas improving HDL functionality may have the potential to lessen intragraft atherosclerosis and preserve kidney function. Considering that HDL and its major apoproteins are metabolized in the kidney, HDL may directly modulate different types of renal resident cells, which express its transporters and receptors and thus, directly contribute to allograft vasculopathy. Interestingly, cholesterol loading and downregulation of the ATP-binding cassette transporter, which removes excess cellular lipids, have been observed in podocytes exposed to sera of patients with diabetes who are albuminuric compared with sera of patients with diabetes who are normalbuminuric, although both groups had similar lipid profiles and duration of diabetes. The expansion in the cellular lipid pool was not because of increased cholesterol uptake or synthesis but was, instead, linked to impairment in cholesterol efflux. Notably, induction of cholesterol efflux with cyclohexim in cultured cells and diabetic mice preserved podocyte functions and lessened albuminuria. These results support a link between impairment of cholesterol efflux from kidney cells and kidney dysfunction and illustrate injurious effects of cholesterol accumulation as well as possible benefits of increasing cholesterol efflux on renal cellular integrity and kidney function, including grafted kidneys. The study by Annema et al. raises important questions regarding the mechanistic link between HDL efflux capacity and graft failure that suggest the need for further inquiry into the lipid content of cells within the graft and determination of which cells and pathways are affected as the graft fails.

Alloimmunity plays a central role in the pathophysiology of graft failure, and HDL has extensive cross-talk with the immune system. For example, HDL downregulates the immune response by reducing activation, adhesion, and migration of neutrophils and also, decreasing synthesis of inflammatory mediators by macrophages and dendritic cells. HDL inhibits monocyte to dendritic differentiation and reduces antigen
presentation, and HDL regulates the principal cellular components of adaptive immunity: B and T lymphocytes. Relevant to the study by Annema et al., activation and function of the immune cells have specifically been linked to cholesterol efflux. HDL-stimulated efflux decreases cholesterol content in macrophages, which in turn, affects antigen presentation and T cell receptor signaling. Importantly, it is not only the capacity to affect the cellular cholesterol content but modulation of specific cellular cholesterol pools that seems critical to effect immunomodulation. Thus, membrane microdomains known as lipid rafts contain high concentrations of cholesterol, sphingolipids, and proteins and are integral to cellular signaling, protein transport, and adhesion. Lipid rafts are also the sites for key receptors of B and T cells, and changes in the composition and structure of these membrane microdomains affect both the localization of receptors to the microdomains and the activation and function of these receptors. For example, although HDL-induced depletion of cholesterol in lipid rafts of antigen-presenting cells inhibits T cell activation, cholesterol repletion reverses the inhibitory effects of HDL. Such observations suggest the possibility that graft loss in individuals with the most profoundly impaired HDL CEC observed by Annema et al. may reflect disruption in the cholesterol content of lipid rafts that may activate immune cells, including monocytes/macrophages, dendritic cells, and lymphocytes, and drive the autoimmune response. Such possibilities encourage additional elucidation regarding the structure and composition of lipid rafts in immune cells in the cohort of recipients of renal transplants.

A key unresolved issue in interpreting the results is the uncertainty of whether reduced cholesterol efflux is causative in the graft loss. It is possible that some factors in the systemic or intrarenal milieu rendered HDL ineffective and that dysfunctional HDL is rather a biomarker and not the actual mechanism for graft loss. Nonetheless, although the clinical and biologic importance of measuring HDL-C to predict adverse events is waning, the report by Annema et al. underscores the increasing range of HDL actions and provides new incentive to develop targeted interventions beyond atherosclerotic coronary artery disease to change not only HDL levels but its functions.

DISCLOSURES
None.

REFERENCES