Can Preservation Fluid Biomarkers Predict Delayed Graft Function in Transplanted Kidneys?

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The need for kidney transplantation far outstrips the organ supply. To address this growing problem, the field continues to push the envelope by procuring and transplanting kidneys from deceased donors who may be considered suboptimal. Donation after circulatory determination of death (DCD), in particular, has become increasingly common. Between 2005 and 2015, the percentage of deceased donors who were DCD in the United States grew from 8% to 18% (1), whereas some countries use DCD donors to even greater extents (*e.g.*, the current Transplant Registry Activity Report noted that 42% of deceased donors in the United Kingdom are DCD). Several studies have shown that DCD kidney transplants are at increased risk for primary nonfunction (PNF) and delayed graft function (DGF) (2). In addition to avoiding the devastating outcome of PNF, the transplant community has sought to better predict, prevent, and even ameliorate the potential negative effects of DGF given its associations with worse long-term allograft function and survival. Specific data discussed below, however, provide insights into the complexities of long-term outcomes after DGF in the DCD setting.

Compared with living donation, sudden brain death often causes a catecholamine surge followed by hypothalamic-pituitary-adrenal axis dysregulation that may contribute to worsening organ function, despite continued perfusion by the beating heart (3). Although this autonomic storm typically does not occur with DCD, progressive organ/tissue hypoxia and stasis of blood after withdrawal of life support and circulatory arrest (*i.e.*, warm ischemia) are the processes that likely predispose DCD kidneys to PNF and DGF. However, nearly all published data have shown that long-term outcomes are similar between adult DCD and non-DCD donor kidneys and that DCD-related DGF does not convey the same deleterious effects on long-term outcomes as DGF in non-DCD settings (4–6). A study of the United States transplant registry found that 5-year death-censored graft survival was worse with DGF (73% versus 83% without DGF; $P<0.001$) (7). Compared with DGF in non-DCD donor settings, DGF in the setting of DCD donors <50 years old was surprisingly associated with less graft loss. Causes for these counterintuitive associations are unclear but may be attributed to complex immunologic responses after renal recovery from warm ischemia in the setting of DCD kidneys, which may differ from responses to severe ischemia-reperfusion injury leading to DGF in non-DCD kidneys. Regardless of these complexities relative to DCD status and given the effect that DGF seems to have on later kidney transplant outcomes in general, many transplant clinicians would embrace the ability to accurately predict DGF to better inform recipients, prepare for dialysis needs, or even modify therapy in hopes of shortening or reducing DGF and its associated peritransplant costs.

In a series of analyses for DGF in 56 DCD kidneys at two centers in The Netherlands, van Balkom *et al.* (8) report in this issue of the *Clinical Journal of the American Society of Nephrology* that a combination of two protein biomarkers (leptin and GM-CSF) measured from samples of flushed kidney preservation fluid just before transplant predicted DGF with an area under the receiver operating characteristic curve (AUC) of 0.87. Adding a single recipient characteristic, body mass index, to these biomarkers improved the AUC to 0.89, which was superior to the utility of the clinical risk prediction model for DGF developed by Irish *et al.* (9)—AUC of 0.59 in this cohort—as well as the kidney donor risk index (KDRI) (10)—AUC of 0.55.

The authors described an innovative sample collection method and a stepped approach for biomarker measurements in sequential patient groups (8). Others have investigated potential biomarkers in fluid samples collected from kidneys preserved with hypothermic machine perfusion (HMP) (11–16), but this is the first report of preservation fluid biomarkers using a noninvasive flush to collect samples from statically cold-stored kidneys before implantation. Although not technically exploratory as in unbiased proteomics for biomarker discovery (17), the authors did use a multiplex immunoassay platform (Luminex) to simultaneously quantify 158 different proteins in an initial group of 16 kidneys (eight with DGF matched to eight without DGF) (8). The five proteins with significantly different concentrations by DGF status were then measured *via* Luminex in a verification group of 40 additional kidneys, of which 27 (68%) developed DGF. Stepwise multivariable regression was then used to identify the best predictive model with various combinations of these biomarkers along with recipient body mass index and dialysis duration, which were the only donor or recipient characteristics different (at $P<0.10$) by DGF status in the verification

group. The study provides interesting and novel results, but it also highlights important issues to consider when using pretransplant biomarkers to predict outcomes.

First, what is the most appropriate post-transplant clinical outcome that we should predict? As stated above, there are justifiable reasons to better understand and predict DGF; however, there are stronger arguments to develop tools to more precisely predict harder outcomes, like allograft survival or long-term allograft function. In the DCD setting in particular, in which the two highest-quality HMP trials to date provide arguably conflicting results about DGF prevention (18,19), the data do not support the use of DGF as an acceptable surrogate outcome relative to subsequent DCD allograft failure.

Second, would adding a pretransplant biomarker panel with a moderately high AUC for an appropriately hard post-transplant outcome sufficiently address the organ supply-demand problem and increase life-years saved? Investigators could perform simulations on the basis of necessary assumptions about the effects of using such a biomarker panel during organ allocation. In the absence of simulation studies, the field may glean some insights from the December 2014 addition of KDRI to the new kidney allocation system. Several outcomes shifted abruptly, including a realignment of kidney-recipient longevity matching and increases in kidney discard, transplant, and DGF rates as well as increases in regional kidney sharing and transplants to higher-risk recipients (20). It is important to realize, however, that the initially described *c* statistic (AUC) for predicting allograft failure *via* KDRI was only 0.62 (10). As such, it remains very difficult to anticipate how other novel pretransplant risk predictors will affect the fundamental goals of deceased donor kidney transplantation in clinical practice. Thus, we should remain circumspect about implementing biomarkers (even those that seem to optimize donor potential in initial studies) without pursuing adequately large prospective studies and proper due diligence.

Expanding on the need for due diligence, a major problem in interpreting AUCs involves the failure to recognize when the statistic is reported for the same group in which the classifier was developed. This may be less of a concern when the classifier is a raw biomarker value (*e.g.*, protein concentration), but when a regression model is involved, separate cohorts are needed to describe the predictive utility of the classifier. Ideally, a predictive model is developed/trained on a discovery cohort and then assessed without additional training in separate validation sets. Such validation sets should not be confused with the verification set used in this study to develop the biomarker panel. This is not to say that promising pretransplant biomarker panels (including novel preservation fluid biomarkers) should not be reported and actively discussed. However, the transplant community must exercise caution when interpreting the reported performance of any prediction tool and insist on appropriate validation studies that attempt to limit selection and other biases, which become ingrained when physicians prematurely begin to rely on such tools for clinical decision making (*e.g.*, whether to accept or decline particular organ offers).

Ultimately, reports like that by van Balkom *et al.* (8) represent necessary and exciting proof of concept studies of noninvasive biomarker assessment before kidney transplantation. With expanding use of HMP, especially in clinical settings of increased DGF risk, this novel method of collecting flushed samples of static preservation fluid may prove to be most helpful in settings traditionally considered lower risk for DGF, where the continued use of static cold storage is more likely. If certain biomarkers that can be rapidly measured *via* Luminex or other platforms are found to accurately predict meaningful outcomes (*e.g.*, PNF or DGF mechanistically linked with premature allograft failure), clinicians would undoubtedly incorporate the biomarkers into organ offer decisions. Other biomarkers with strong associations with important outcomes but relatively poor predictive utility may still prove useful clinically. Such biomarkers should not be used in isolation to reject organ offers, but they could help inform recipients of potential risks or be used to enrich clinical trial enrollment for higher-risk participants. Although the promise of preservation fluid biomarkers has yet to be realized with regard to predicting DGF, investigators should continue to pursue exploratory research in this area coupled with appropriate validation studies and a focus on expanding transplantation while improving long-term outcomes.

Disclosures

None.

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