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Serum aminoacylase-1 is a novel biomarker with potential prognostic utility for long-term outcome in patients with delayed graft function following renal transplantation

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Early identification and prognostic stratification of delayed graft function following renal transplantation has significant potential to improve outcome. Mass spectrometry analysis of serum samples, before and on day 2 post transplant from five patients with delayed graft function and five with an uncomplicated transplant, identified aminoacylase-1 (ACY-1) as a potential outcome biomarker. Following assay development, analysis of longitudinal samples from an initial validation cohort of 55 patients confirmed that the ACY-1 level on day 1 or 2 was a moderate predictor of delayed graft function, similar to serum creatinine, complementing the strongest predictor cystatin C. A further validation cohort of 194 patients confirmed this association with area under ROC curves (95% CI) for day 1 serum (138 patients) of 0.74 (0.67–0.85) for ACY-1, 0.9 (0.84–0.95) for cystatin C, and 0.93 (0.88–0.97) for both combined. Significant differences in serum ACY-1 levels were apparent between delayed, slow, and immediate graft function. Analysis of long-term follow-up for 54 patients with delayed graft function showed a highly significant association between day 1 or 3 serum ACY-1 and dialysis-free survival, mainly associated with the donor–brain–dead transplant type. Thus, proteomic analysis provides novel insights into the potential clinical utility of serum ACY-1 levels immediately post transplantation, enabling subdivision of patients with delayed graft function in terms of long-term outcome. Our study requires independent confirmation.

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Renal transplantation provides clear benefits for patients with end-stage kidney disease,[1,2] and significant cost savings compared with dialysis.[3,4] In 2010, 16,151 renal transplants were performed in the United States (http://optn.transplant.hrsa.gov), and 2687 in the United Kingdom (http://www.uktransplant.org.uk). However, early complications can significantly impact clinical and economic outcomes, such as delayed graft function (DGF) that affects ~20% of patients in the United States.[5] A number of definitions of DGF have been proposed[6–8] with one commonly used being the need for dialysis in the first week after renal transplantation, other than for isolated hyperkalemia. Although there are parallels with acute kidney injury, the pathology underlying DGF is complex with contributions from donor-derived factors, such as donor age and duration of ischemia, and recipient factors such as ischemia–reperfusion injury (IRI), immunological responses, and immunosuppressant medications.[9] Acute tubular necrosis secondary to IRI is the predominant histological finding but acute cellular or humoral rejection may occur concurrently, and other pathologies are sometimes apparent histologically, e.g., calcineurin inhibitor toxicity. Increasing use of organs donated after circulatory death (DCD) and from extended criteria donors[10] has corresponded with an increase in the incidence of DGF. DGF increases the risk of graft failure, patient death, and death-censored graft failure by two- to three-fold,[11,12] and is associated with a number of complications that contribute to reduced longer-term graft survival, such as a poor transplant function at 1 year, arterial hypertension, and acute rejection.[13] Overall, DGF has been associated with a 41% increased risk of graft loss at just over 3 years.[14]

Early identification, stratification, and increased understanding of DGF has significant potential to improve patient management and outcomes,[15] allowing fluid volume status optimization, timely appropriate dialysis, tailoring of therapies, and avoidance of unnecessary investigation and treatment. There is increasing excitement about the potential of clinical proteomics in identifying new biomarkers with clinical impact,[16] complementing promising markers emerging from genomic-based studies. Urinary markers currently under investigation in renal transplantation include interleukin 18 and neutrophil gelatinase lipocalin,[17] with tissue-associated markers including ICAM-1 and VCAM.[15,18] Unfortunately, in the majority of cases of DGF, urine is not produced or may be mixed with residual native renal output confounding analysis of any results, and biopsied tissue is often only available once DGF is established. Although serum neutrophil gelatinase lipocalin and interleukin 18 have not shown promise,[19] blood-borne biomarkers would be ideal being readily accessible and routinely used in hospital laboratories. However, biomarker discovery with serum or plasma is challenging with only 22 proteins comprising ~99% of the total protein mass, and the wide dynamic range of protein abundances spanning >10 orders of magnitude.[20]

In this study (Figure 1), we have compared serum proteins pre- and postoperatively from patients undergoing renal transplantation, with and without DGF, using our previously optimized immunodepletion followed by label-free single-dimensional liquid chromatography-tandem mass spectrometry analysis strategy.[21] A key candidate marker of DGF was identified as aminoacylase-1 (ACY-1). Following assay development and validation, allowing the measurement of ACY-1 in serum for the first time, the predictive use of ACY-1 for DGF as early as day 1 post transplant was confirmed. Using follow-up data from a cohort of 194 transplant patients where as expected DGF was associated with poor prognosis, serum ACY-1 day 1 post transplant was shown to further subdivide the 54 patients with DGF in terms of their long-term outcome.

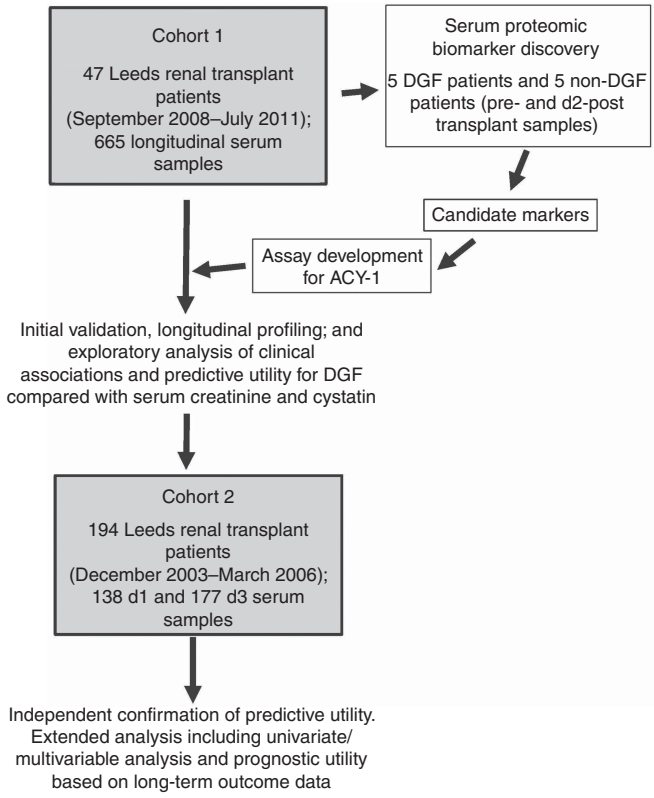

Figure 1 | Schematic showing the study design using samples undergoing renal transplantation at the St James's University Hospital in Leeds. Following initial discovery of aminoacylase-1 (ACY-1) as a novel potential serum marker for delayed graft function (DGF) using mass spectrometry, this was validated using an immunoassay for ACY-1, and the performance as a predictive marker assessed. Using serum samples from a further independent cohort of patients, these results were confirmed and additionally the association with long-term outcome in those patients with DGF was examined.

RESULTS

Patient groups

Examination of the patient groups (Figure 1, Table 1) shows similar characteristics of cohorts 1 (the discovery and initial validation group) and 2 (the larger validation group with long-term outcome data), with the exceptions of proportion of DCD transplants and the induction regimen, reflecting changing clinical practice. DGF was diagnosed in 31.9% of patients in cohort 1 and 28.4% of patients in cohort 2. Mean age and cold ischemic time (CIT) were significantly higher in the DGF groups in each cohort, as was warm ischemic time (WIT) in cohort 2. The five DGF and five non-DGF patients used for the initial proteomic discovery had no evidence of calcineurin inhibitor toxicity or acute rejection and were matched as closely as possible in terms of mean age, ethnicity mix, CIT, WIT, and mean HLA mismatches at the A, B, and DR loci, and immunosuppression regimens, differing slightly in donor type (five DCD in the DGF group compared with three DCD/two donation after brain death (DBD) in the non-DGF group).

Mass spectrometry and candidate biomarker selection

Across all 20 samples (10 patients, two time points) used for the initial mass spectrometry screen, 553 proteins with at least two peptides (at least one of which was unique) were identified and relative quantification determined (all proteins listed and quantification data shown for selected proteins in Supplementary Data—Proteins 1 and 2). On the basis of the statistical significance ($P<0.05$), 34 candidates differentiated between DGF and non-DGF groups either preoperatively, postoperatively, or by pattern of change. These included cystatin C demonstrating proof-of-principle. ACY-1 was prioritized for further investigation, being undetectable preoperatively but increasing markedly postoperatively, particularly in the DGF group (Figure 2a).