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# Perfusate Proteomes Provide Biological Insight Into Oxygenated Versus Standard Hypothermic Machine Perfusion in Kidney Transplantation

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**Objective:** To provide mechanistic insight into key biological alterations in donation after circulatory death kidneys during continuous perfusion we performed mass spectrometry profiling of perfusate samples collected during a phase 3 randomized double-blind paired clinical trial of hypothermic machine perfusion with and without oxygen (COMPARE).

**Background:** Despite the clinical benefits of novel perfusion technologies aiming to better preserve donor organs, biological processes that may be altered during perfusion have remained largely unexplored. The collection of serial perfusate samples during the COMPARE clinical trial provided a unique resource to study perfusate proteomic profiles, with the hypothesis that in-depth profiling may reveal biologically meaningful information on how donor kidneys benefit from this intervention.

**Methods:** Multiplexed liquid chromatography-tandem mass spectrometry was used to obtain a proteome profile of 210 perfusate samples. Partial least squares discriminant analysis and multivariate analysis involving clinical and perfusion parameters were used to identify associations between profiles and clinical outcomes.

**Results:** Identification and quantitation of 1716 proteins indicated that proteins released during perfusion originate from the kidney tissue and

blood, with blood-based proteins being the majority. Data show that the overall hypothermic machine perfusion duration is associated with increasing levels of a subgroup of proteins. Notably, high-density lipoprotein and complement cascade proteins are associated with 12-month outcomes, and blood-derived proteins are enriched in the perfusate of kidneys that developed acute rejection.

**Conclusions:** Perfusate profiling by mass spectrometry was informative and revealed proteomic changes that are biologically meaningful and, in part, explain the clinical observations of the COMPARE trial.

**Keywords:** COMPARE, DCD, extended criteria donors, kidney, LDH, machine perfusion, mass spectrometry, oxygenated perfusion, perfusate proteomic profiling, transplantation

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Kidney transplantation is the gold standard treatment for patients with end-stage renal disease reducing morbidity and mortality while improving quality of life. The required need for kidney transplantations continues to exceed the supply of suitable kidneys from deceased donors, especially when many organs are currently discarded as deemed untransplantable. To maximize organ utilization without compromising transplantation outcomes, machine perfusion technologies have been developed to better assess, preserve, and potentially improve donor organ quality before transplantation.<sup>1</sup> We demonstrated in a randomized controlled trial comparing static cold storage preservation of deceased donor kidneys, with continuous hypothermic machine perfusion (HMP), that the latter improved short-term kidney function and survival.<sup>2</sup> Using HMP showed superior outcomes not only in donation after brain death (DBD) but also in extended criteria donor (ECD) and donation after circulatory death (DCD) donor kidneys.<sup>3</sup>

As preclinical studies repeatedly have suggested a clear benefit of oxygenation during cold preservation,<sup>4–11</sup> we developed a novel HMP device that allowed continuous oxygenation of the perfusate during perfusion and tested this modality in a clinical trial<sup>4</sup>. The COMPARE trial was a phase 3 randomized double-blind paired trial performed by the Consortium for Organ Preservation in Europe and has demonstrated the clinical benefit of oxygenated HMP (HMPO<sub>2</sub>) compared with standard HMP of older DCD kidneys. The trial showed improved graft function of oxygenated donor kidneys at one year after transplantation when accounting for decreased graft failure. It also found fewer severe complications and a lower rate of acute graft rejection when HMPO<sub>2</sub> was used.<sup>4</sup>

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J.F.M. and S.S. contributed equally as first authors; M.K., and R. J.P. contributed equally as senior authors to this study.

Raw files from the proteomic analysis along with all metadata were deposited in PRIDE with the project accession number PXD041078. The code required to reproduce our analysis, as well as all the underlying figure data, will be made available at 10.5281/zenodo.7638684.

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Although the clinical outcome of the COMPARE trial showed clear benefit for patients, it has remained unclear which biological changes take place during HMP. In this study, we have aimed to reduce the translational gap between the pre-clinical model and clinical scenario, contributing at least, in part, by analyzing all perfusate samples collected from donor kidneys during both nonoxygenated and oxygenated HMP as we hypothesized that the perfusate contains combination of signals released from donor kidneys. As we wanted to observe preexisting, as well as more definitive alterations and mechanistic changes of injury and/or repair, we focused on the profiling of the proteomes in perfusate samples reflecting the condition of the kidneys, as well as potentially informing us about future beneficial interventions to enhance donor kidney quality during machine perfusion.

## METHODS

### Perfusate Samples

Perfusate samples were obtained as part of the Consortium for Organ Preservation in Europe (COPE) COMPARE clinical trial<sup>4</sup> (Fig. 1) according to trial protocol using the Kidney Assist Transport device (Organ Assist BV). All participants provided written consent for the collection and storage of biological samples and the use of follow-up clinical data. This study was approved by the relevant Institutional Review Board and ethics committee. Perfusate samples were collected at 3 time points: 15 minutes after the start of perfusion, immediately before the kidney left the donor hospital on the device ( $3.8 \pm 3.4$  hours), and at the end of perfusion ( $7.8 \pm 5.4$  hours). Perfusate samples were stored on ice until transfer to the processing laboratory in a central hub in the Netherlands, where they were centrifuged (1300 g for 15 minutes at room temperature), temporarily stored at  $-80^{\circ}\text{C}$ , and subsequently transferred for long-term storage at  $-80^{\circ}\text{C}$  in Oxford, UK, according to the COMPARE protocol (ISRCTN32967929). All perfusate samples containing sufficient biological material for proteomic analysis were included.

Clinical characteristics of donor and recipient populations (Supplemental Data File, Supplemental Digital Content 1, <http://links.lww.com/SLA/E779>).

### Untargeted Proteomics

Detailed methodology of proteomics analysis is described in Supplemental Data (Supplemental Digital Content 1, <http://links.lww.com/SLA/E779>). Samples were analyzed by liquid chromatography-tandem mass spectrometry using an isobaric tandem mass tag (Thermo Fisher Scientific) labeling approach to allow sample multiplexing and high-precision relative quantitation (Fig. 1) as described in detail in Supplemental Methods (Supplemental Digital Content 1, <http://links.lww.com/SLA/E779>). Samples were allocated nonrandomly across liquid chromatography-mass spectrometry runs to minimize confounding between the relevant clinical and technical variables (Supplemental Digital Content Table S1, <http://links.lww.com/SLA/E779>).

### Statistical Analyses

Detailed description of the statistical analysis can be found in Supplemental Files (Supplemental Digital Content 1, <http://links.lww.com/SLA/E779>). Data analyses were performed using R. Unless otherwise specified, data are presented as the median  $\pm$  interquartile range.  $P < 0.05$ , or where appropriate, a false

discovery rate of 5% was controlled using the method of Benjamini and Hochberg.<sup>12</sup>

## RESULTS

Proteomic profiles were obtained for 210 perfusate samples that were longitudinally obtained across oxygenated HMP ( $n = 101$ ) and nonoxygenated HMP ( $n = 109$ ) from the perfusion of 67 paired DCD kidneys ( $n = 137$  total kidneys) (Fig. 1). The median perfusion duration was 7.40 hours (4.8–9.9) for HMP and 6.85 hours (4.5–9.1) for HMPO<sub>2</sub>. The clinical characteristics of the donors are shown in Table 1.

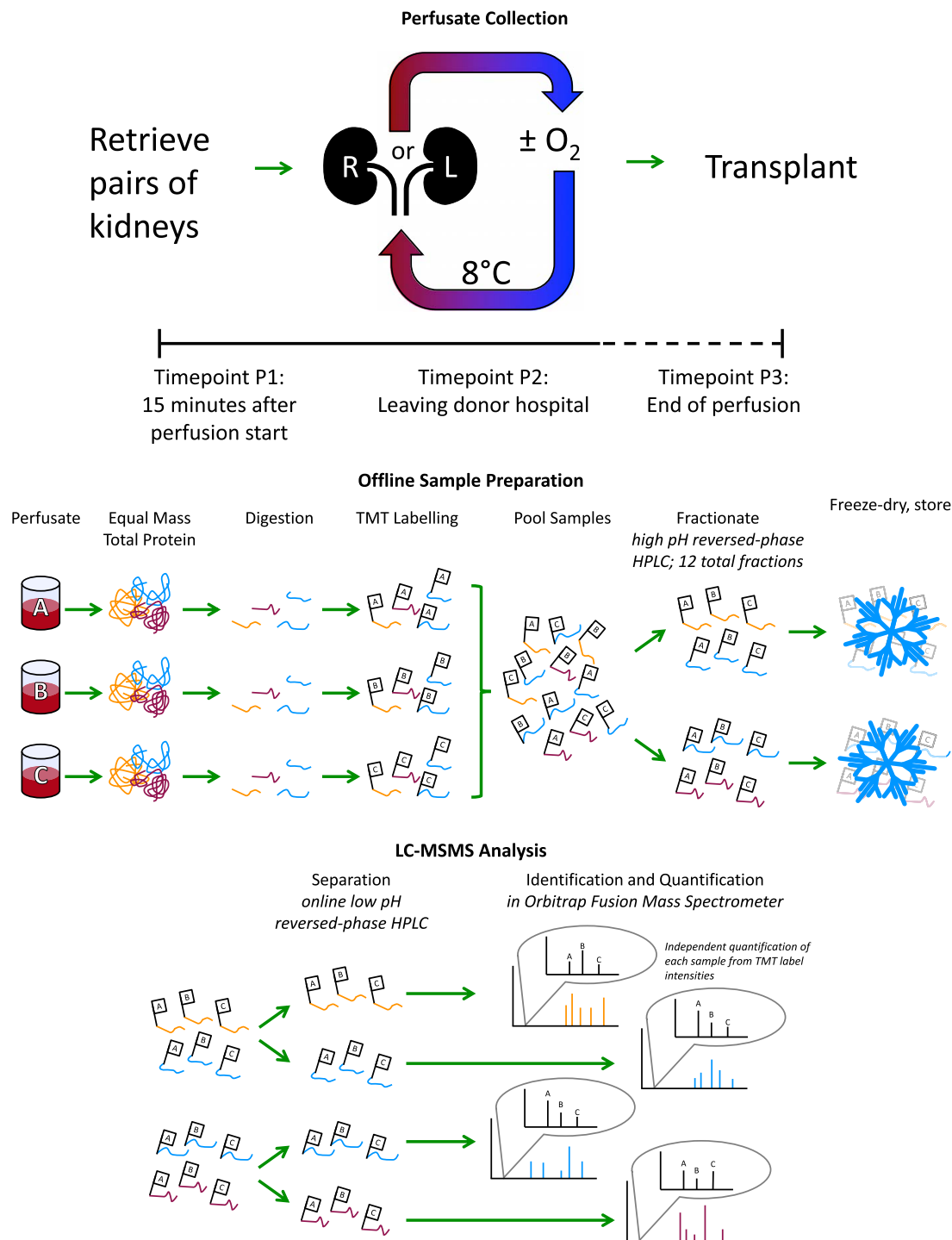
### Perfusate Contains Proteins From Both Kidney Tissue and Blood

We identified and quantified 1716 proteins in the perfusate samples. Next, we searched for the identified proteins across the atlas data sets and classified them according to their origin. While many of these proteins originated from the kidney tissue, a large number of proteins could also originate from blood components released in the vascular compartment of the donor kidney. Therefore, plasma and red blood cell proteomes were used as a proxy (Supplemental Material, <http://links.lww.com/SLA/E815>). The most abundant proteins identified were complement C3, apolipoprotein A1, and fibrinogen alpha chain. Taking abundance into account, blood proteins were quantitatively dominant over those that originated unambiguously from the kidneys (Supplemental Material, <http://links.lww.com/SLA/E815>).

A Reactome pathway enrichment analysis of proteins overrepresented in the perfusate highlighted the enrichment of a number of biological functions (Supplemental Material, <http://links.lww.com/SLA/E815>), including complement cascade, platelet aggregation, and detoxification of reactive oxygen species (Supplemental Material, <http://links.lww.com/SLA/E815>).

### Perfusion Duration and Effect on Protein Abundance

The perfusion duration had a major influence on protein abundance and identified a relationship between high-density lipoprotein (HDL) during perfusion and recipient outcomes after 12 months. To quantitatively understand the abundance of proteins within the perfusate, we used an unsupervised approach to identify relevant relationships between clinical variables and the major sources of variation in the abundance of proteins in the perfusate (Supplemental Digital Content Fig. S1A–C, <http://links.lww.com/SLA/E779>; Supplemental Digital Content Table S2, <http://links.lww.com/SLA/E779>). Significant correlations were observed with perfusion duration (Pearson  $r = 0.34$ ,  $P = 0.0002$ , Supplemental Material, <http://links.lww.com/SLA/E816>) and the total protein concentration of samples as determined by the Bradford assay. Oxygenation of the perfusate by itself, during organ preservation, appeared not important in determining protein abundance in the perfusate (Supplemental Digital Content Fig. S1A–C, <http://links.lww.com/SLA/E779>). We did not observe a relationship between the duration of perfusion and the 12-month estimated glomerular filtration rate (eGFR; Supplemental Digital Content Fig. S2A, <http://links.lww.com/SLA/E779>). At the level of individual proteins, 137 proteins correlated significantly with perfusion duration. Notably, this includes both those that increase (eg, Vimentin, Spearman  $\rho = 0.69$ ,  $P < 2e-25$ ), as well as those that decrease (eg, cystatin C, Spearman  $\rho = -0.41$ ,  $P < 2e-7$ ) with the duration of perfusion (Supplemental Material, <http://links.lww.com/SLA/E816>). Multivariate methods were used to identify proteins that changed in a coordinated manner across perfusion



**FIGURE 1.** Experimental workflow of perfusate proteomic analysis. In the COMPARE trial, pairs of kidneys donated after circulatory death were randomized into HMPO<sub>2</sub> or HMP. Perfusate samples were collected during the perfusion. Perfusate samples were collected 15 minutes after the start of perfusion, just before the departure of the kidney from the retrieving hospital, and at the end of perfusion before transplantation. Equal amounts of protein that had previously precipitated from perfusate samples (n = 210) were digested to tryptic peptides, isolated by HPLC, and subsequently, each sample was individually labeled by isobaric mass tagging of every peptide, using TMT. LC-MS/MS analysis of TMT-labeled tryptic peptides was performed using LC-MS<sup>n</sup>. Each sample has an individual tag that allows the analysis of individual samples and comparison across samples. HPLC indicates high-performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; TMT, tandem mass tag.

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**TABLE 1.** Clinical Metadata That Associates to the Perfusate Samples Analyzed, Grouped According to the Standard and Oxygenated Perfusion

Characteristics	Overall (N = 210)*	Treatment received		
		HMP -02 (N = 107)*	HMP +02 (N = 101)*	Unknown (N = 2)*
Donor sex				
Female	86 (41)	45 (42)	41 (41)	0
Male	124 (59)	62 (58)	60 (59)	2 (100)
Donor BMI (kg/m <sup>2</sup> )	25.1 (22.8, 27.7)	24.8 (22.6, 27.5)	25.1 (22.9, 27.7)	38.7 (38.7, 38.7)
Donor age (yr)	59 (54, 62)	59 (54, 62)	59 (56, 62)	72 (72, 72)
Donor cause of death				
Cerebrovascular accident	79 (38)	43 (40)	34 (34)	2 (100)
Hypoxia	79 (38)	38 (36)	41 (41)	0
Other	20 (9.5)	9 (8.4)	11 (11)	0
Trauma	32 (15)	17 (16)	15 (15)	0
Donor last creatinine (μmol/L)	60 (41, 71)	59 (41, 70)	60 (41, 69)	105 (105, 105)
Unknown	2	1	1	0
Total warm ischemia (min)	27 (17, 35)	27 (22, 35)	26 (16, 35)	28 (28, 28)
Recipient eGFR at 12 mo (mL/min/1.73 m <sup>2</sup> )	41 (29, 49)	40 (25, 52)	41 (30, 46)	34 (31, 36)
Biopsy confirmed acute rejection				
False	164 (78)	84 (79)	80 (79)	0
True	46 (22)	23 (21)	21 (21)	2 (100)

\*n (%); median (interquartile range).  
BMI indicates body mass index.

duration and in relation to 12-month eGFR (Supplemental Digital Content Fig. S2B, <http://links.lww.com/SLA/E779>), and an enrichment test was then used to identify functional gene sets (Supplemental Material, <http://links.lww.com/SLA/E816>). We then examined the network of known protein-protein interactions in the enriched protein networks. This analysis revealed a pertinent role for HDL, as well as the complement cascade (Supplemental Material, <http://links.lww.com/SLA/E816>).

### Perfusate Proteomes Provide Mechanistic Insights Into Acute Rejection of Kidneys

To explore the biological process(es) associated with acute rejection, we tested for differences in the composition of the perfusate proteome at the end of perfusion between kidneys that developed acute rejection within the first year and those that did not use a partial least squares discriminant analysis approach. The first latent variable separated kidneys that developed acute rejection from those that did not (Fig. 2A). Performing a gene enrichment analysis, we found that pathways related to “regulation of response to wound healing,” “metabolic processes that contribute to adenosine triphosphate generation,” and “nucleotide phosphorylation,” were all associated with acute rejection (Fig. 2B). Calculating the enrichment of proteins that might originate from the kidney tissue (Fisher exact test,  $P = 0.90$ , 1-tailed) or blood compartment (Fisher exact test,  $P = 0.025$ , 1-tailed) suggested that this signal originated primarily from the vascular compartment rather than the kidney tissue. Finally, we intersected our proteomics data with atlas single-cell transcriptomics data sets from both kidney and peripheral blood mononuclear cells (Supplemental Digital Content Fig. S3A, B, <http://links.lww.com/SLA/E779>). We tested for enrichment of cell types classified by their transcriptomic profile and found a significant enrichment of proteins originating from platelets (Fig. 2C).

## DISCUSSION

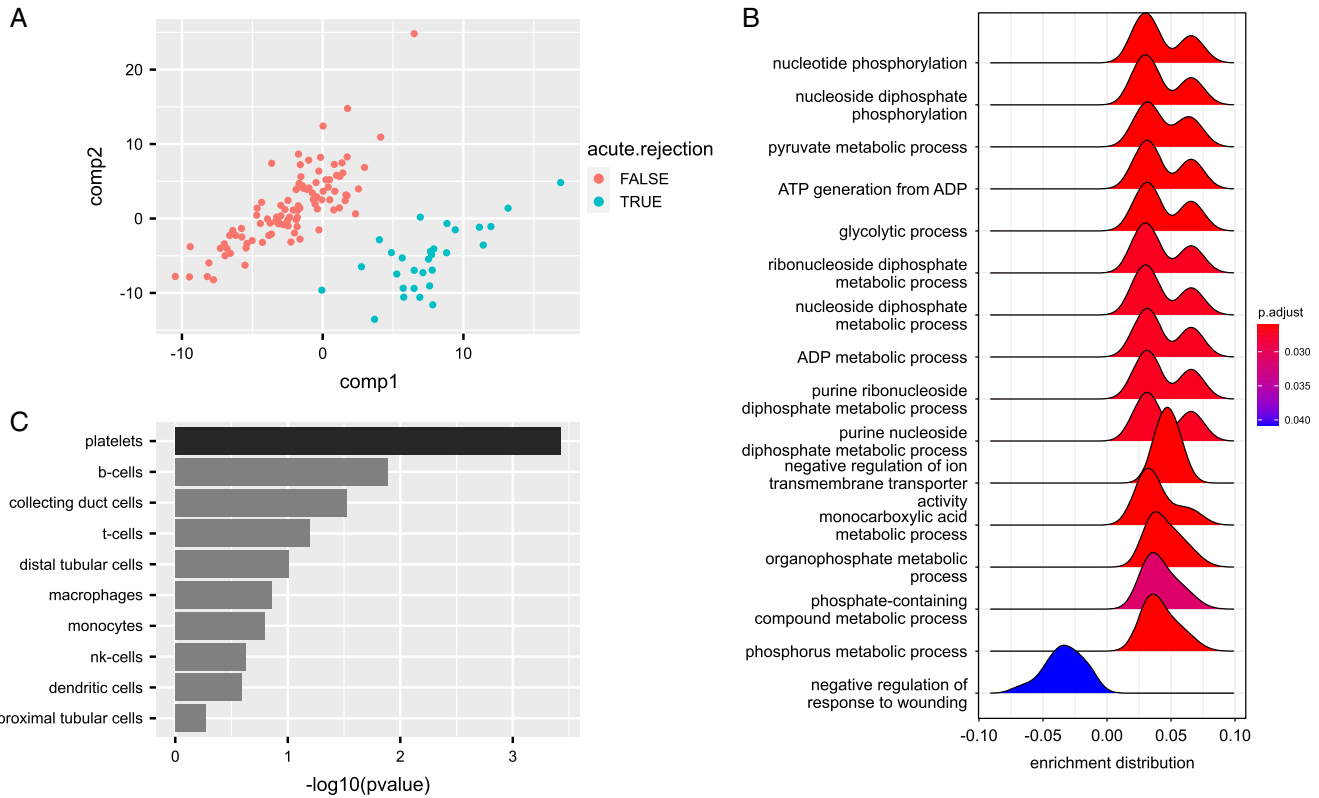
In this study, we have focused on the alterations and mechanisms that take place in deceased donor kidneys from DCD donors during standard nonoxygenated and oxygenated HMP. We have analyzed serial perfusate samples of donor kidneys obtained between organ retrieval and transplantation.

We have performed an unbiased characterization of the proteome profiles in clinical perfusate samples from both nonoxygenated and oxygenated HMP. We quantified 1716 proteins across 210 samples, representing both the largest and most comprehensive measurements of proteins in the HMP perfusate to date.

We identified many proteins originating from kidney tissue and blood in the vascular compartment of the donor kidney with high overlap between enriched pathways and blood-centric mechanisms. This was despite the fact that all kidneys had been flushed according to clinical standards before the initiation of perfusion. This observation is consistent with previous work that has performed proteomics on perfusate during HMP, that is, the enrichment of complement and coagulation cascades in samples both early and late in perfusion as reported by van Leeuwen et al.<sup>13</sup> Coskun et al<sup>14</sup> similarly observed blood proteins in their proteomic data set but suggested that they are artefactual. In contrast, the data presented here indicate that the blood in the vascular compartment contributes to pathophysiological mechanisms and should be considered as part of the biologically relevant signals. In our data set, for most proteins, it was difficult to unambiguously determine their origin between kidney tissue and blood, which necessitates careful inference about the tissue or cell types of origin.

Based on our data and those of others, we suggest that these blood proteins may originate from the occult nonperfused vasculature of the kidney. Histologically detectable fibrin and/or fibrinogen have been reported in biopsies taken after static cold storage.<sup>15,16</sup> We are aware of reports that cold storage of kidneys may also result in the accumulation of fibrinogen within the tubular epithelium,<sup>15</sup> but note that the two mechanisms are not mutually exclusive.

Nonperfused vasculature results in regions of the tissue undergoing warm ischemia on reperfusion. This may be of particular importance in the kidney in comparison to other organs: the kidney has a high metabolic rate<sup>17</sup> but does not regulate its own blood flow to match metabolic requirements.<sup>18</sup> Furthermore, certain regions also operate at low oxygen tensions to enable the concentration of urine.<sup>19</sup> Therefore, we hypothesized that pathophysiological signals related to organ damage



**FIGURE 2.** Machine learning identifies proteins associated with the incidence of acute rejection posttransplantation. **A**, PLSDA of perfusate proteomes separated kidneys that developed acute rejection posttransplantation from those that did not. **B**, Gene set enrichment analysis of proteins associated with acute rejection identified upregulation of biological processes related to cellular respiration and oxidative phosphorylation, as well as downregulation of tissue injury response and wound healing. **C**, Intersection with single-cell RNA-sequencing data from both kidney tissue and peripheral blood mononucleocytes shows a significant enrichment of platelet-derived proteins, but with no other cell type reaching significance. ADP indicates adenosine diphosphate; ATP, adenosine triphosphate; NK, natural killer; PLSDA, partial least squares discriminant analysis.

and transplantation outcomes may originate from both the kidney tissue and blood.

Our unbiased analysis of perfusate samples detected proteomic changes longitudinally over the duration of perfusion. Notably, these changes show strong associations with transplantation outcomes. Specifically, we identified that the HDL complex decreases in abundance over perfusion duration, and these changes over time related to a higher recipient eGFR at 12 months posttransplantation. Previous work has highlighted the release of lipids into the perfusate of the kidneys during HMP.<sup>20</sup> Further studies are required to confirm the extent to which HDL complexes remain intact during perfusion.

We also observed that proteins from the complement cascade decreased during more extended durations of perfusion and in relation to a higher 12-month eGFR. Complement has already been shown to be activated within the plasma of circulatory death donors,<sup>21</sup> but equally preclinical research indicates that local activation within the graft can be detrimental.<sup>22</sup> These findings suggest that a better understanding of the proximal consequences of changes during HMP and the relationship to systemic activation in the donor might allow better experimental designs in future HMP trials.

At the end of perfusion, in kidneys that developed acute rejection, we found an increased abundance of a subset of proteins originating specifically from platelets. We speculate that

this may be at least partially responsible for (micro) vascular obstructions. HMP demonstrably causes at least partial clearance of obstructed vasculature, as per our own findings regarding the detection of blood proteins within the perfusate. This speculation is consistent with our own practical experiences in the laboratory, where a period of HMP before subsequent warm reperfusion improves the perfusion characteristics of porcine kidneys.<sup>23</sup> Partial clearance of obstructed vessels is also supported by preclinical studies in human kidneys, where marked heterogeneity in initial perfusion was observed during reperfusion (either in the recipient or during normothermic machine perfusion), even after HMP.<sup>24–26</sup> We note that there is also mechanistic evidence for nonenzymatic fibrinolysis from in vitro assays assessing thrombolysis, where small amounts of lytic activity are observed, even in the absence of fibrinolytic enzymes.<sup>27–29</sup> After preservation in cold conditions, there is an emerging consensus that these obstructions are aggravated on warm reperfusion, either by normothermic machine perfusion<sup>15,30</sup> or by reperfusion at the time of transplantation in the recipient.<sup>31</sup>

Among our mechanistic findings, we observed no difference between the perfusate proteomes of oxygenated and non-oxygenated HMP. However, in preclinical models, oxygenated HMP has been shown to result in higher adenosine triphosphate levels<sup>10</sup> and an increased rate of aerobic metabolism.<sup>8</sup> It is logical

to anticipate that such metabolic differences are also reflected in the perfusate proteome, but our results do not support this conclusion. A lack of difference suggests that the energy required for biological processes during HMP is limited by the temperature irrespective of oxygenation, that there is enough alternative energy supplied through nonaerobic processes such as glycolysis, or that in our study, the metabolic-associated signal was potentially obscured by biological variance. Whether perfusate metabolomic profiling will be more informative in detecting metabolic changes, is to be determined.

We acknowledge the limitations of this study. We report an association between the presence of complement proteins and recipient eGFR at 12 months, indicative of immune-related processes. However, this does not provide proof of actual complement activation, which would require either immunostaining on pretransplantation biopsies, or specific immunoassays for complement activation fragments. Proteomic profiling typically has poor coverage of small, low-abundance proteins and peptides, which include many likely relevant species, including inflammatory regulators, such as cytokines. While reporting mechanistic associations between the perfusate proteome and clinical outcomes, we must stress that, despite using an experimental design that minimized the effects of confounding experimental effects, practicality limited the cohort size and, therefore, its power to identify individual biomarkers. Finally, parallel proteomic profiling of perfusate and tissue biopsies of DBD, DCD kidneys to include HMP, and a cohort of cold stored kidneys will answer any additional questions regarding associations of kidney tissue molecular mechanisms and released proteins during perfusion, although such an analysis would require considerable analytical resources to perform.

## CONCLUSIONS

In this study, we have identified associations between the perfusate proteome and clinical outcomes, also highlighting the underappreciated role of proteins originating from the blood compartment versus those from the kidney tissue. These associations suggest that the dismissal of blood proteins as artifacts risks ignoring pathologically relevant signals. In contrast, the large overlap that we observed between kidney and blood proteins necessitates further experimental investigations to trace the origin of identified proteins and describe their biological significance. Future work on this will enrich our understanding of key determinants of pretransplantation kidney assessment. Our findings reinforce previous suggestions that transplantation outcomes are by no means predetermined at retrieval and highlight the importance of further studies on new interventions during kidney preservation to resuscitate organs.

## ACKNOWLEDGMENTS

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## DISCUSSANT

### Wolf O. Bechstein (Frankfurt am Main, Germany)

Thank you for your interesting presentation, and to the Society for the privilege of the floor. I congratulate you and your coworkers on this tremendous translational work. During the latter half of the 20th century, the cold storage of kidneys after organ retrieval, during transport, and before transplantation has been the gold standard. More than a decade ago, randomized controlled trials (RCTs) demonstrated that continuous machine perfusion may improve the function of donor kidneys retrieved from donors after circulatory death (DCD).

The paper presented here is a translational subproject of an RCT, which compares oxygenated versus standard cold machine perfusion in kidney transplantation. The results of the main trial, the COMPARE study, were published more than 2 years ago. While mean eGFR was not significantly different between the groups, the incidence of severe complications and graft failure was significantly lower in the group of kidneys that underwent continuous oxygenated machine perfusion.

The strength of the paper presented today is that, as a translational study, the authors were able to match extensive laboratory analyses with well-documented clinical data from a large international, multicenter RCT. One of the weaknesses of the study is that it was limited to older donors whose kidneys were retrieved after circulatory death (DCD); in other words, the results may not be easily extrapolated to brain-dead donors (DBD) or younger kidneys.

Some questions remain: First, in the COMPARE study, which had graft function as a primary endpoint, as measured by eGFR, 23 kidney pairs had been excluded from primary analysis because of kidney failure or patient death. Were the perfusates of these kidney pairs analyzed in the study presented today or were they excluded?

Second, could the analysis of the perfusate proteomes help to identify kidneys that never function after transplantation, thus sparing these often multimorbid recipients, the risk of an – in the end – futile procedure?

Finally, having identified perfusate proteomes predicting an increased risk of rejection, could you envisage tailored immunosuppression for those grafts and recipients with the respective proteosome markers?

### Response from Maria Kaiser (Oxford, United Kingdom)

Thank you for your insightful comments. I would like to thank you for highlighting the strength of our study, which is that perfusate proteomes can convey meaningful biological data. Let me take a closer look at the point you raised, regarding whether the findings of this DCD study can be extrapolated to DBD or younger donors. I agree that our observations on biological mechanisms and pathways may not extrapolate to DBD or younger donors; our group has demonstrated the differences in injury mode between DBD and DCD, as have others. Also, we think that younger donors may have higher metabolic resilience, and for this reason, they might be less affected by the challenges of ischemia or other injuries during donor management. However, as you mentioned, the study does link to the COMPARE trial, which showed a significant clinical benefit of oxygenated HMP for one year glomerular filtration rate and graft survival in older DCD donors, which is the fastest-growing source of deceased organs in transplantation. Undoubtedly, the ideal situation would have been to compare both older and younger DBD and DCD. Even if we wanted to repeat this analysis, the perfusate cohorts are not currently available from these groups; however, we agree that this is an important follow-up analysis.

I will now move on to answer your specific questions. First, I can confirm that there was no exclusion of kidney pairs from the selection of samples. There were only two criteria for sample exclusion: (1) if perfusate samples had not been collected due to logistical challenges and (2) if the protein levels in the perfusate were below the detection level.

Second, our study indicates that perfusate proteomes provide biologically meaningful information related to perfused kidneys. In addition, our current research on pretransplant biopsies that were obtained from the UK-wide “Quality in Organ Donation (QUOD)” biobank reveal associations between tissue protein profiles and primary nonfunction, and short and long-term transplantation outcomes. To this end, when we combine pretransplant proteomic data and data from the perfusate proteomes, we think that we could improve the prediction of posttransplant function.

Finally, understanding how the identified proteomes associate with kidney immunophenotypes could lead to the development of immunomodulation strategies during perfusion and personalized immunosuppression, with the potential to extend graft longevity and improve transplantation outcomes.