

# The Extracorporeal Proteome—The Significance of Selective Protein Removal During Dialysis Therapy

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Dialysis as renal replacement therapy aims excess water and waste solutes from the uremic patient while retaining proteins in the plasma. Irrespective of the dialysis modality, hemodialysis (HD) or peritoneal dialysis (PD), the amount and composition of proteins that are removed are important determinants of the biocompatibility of the therapy. Although hemodialysis membranes would ideally be biologically inert filtration tubes, they are known to adsorb proteins. The part of the plasma proteome that is thereby removed during every dialysis session may be regarded as the extracorporeal proteome, which has to be kept in balance with the plasma proteome, regarding the individual proteins' biological roles and activation states. In a recent study, Ronci et al. (*Proteomics Clin. Appl.* 2018, e1700140) comprehensively compare two hemodialyzer membrane materials by shotgun LC–MS proteomic analysis of adsorbed proteins and ultrafiltrates from four HD patients. While pathway analysis is an attractive tool to compare different proteomes on an abstract level, some challenges remain regarding the adaptation for such tools for special proteomes and the interpretation of relative changes compared absolute changes regarding their biological importance in dialysis techniques. In summary, selective protein removal may represent a yet unexploited therapeutic opportunity if the “right” proteins are removed from the blood.

function of an essential organ can be replaced by an allegedly simple technical procedure. The goal of dialysis is to remove excess water and waste solutes from the uremic patient while retaining essential solutes, such as nutrients and proteins, in the organism. Protein loss is regarded as one of the major problems during dialysis therapy, as the already weakened patient needs to replace lost proteins through metabolism which in turn generates uremic toxins that need to be removed through more dialysis.

In hemodialysis (HD), proteins may get lost “through” the membrane—a problem that can be tackled by selection of the membrane pore size distribution—or they might be removed from the blood by adhesion to the large surface of the dialyzer membrane. It has long been suggested that the hemodialyzer membrane is not biologically inert and that the specific composition of adsorbed and filtered proteins will likely cause differential biological effects. Although the repertoire of proteins that are adsorbed may be influenced by chemical properties of the

Renal replacement therapy by dialysis techniques represents a life-saving medical procedure and a rare case where major

membrane surface as well as by flow conditions (high flux/low flux), it is not yet clear if this process is selective. Since the advent of proteomic techniques, a few research groups have invested considerable effort into investigating the characteristics of protein adsorption to dialyzer membranes.<sup>[1–5]</sup> Technical advances regarding sensitivity of mass spectrometric methods now allow unprecedented characterization of the “extracorporeal proteome” that is removed in the dialysate or adsorbed to the dialyzer but the systemic effects of protein removal, adsorption, and activation on the dialyzer surface are still incompletely understood.<sup>[6]</sup>

In their recent study, Ronci et al. comprehensively compare two hemodialyzer membrane materials by shotgun LC–MS proteomic analysis of adsorbed proteins and ultrafiltrates from four HD patients.<sup>[7]</sup> The used label-free quantification methodology is state of the art and the tools used for interpretation (GO, STRING, and IPA) are widely used and accepted. The question that might be asked is of course if these GO and pathway enrichment tools really work in this type of sample material as they are optimized for cell-culture and tissue samples. Pathway enrichment is based on a statistical overrepresentation test, taking into account the number of pathway members and the observed number of differentially abundant candidate proteins. The

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significance is usually calculated from the expected number in each pathway resulting from the background list (with standard settings the known human proteome) and the total number of candidates that entered the analysis.

One of the challenges of pathway analysis from proteomics experiments is that the background list is usually unknown. This is in contrast to transcriptomic techniques where, at least in theory, each probe on a microarray and each mRNA in an RNAseq experiment has the same probability of being detected and the background is therefore the whole genome or transcriptome.<sup>[8]</sup> In current state-of-the-art proteomics experiments 5000 to 10 000 proteins can be identified from cell lysates. The plasma proteome is infamously resistant to full coverage and thus the extracorporeal proteome, which features challenges of the plasma proteome, certainly requires adaptation of these tools. This in turn would allow exploration of unknown territory and avoid the common conception that more or less “the same” proteins and thus pathways are seen in all plasma proteomics experiments.

In this study, Ronci et al. used 1D LC–MS without fractionation, a straightforward way of comparing two membranes. However, low abundance proteins, such as cytokines and transcription factors are known to be tremendously important, and their removal might have biological consequences far beyond the removal of bulk proteins. Only recently, in a proteomic study, a combination of label-free and isobaric labeling strategies was applied to effluent samples from peritoneal dialysis (PD) patients after depletion of highly abundant plasma proteins and enrichment of low-abundance proteins.<sup>[9]</sup> In PD (an alternative to HD) the peritoneal wall has the role of the dialysis membrane and biocompatibility is more determined by the dialysis solution that is instilled into the peritoneum (in contrast to extracorporeal HD where the body fluid only comes in contact with the artificial hemodialyzer membrane). The study in PD effluent identified roughly 2500 unique proteins, more than 10 times the number of previously reported proteins. It also showed that based on these low abundant markers characteristics of the therapy and the underlying disease can be monitored. In PD, the composition of the dialysate and its contained low abundance proteins might be even more important as it is “incubated” in the peritoneum for up to a few hours before it is removed from the body. Similarly, in HD, beyond the highly abundant proteins that “stick” to the hemodialyzer membrane, low abundant biomarkers may be scavenged that could be converted into clinically relevant information on the status of the disease and the therapy.

Another important point is the biological relevance of relative changes of removed or adsorbed proteins. In case of a new dialyzer membrane that removes only a third of the proteins compared to the competitor product, as showcased by Ronci et al., an individual protein would have to be threefold increased in relative abundance in order to reach an equal absolute amount being

removed. In shotgun LC–MS proteomic analyses equal amounts of protein of each sample are loaded for accurate quantification of relative changes. The question remains if these relative changes are more relevant, or in the end the absolute amount of any given protein is the ultimate determinant of biocompatibility. Regarding the study by Ronci et al., the combination of decreased total amount and shift in relative abundance might even strengthen the authors’ case for better biocompatibility of the ATA material.

In both cases, HD and PD, the unwanted effect of protein loss is a biological factor that has long been known but only recently came into focus of proteomic investigation. In summary, the discrepancy between relative and absolute quantification may be more relevant in dialysis than in other proteomic studies, because it might not only be important that less total protein is removed from the patient, but that these proteins, that comprise the extracorporeal proteome, are in balance with the ones remaining in the plasma proteome, regarding their biological roles and activation states. This may very well be a yet unexploited therapeutic opportunity, as selective removal of proteins could be exploited for therapeutic benefits (similar to apheresis techniques) but could also represent a so far neglected risk, when the “wrong” proteins are being preferentially removed from the blood.

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