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Proteome-Wide Changes in Blood Biomarkers During Hemodialysis

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INTRODUCTION

Patients receiving hemodialysis experience significant treatment-related symptoms and are at high risk for cardiovascular disease and death. The goal of hemodialysis is to remove water-soluble toxins of low- and middle-molecular weight, restore the balance of electrolytes and acid-base status, and remove excess water. However, hemodialysis itself can induce organ hypoperfusion, inflammation, and fibrosis. Intradialytic hypotension is common occurring during 3 in 100 hemodialysis treatments and affecting all organs.[1]

Hemodialysis can reduce the blood concentration of a protein through diffusion down its concentration gradient between the blood and dialysate, convection where the protein is pulled by the solution in which it is dissolved (solvent drag), or by adsorption to the hemodialysis filter (Figure 1). Conversely, ultrafiltration increases the concentration of proteins due to water removal. Proteins can also increase in the blood due to an increase in production or shift from the intracellular to intravascular space, or decrease due to a movement into cells. Protein characteristics, including size and molecular weight, charge and protein-binding, volume of distribution, and baseline concentration can all impact their change during hemodialysis. Furthermore, covariates such as age, genetically determined sex, gender, ancestry, weight, comorbidities, and residual kidney function explain some variation in concentration between individuals, whereas cointerventions, treatment time, dialyzer characteristics, vascular access type, ultrafiltration volume, the quantity of blood processed on dialysis, and occurrence of intradialytic events such as organ hypoperfusion or ischemia lead to differences during hemodialysis (Figure 1).

Technological advancements have exponentially expanded the number of proteins that can be simultaneously measured in body fluids. Proteome-wide measurement could provide quantitative assessments of the effects of hemodialysis. Biomarkers can quantitatively assess pathological processes, improve diagnostic accuracy and risk stratification, and evaluate response to therapy. In contrast, patients are often surprised by the lack of measurable targets driving dialysis dose decisions, including treatment frequency and duration.

Patients receiving hemodialysis may never be in metabolic equilibrium, and the timing of biomarker measurement relative to hemodialysis treatment can have significant ramifications. Before proteome-wide assessment can be incorporated into research, the first step is to understand proteomic changes observable during hemodialysis.

METHODS

See supplemental methods for details. Briefly, 44 patients were randomly selected from the Hemodialysis Outcomes and SympToms assessment cohort,[2] stratified to include 2/3rds with intradialytic hypotension episodes, for proteomic assessment of 1163 proteins between 5 and 500 kilodaltons in size using the Olink platform (www.olink.com).

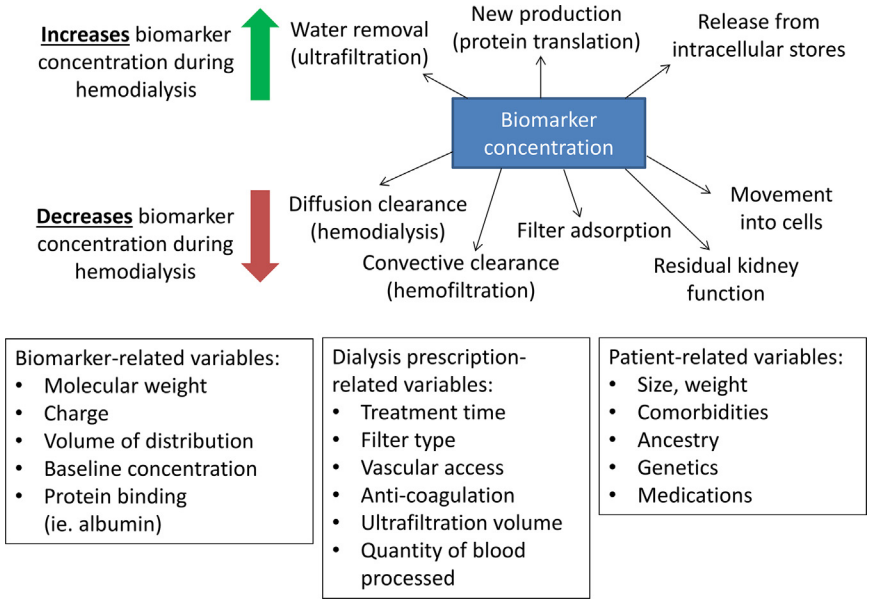

Figure 1. Factors impacting biomarker concentration during a hemodialysis session.

RESULTS

The average age of participants was 67 years old, 57% were male, with an average of 3 years on dialysis (Supplementary Table S1). Across the 42 participants with predialysis and postdialysis measurements, 189 proteins (16%) decreased and 54 (5%) increased in concentration ($P < 4.3 \times 10^{-5}$, Supplementary Figure S1 and Supplementary Table S2). We observed a halving of 2 small molecule positive controls: cystatin C (mean change −1.11 log2 normalized protein expression [NPX] value, $P = 3.2 \times 10^{-22}$) and trefoil factor 3 (−0.75 log2 NPX value, $P = 5.0 \times 10^{-20}$).[3] Growth hormone (−1.79 log2 NPX value, $P = 3.8 \times 10^{-9}$) and C-type natriuretic peptide (−1.21 log2 NPX value, $P = 7.0 \times 10^{-22}$) were the 2 proteins with the largest decrease in concentration. The protein with the largest increase in concentration was brain-enriched hyaluronan-binding protein (+0.54 log2 NPX value, $P = 3.1 \times 10^{-14}$). Erythropoietin showed interindividual differences; most individuals had no change but 5 had a doubling in concentration (Figure 2). Unfortunately, data regarding who received exogenous erythropoietin with each specific treatment was unavailable. As previously evaluated in the Hemodialysis Outcomes and SympToms cohort using the Abbott Architect assay, we observed no change in high sensitivity troponin I (+0.01 log2 NPX value, $P = 0.94$) and a decrease in galactin-3 levels (−0.11 log2 NPX value, $P = 0.001$).

Proteins were more likely to decrease in concentration if they were smaller in size (r = 0.37, 95% confidence interval: 0.31–0.43, $P = 2.2 \times 10^{-16}$), positively charged (r = −0.26, 95% confidence interval: −0.32 to −0.19, $P = 6.4 \times 10^{-14}$), and had a greater baseline concentration (r = −0.21 95% confidence interval: −0.27 to −0.14, $P = 3.0 \times 10^{-9}$, Supplementary Figure S2). In univariate regression, no single protein surpassed the Bonferroni-adjusted association threshold with intradialytic hypotension. An increase in thrombomodulin, which decreased during dialysis on average (−0.04 log2 NPX value, $P = 0.01$), was the strongest association with intradialytic hypotension ($\beta_{\text{Systolic BP drop}} = 0.021$, $P = 0.00011$; $\beta_{\text{occurrence of SBP < 70 mm Hg}} = 0.11$, $P = 0.003$). There was an excess of cardiovascular biomarkers among the top 20 nominally associated with intradialytic hypotension (13 of 90 proteins on the Olink cardiovascular disease panel in the top 20, $P < 0.05$, $P_{\text{binomial}} = 2.8 \times 10^{-8}$, Supplementary Figure S3) and all increased in concentration with intradialytic hypotension.

DISCUSSION

We evaluated the concentration of 1163 blood proteins before and after hemodialysis using an antibody-based proteomics platform with Bonferroni-significant changes observed in 21% of proteins. Despite most proteins being larger than 40 kilodaltons in size and not expected to pass through the dialysis filter, protein characteristics including smaller size, positive charge, and higher baseline concentration were associated with larger drops in concentration during hemodialysis. Perhaps more interesting than proteins that drop in concentration are those that increase because they must come from

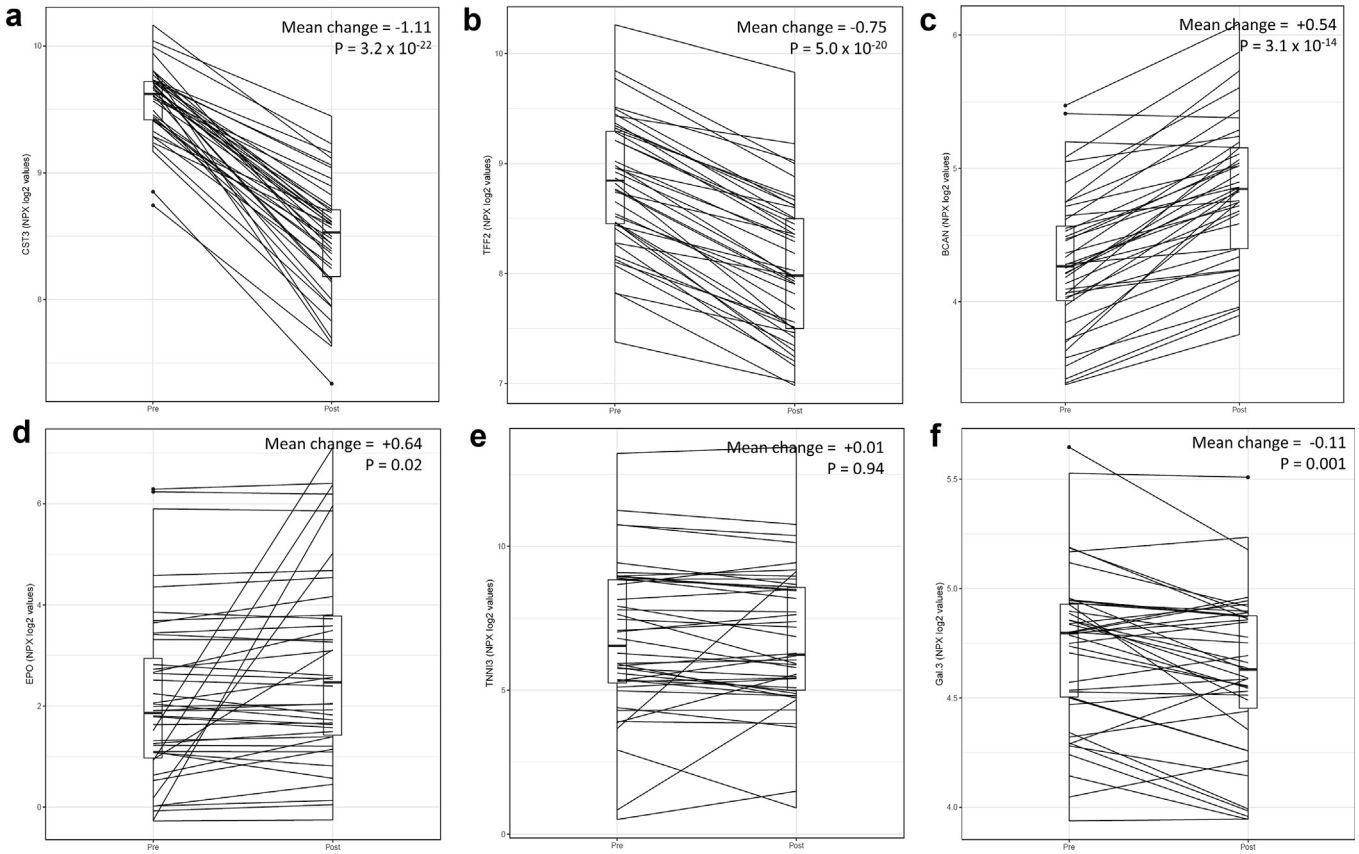

Figure 2. Changes in the concentration of selected biomarkers during a single hemodialysis session measured in a high-throughput assay. Paired pre- and post-dialysis concentration of (a) Cystatin C, (b) Trefoil factor 3, (c) Brain-enriched hyaluronan-binding protein, (d) Erythropoietin, (e) Troponin I, and (f) Galectin-3.

an exogenous or intracellular source. We did not find an individual protein associated with intradialytic hypotension beyond a Bonferroni-corrected multiple testing threshold; however, our sample size was limited in this exploratory pilot study. There was significant enrichment for cardiovascular biomarkers among those nominally associated with intradialytic hypotension, and all of them increased in concentration.

Whether intradialytic hypotension is a cause or effect of cardiovascular disease, recent evidence underscores its importance as a negative prognostic event portending future adverse outcomes.[1] Previous proteomic studies of dialysis patients utilized mass spectrometry,[4,5] and multi-omic investigations such as proteome-wide association studies and proteome-wide Mendelian randomization using antibody-based or aptomer-based protein quantification for studying kidney disease are growing in popularity.[6] How to adapt these methods to patients treated with hemodialysis is an open question. Evaluating the impact of 436 Olink-measured proteins on COVID-19 outcomes in 97 patients on hemodialysis, samples were drawn prior to treatment and 48 to 72 hours from the preceding treatment.[7] Similarly, 92 Olink cardiovascular biomarkers were measured in 369 patients on hemodialysis, with samples collected immediately before commencement of dialysis.[8] We show that proteome-wide measurement is sensitive to identify changes during hemodialysis and before-dialysis and after-dialysis measurement could be helpful in unravelling mechanisms behind intradialytic symptomatology.

Patients often ask why 12 hours of hemodialysis per week is required. Hemodialysis can leave patients feeling "washed out" or symptomatic. Encouraging adherence to treatment time can be difficult. Shifting the assessment of dialysis adequacy from a single small molecule, exemplified by Kt/V_{urea} or urea reduction ratio, to a comprehensive assessment of adequacy that improves quality of life and overall survival is needed.[9] Quantitative assessment of numerous biomarkers, in addition to traditional small molecule kinetic modeling, clinical measures, symptoms, and goals of care could be combined into a precision dialysis prescription. Providing patients with quantitative measurement of changes during hemodialysis, and targets to reach, could improve treatment satisfaction and quality of life.

Although our dataset is broad, evaluating 1163 proteins, the number of participants was small, limiting the ability to evaluate patient-related or dialysis prescription-related variables in multivariate models. Similarly, the power to test individual proteins with intradialytic symptomatology or subsequent cardiovascular events was inadequate. In addition, participants were limited to European ancestry and recruited at a single center, only 1 membrane was evaluated, and slow low-efficiency dialysis, continuous renal replacement therapy, hemodiafiltration, or isolated ultrafiltration were not evaluated.

In conclusion, blood proteins change in concentration during a single hemodialysis treatment that can be measured on a proteome-wide scale. Changes are related to protein characteristics; however, significant interindividual differences exist, especially in the context of intradialytic events. Further high-throughput proteomic studies are needed to assess dialysis adequacy, test biomarker-symptom associations, and improve risk prognostication of patient important adverse events.

DISCLOSURE

GP has received consulting fees from Bayer, Sanofi, Bristol-Myers Squibb, Lexicomp, and Amgen; and support for research through his institution from Sanofi and Bayer. MBL has received speaker and advisory fees from Otsuka, Reata, Bayer, and Sanofi Genzyme. All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

MBL, GP, and MW conceived the study. ML performed data analysis and drafted the manuscript. All authors contributed to study design, data interpretation, critical revisions, and provided approval of the final draft.

SUPPLEMENTAL MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary Data (.xls) Biomarker changes during a hemodialysis session as measured on the Olink platform. The mean concentration and changes during dialysis for all measured biomarkers, as well as the association of changes with dialysis variables, are provided in the data supplement.

Figure S1. Change in biomarkers during one session of hemodialysis. Distribution of the concentration change of 1061 biomarkers during a single hemodialysis session of 42 study participants. Whereas 189 significantly *decreased* in concentration, 54 significantly increased in concentration ($P < 5 \times 10^{-5}$).

Figure S2. Biomarker characteristics including size (A), charge (B), and baseline concentration (C) impact the change in concentration on dialysis. Each point represents the change in a single biomarker during a single dialysis session.

Figure S3. Enrichment of cardiovascular biomarkers in those associated with intradialytic hypotension. Thirteen of the 20 biomarkers associated with hypotension were cardiovascular biomarkers (all had an increase in concentration with hypotension; $P_{\text{individual marker}} < 0.01$; $P_{\text{enrichment}} = 2.8 \times 10^{-10}$).

Table S1. Cohort data. Descriptive statistics of nested case-control cohort evaluated for changes in biomarker concentration during a single session of hemodialysis.

Table S2. Biomarker changes during a hemodialysis session as measured on the Olink platform.

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