A quantitative proteomics workflow for urine and spent dialysate using data-dependent and data-independent analyses on a timsTOF Pro instrument

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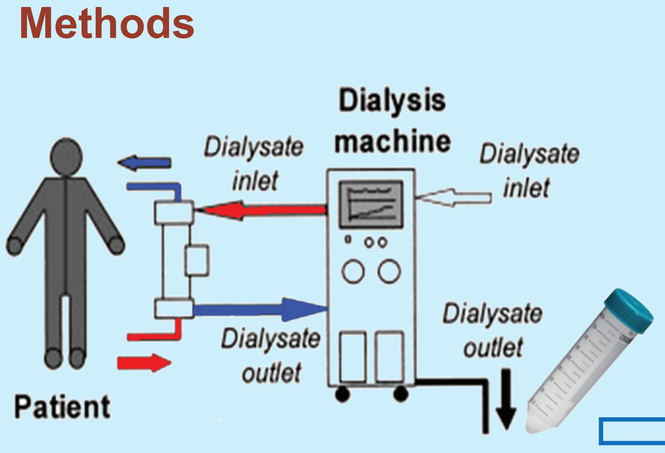
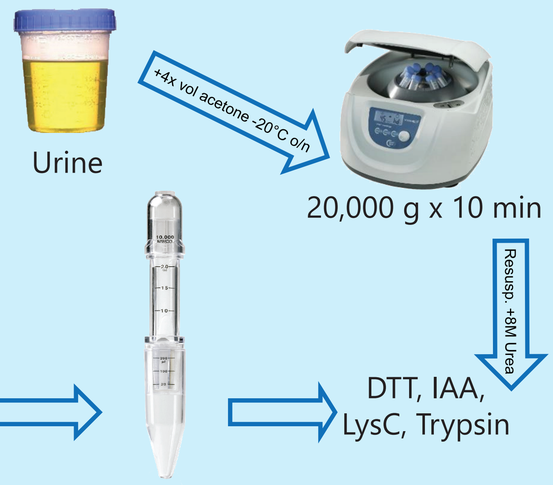
Introduction

Despite hemodialysis being immediately life saving for patients with kidney failure, the prognosis of patients on hemodialysis is generally poor with median survival rates of approximately five years. Interestingly, mortality of hemodialysis patients who retain RKF (residual kidney function) is lower.[1,2)] While hemodialysis effectively removes small molecules, the clearance of medium-size molecules appears less efficient. It was hypothesized that toxic medium-size proteins, which can still be excreted into urine by hemodialysis patients with RKF, might accumulate in patients without RKF.[3)] Alternatively, proteins that are normally reabsorbed in the kidney tubulus and that help maintain stable homeostasis, could be lost in hemodialysis. To evaluate these hypotheses, we established a high-throughput quantitative proteomics workflow for the analysis of spent dialysate and urine using a timsTOF Pro instrument.[4)] We here present the results of a proof-of-concept study.

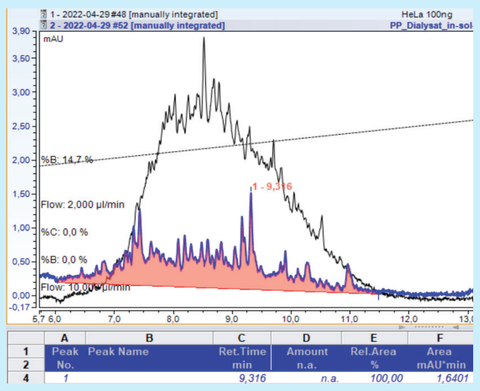
Methods



Dialysate flow was 500-800 mL/min i.e. 100-200 L during 3-4 hr dialysis session. Spent dialysate was collected by slow aspiration with a syringe from an adapter mounted on the dialysate outlet hose.

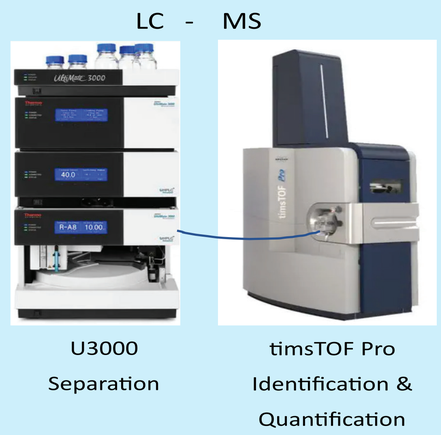


400 µL residual urine +4x vol acetone was precipitated o/n and centrifuged @20.000g for 10 min, followed by resuspension in 8M Urea. 10 mL of spent dialysate +8M Urea was concentrated to 200µL by repeated ultrafiltration using 2mL spin columns with 10kD cut-off



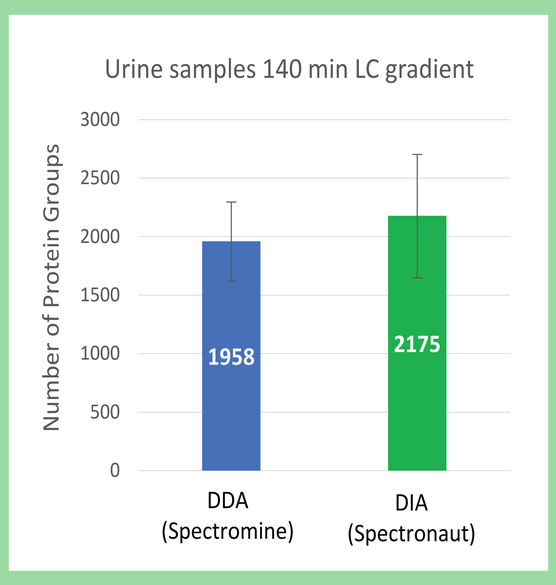
	A	B	C	D	E	F
1	Peak No.	Peak Name	Ret.Time min	Amount n.a.	Rel.Area %	Area mAU*min
2	1		9,316	n.a.	100,00	1,6401

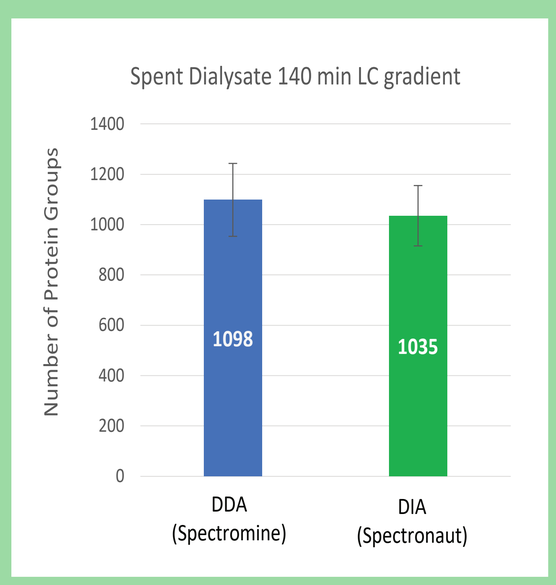
Digested samples were quantified by AUC @ 214 nm compared to 100ng HeLa on an monolith LC. This permitted an estimation of the protein concentration of the original urine and spent dialysate samples.

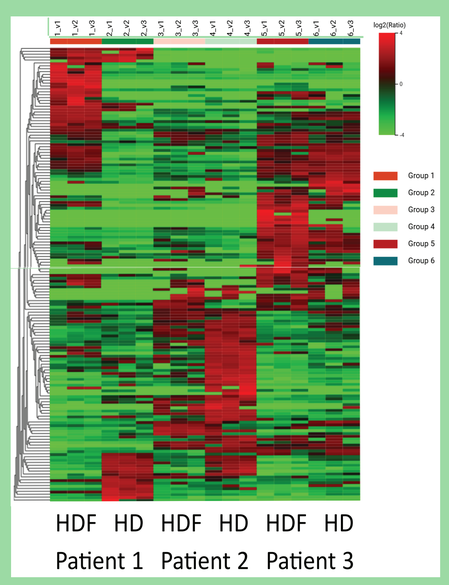


Samples were separated on a U3000 system with PepMap 50cm or Pharmafluidics 110cm columns (140 min gradient time). Spectra were acquired on a timsTOF Pro instrument using DDA and DIA. Spectromine 3.2 and Spectronaut 16 were used for data analysis.

Results







Protein	average mg HD	95% KI	average mg HDF	95% KI
Albumin	27.02	[24.6;29.4]	768.88	[765.5;772.3]
Serotransferrin	1.59	[1.5;1.6]	85.57	[74.9;96.3]
Complement C3	0.02	[0;0]	0.87	[0.7;1]
Alpha-1-antitrypsin	2.47	[2.4;2.5]	136.01	[119;153.1]
Complement factor H	0.07	[0.1;0.1]	0.56	[0.5;0.6]
Fibrinogen alpha chain	2.32	[1.8;2.9]	14.26	[11.9;16.6]
Vitamin D-binding protein	1.42	[1.4;1.5]	23.19	[21.8;24.6]
Apolipoprotein A-IV	1.88	[1.4;2.3]	0.66	[0.5;0.8]
Beta-2-glycoprotein 1	1.17	[1.1;1.2]	21.24	[19.4;23.1]
Transthyretin	1.69	[1.4;2]	22.81	[19.4;26.2]
Beta-2-microglobulin	27.03	[22.7;31.3]	79.40	[61.6;97.2]
Immunoglobulin kappa constant	7.90	[6.8;9]	52.41	[43.4;61.4]
Alpha-1-acid glycoprotein 1	3.01	[2.7;3.4]	69.83	[52.3;87.4]
...	1.86	[1.37;2.28]	24.62	[21.72;21.72]
Haptoglobin	0.01	[0;0.02]	0.48	[0.4;0.6]
Myoglobin	0.09	[0.1;0.1]	0.19	[0.1;0.3]
Neutrophil gelatinase-associated lipocalin	0.17	[0.2;0.2]	0.59	[0.3;0.9]
Major prion protein	0.14	[0.1;0.2]	0.30	[0.2;0.4]

On average 1958 protein groups could be identified in triplicates of four urine samples using DDA (data-dependent analysis), as compared to 2175 protein groups using DIA (data-independent analysis). Data completeness was superior with DIA. This indicates that a level of sensitivity that required two-dimensional separation of samples only a few years ago, can now be achieved in 1D LC-MS runs.

With regard to spent dialysate, on average 1035 protein groups could be identified with DDA in triplicates of six dialysate samples from three patients using two dialysis modes (hemodialysis HD and hemodiafiltration HDF), as compared to 1098 groups with DIA. The protein list comprised several known uremic toxins. Results showed significant differences in the concentrations of proteins, depending on the type of dialyzer membrane and whether HD or HDF was performed. This suggests a possibility to tailor hemodialysis to individual patients' needs.

References

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Acknowledgements

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