profiling using patients' PD effluents and HR/AM. Therefore, we investigated the abundance of metabolites in a non-targeted approach in timed PD effluents from stable patients on chronic PD therapy undergoing a peritoneal equilibration test (PET).

METHODS

The study was approved by the local ethics committee. Peritoneal dialysis effluents were collected during a PET with 2 L of a conventional dialysis fluid (Dianeal 3.86% Glucose, Baxter Healthcare Corporation, Deerfield, IL, USA) in 8 consenting clinically stable adult patients who were on chronic PD (Supplementary Table 1). Aliquots of 2 mL effluent were obtained after completion of instillation of the dialysis fluid into the patients' cavity (= time point 0) and 4 hours later (= time point 4 h) and used for both untargeted metabolomics (HR/AM) and for routine amino acids. Samples were centrifuged at 2,500 U/min for 8 min, and the supernatant was stored at -80°C until further analysis (see Supplementary Methods for full description). In brief, for metabolomic measurements, a state-of-the-art HR/AM spectrometer, Q Exactive Plus Hybrid Quadrupole-Orbitrap in combination with a multidimensional Transcend UHPLC system with Allegro quaternary pumps (Thermo Fisher Scientific, San Jose, CA) was used (4). A non-parametric Wilcoxon-Rank test was carried out and a *p* value < 0.05 was considered statistically significant.

Figure 1 demonstrates the metabolomics workflow. Mass spectrometry (MS) data were recorded in MS¹ (measuring initial mass-to-charge-ratio) and MS² (measuring fragment ions) mode following direct-injection high performance liquid chromatography (HPLC) and on-line sample cleanup. By automatic chromatographic alignment of retention time, peak picking, and framing, raw data were converted into an intensity matrix of masses determined with high precision. Highly accurate measurement of the apparent molecular weight allowed confinement of the set of putative identifications for *in-silico* fragmentation. In total, 200 masses were detected in PD effluents using the Sieve software 1.3 (Thermo Fisher Scientific, San Jose, CA). Human metabolome database (HMDB) and Kyoto encyclopedia of genes and genomes (KEGG) database were queried, resulting in 418 ChempSpider IDs. Out of all masses, 29 showed a significantly increasing abundance between time point 0 and 4 h, and were detected in at least 5 of 8 patients at time point 4 h. Those 29 linking to 41 putative entities were forwarded to *in-silico* fragmentation using the Mass Frontier 6.0 software (HighChem, Ltd., Bratislava, Slovakia). The resulting fragments were matched with those obtained in MS² identification of chemical entities, detailed in Supplementary Table 2.

RESULTS AND DISCUSSION

We could unambiguously identify 13 of 29 masses, whereas in the remaining, more than 1 putative chemical entity was assigned. The list of identified metabolites comprises amino acids, metabolites of the fatty acid metabolism and

Feasibility of Metabolomics Analysis of Dialysate Effluents From Patients Undergoing Peritoneal Equilibration Testing

Metabolomics systematically measures multiple metabolites directly from complex biological samples like bio-fluids (1). High-resolution accurate mass spectrometry (HR/AM) and full-scan capabilities, capturing all sample data, has the potential to be used in untargeted high-throughput approaches for detecting and analyzing a great number of molecules per measurement. As identified metabolites represent the intermediates and end products of active biological processes, the metabolome reflects the sum of biotic and abiotic perturbations, giving a real time stature ("snapshot") of an organism's phenotype (2). Although peritoneal dialysis (PD) effluents are particularly easily available bio-fluids, metabolomics in PD is still a nearly unexplored field (3). The aim of our study was to demonstrate the feasibility of dynamic metabolic

Figure 1 — Metabolomics workflow, starting with chromatographic separation of the supernatant obtained from the PD effluents. Processing of raw data using the software Sieve 1.3 resulted in 200 exact masses. These masses were used to search for Chemspider IDs by querying the HMDB and KEGG databases. Out of those 200 masses linked to 418 Chemspider IDs, in 41 a significant increase between time point 0 and 4 h could be found. Those molecules were forwarded to *in-silico* fragmentation using the Mass Frontier 6.0 software and the resulting fragments were matched with those obtained on the MS² mass analyzer. Using this workflow we could unambiguously identify 13 metabolites, in all the others more than 1 putative chemical entity was assigned. PD = peritoneal dialysis; HMDB = human metabolome database; KEGG = Kyoto encyclopedia of genes and genomes; MS = mass spectrometry; HCD = higher-energy collisional dissociation.

other substances of endogenous or exogenous origin; some metabolites without special classification were also found. Our comparative approach identified good agreement between results of the untargeted approach and those of a validated targeted method, which is routinely used in clinical practice (4) in 5 canonical amino acids increasing over time in the effluent (Supplementary Figure 1).

This study demonstrated the feasibility of metabolomics analysis of timed peritoneal dialysate effluents to provide “snapshots” of the metabolic phenotype of 8 PD patients undergoing a PET. With our untargeted method, it was possible to measure a wide range of small molecules, largely unbound metabolites, that can be cleared by PD or were locally produced by peritoneal cells. The lack of systemic metabolic measurements in plasma allowed no correlation with the values of the effluents, prohibiting relative discrimination between these compartments.

As expected, our untargeted approach also detected intermediates that have not been described in this setting before, the majority related to the tryptophan metabolism (Supplementary Table 2). Uremia induces increased formation of tryptophan metabolites related to the kynurenine pathway (5). Kynurenines are precursors of a number of toxic substances that can induce neurological symptoms and have carcinogenic properties. Further, levels of kynurenines and

their metabolites were related to other markers of inflammation and oxidative stress as well as with the presence of coronary artery disease in patients on hemodialysis and on PD (5–7). Thus, future studies in larger cohorts will be needed to test whether peritoneal profiles of kynurenine, indoleacrylic acid, indole-3-acetic acid, and other metabolites of tryptophan during dwell time in patients’ effluents could give essential information about the peritoneal clearance of uremic toxins and reflect dialysis quality. Metabolites detected in effluents could also allow monitoring of the condition of the peritoneum, like identifying patients at risk for developing encapsulating peritoneal sclerosis (3). Low glutamine levels, as present in the peritoneal cavity during PD, have been associated with increased vulnerability, due to inadequate cellular stress response, and impaired metabolic and immunocompetence status (8).

As a major limitation of this study, the small number of patients did not allow any clinical interpretation by comparing the “metabolic snapshots” with the patients’ clinical phenotype. However, our study demonstrates the technical feasibility of an HR/AM approach and shows that metabolomics analysis of clinical PD effluent represents a method that can detect a large number of metabolites with a wide range of small molecules associated with various pathways in a single measurement. This tool may provide potentially relevant information on the uremic status of the patient and dialysis quality as well as on peritoneal cell function. As PD effluents are easily available bio-fluids, further research in larger study populations will help to establish this novel approach for monitoring the clinical phenotype of PD patients.

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DISCLOSURES

DC, AML, RH, and KK are or were employees of Zytrotec GmbH. CA is cofounder of Zytrotec GmbH, a spin-off of the Medical University Vienna that holds the patent ‘Carbohydrate-based peritoneal dialysis fluid comprising glutamine residue’ (International Publication Number: WO 2008/106702 A1).

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