Peritoneal Effluent Biomarker Discovery in Peritoneal Dialysis: The Omics Era

4

Deirisa Lopes Barreto and Dirk G. Struijk

Contents

Abstract

One of the main renal replacement treatment modalities for patients with end-stage renal diseases is peritoneal dialysis (PD). In PD therapy, the peritoneum is used as an intracorporeal dialysis system. The monitoring of intraperitoneal events is hampered by the absence of serial peritoneal biopsies. However, the acquisition of peritoneal effluent is simple and usually occurs after a predefined dwell or if possible after a standardized peritoneal function test. This peritoneal effluent is composed of several proteins and metabolites, which modifies accordingly due to intraperitoneal events. To date, peritoneal effluent biomarker discovery is evolving with a holistic perspective. The rise of applying suffix -omics technologies within PD therapy introduced a more exploratory approach for the

D. Lopes Barreto (✉) • D.G. Struijk
Division of Nephrology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
e-mail: D.LopesBarreto@amc.uva.nl; D.G.Struijk@amc.uva.nl

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identification of candidate effluent biomarkers. The application of genomics, metabolomics, and proteomics with the peritoneal effluent as biospecimen is however still in its infancy.

The emerging field of omics techniques as tools for peritoneal effluent biomarker discovery is presented in this chapter. The high sensitivity of omics technologies requires stringent conditions, and therefore methodological precautions must be undertaken on laboratory technical level, appropriate selection of study design and population, as well as data analysis. For this reason, methodological considerations for conducting omics-based PD research and the current developments with regard to the usage of these disciplines are addressed. Lastly, a summary is given on the available literature concerning the usage of omics techniques with the peritoneal effluent as a liquid biopsy within PD therapy.

Keywords

Biomarker • Discovery • Effluent biomarker • Genomics • Metabolomics • Peritoneal dialysate • Peritoneal dialysis • Peritoneal effluent • Proteomics

Abbreviations

2D-DIGE	Two-dimensional difference gel electrophoresis
Biobank	Biological bank
Biomarker	Biological marker
CAPD	Continuous ambulatory peritoneal dialysis
CKD	Chronic kidney disease
CRP	C-reactive protein
CV	Coefficient of variation
DN	Diabetic nephropathy
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immuno assay
EPS	Encapsulating peritoneal sclerosis
GN	Glomerulonephritis
GWAS	Genome-wide association studies
Ig	Immunoglobulin
IL-6	Interleukin-6
MS	Mass spectrometry
NECOSAD	Netherlands Cooperative Study on the Adequacy of Dialysis
NMR	Nuclear magnetic resonance
NRI	Net reclassification index
PD	Peritoneal dialysis
RNA	Ribonucleic acid
ROC curve	Receiver operating characteristic curve
SNPs	Single nucleotide polymorphisms
SOP	Standard operating procedures
VEGF	Vascular endothelial growth factor

Definitions

Biological bank (biobank) An archive containing human biospecimens that may include blood, effluent, serum, or tissue samples. The storage of these samples occurs preferably under standardized conditions within a single or multicenter study cohort.

Encapsulating peritoneal sclerosis (EPS) EPS is the most devastating complication of PD therapy that occurs in 3–7 % of PD patients. EPS is characterized by a dense cocoon of fibrous tissue that covers the abdomen. High mortality rates and severe morbidity are present for patients diagnosed with EPS.

Free water transport (FWT) Aquaporin-1 mediated transport of water, without dissolved solutes and electrolytes. FWT is one of the peritoneal transport parameters that become impaired in long-term PD patients.

Genomics Laboratory strategy that investigates genes and their functions. Utilized methods comprise DNA sequencing and fingerprinting. Genomics is furthermore subdivided into various disciplines such as epigenomics and metagenomics and functional genomics and structural genomics.

Metabolomics Laboratory method for the identification and quantification of metabolite levels. Chromatography is one of the primary steps used as a separation technique. Thereafter, the metabolites may be detected by means of mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy.

Peritoneal dialysis (PD) therapy One of the renal replacement therapies for end-stage renal disease patients for over almost five decades. The proportion of PD patients in the worldwide dialysis population covers 11 %, and the 5-year survival has increased to 41 %. In many countries, PD therapy is presented as primary dialysis modality choice, as there is a survival benefit over hemodialysis in the first 3 years.

Peritoneal function test A standardized peritoneal test to assess the function status of the peritoneal membrane. Additionally, the tests provide insight into the magnitude of small-solute removal, fluid transport, and ultrafiltration capacity. Several forms of peritoneal function tests are available such as a standardized peritoneal permeability analysis or (modified) peritoneal equilibration test. However, all of them are characterized by a predefined dwell time with or without intermediate sampling of the peritoneal effluent.

Peritoneal membrane The peritoneal membrane is described to be a semipermeable membrane that consists of three main layers. Firstly, the mesothelial cell layer is encountered, followed by the interstitium in which the peritoneal capillaries are imbedded. Long-term and continuous exposure of PD solutions to this membrane may lead to functional and morphological alterations.

Proteomics Laboratory discipline for analyzing structure, conformation, and biological function of proteins. In brief, proteomic analyses encompass separation of the proteins by gel electrophoresis methods, followed by MS-based analyses.

Systems biology A holistic point of view for gaining insight into biological mechanisms within cells, organisms, or species. Systems biology has been applied from the early 1900. Genomics, metabolomics, and proteomics are disciplines within systems biology that seek to qualify and quantify the genome, metabolome, or proteome, respectively.

Introduction

The field of omics is expanding at a rapid pace as novel instruments for biological marker (biomarker) discovery and unraveling pathophysiological mechanisms. Within peritoneal dialysis (PD) therapy, the application of high-throughput technologies is still in its infancy. Especially, the peritoneal effluent as biospecimen, which contains a variety of proteins either due to transperitoneal transport or local production, has not yet been explored extensively (Table 1). Numerous biomarker consortia, working groups, and scientists contributed to the diversity of existing biomarker definitions with different classifications and applications (Atkinson et al. 2001;

Table 1 Key facts of peritoneal dialysis and peritoneal effluent biomarkers

In PD therapy, the peritoneum serves as a biological semipermeable dialysis membrane. Through this membrane, toxic waste products and excess fluid are removed from the circulation into the peritoneal cavity. During the day, 3–5 daily exchanges of PD solutions take place. These exchanges vary in dose and duration of the dwell. After a predefined dwell time, the infused dialysis solution is drained. This drained fluid is called the peritoneal effluent or peritoneal dialysate, which contains several substances
Proteomic profiling and characterization of the peritoneal effluent indicated that the substances represent merely extracellular proteins. In addition, the circulation is found to be responsible for the presence of a majority of peritoneal effluent constituents. Substances or proteins in the peritoneal effluent can only be eligible as effluent biomarker within PD if they are locally produced within the peritoneal cavity
Unfortunately, no direct visualization of the peritoneal membrane is possible without invasive procedures. Furthermore, computed tomography scans are unable to detect anatomical modifications timely. Peritoneal effluent biomarkers are considered as noninvasive instruments for screening or diagnostic purposes. They would allow uncomplicated monitoring of the integrity of the peritoneal membrane. For this reason, the peritoneal effluent is regarded as the most clinically relevant specimen within PD therapy. However, the implementation of the more established peritoneal effluent biomarkers in routine PD patient care is fairly small
The discovery of peritoneal effluent biomarkers was previously based on pathophysiological knowledge and hypothesis driven. The first omics-conducted research within PD therapy originates from 2007. Effluent biomarker discovery through omics technologies is aimed at providing proxies for specific anatomical alterations. This holistic approach is expected to deliver insight into the sequence of intraperitoneal events. Nevertheless, current omics studies within PD require further calibration and standardization

PD peritoneal dialysis

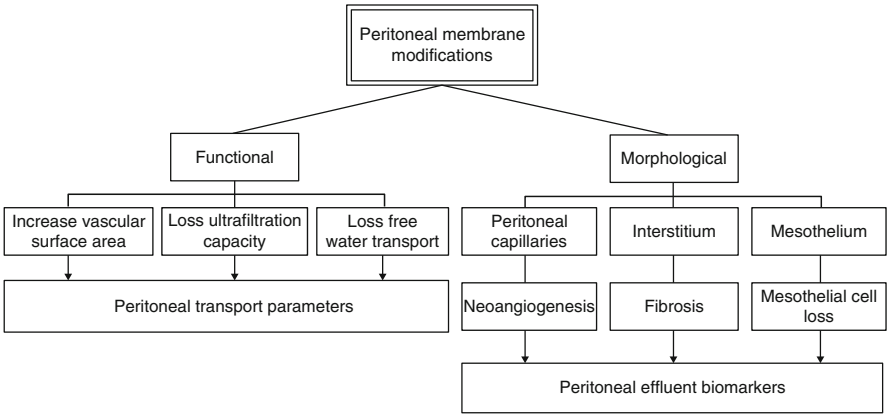

Fig. 1 Peritoneal membrane modifications. The peritoneal membrane consists of three main layers: mesothelium, interstitium, and peritoneal capillaries. In all of these layers, morphological modifications are observed for which effluent biomarkers could be utilized for diagnostic or prognostic purposes. However, not all of these layers are considered as prominent barriers to the peritoneal membrane transport. The increase in perfused peritoneal capillaries leads to a rapid dissipation of glucose and consequently may evolve in a decreased ultrafiltration capacity. In a progressive stage, the fluid transport through the water channels, e.g., aquaporins, may become impaired

Hulka et al. 1990; Perera and Weinstein 2000; Colburn 2000; Jain 2010). Overall, the essence of molecular biomarkers is to provide noninvasive, cost-effective tools for screening or diagnostic purposes and disease prognosis and surveillance. Additionally, molecular biomarkers may also be intended to assess therapeutic responsiveness or offer novel intervention strategies (Atkinson et al. 2001).

The main purpose of PD therapy as renal replacement treatment modality is to remove toxins and excess fluid from the body. For this intention, a permanent catheter is inserted into the peritoneal cavity through which dialysis solutions can be instilled. The removal of these toxic waist products and excess fluid, from the circulation into the peritoneal cavity, occurs mainly by means of diffusion through the peritoneal membrane. Therefore, the efficacy of PD therapy is highly dependent on the biological condition of the peritoneal membrane. The continuous exposure of dialysis solutions to the peritoneal membrane causes however several functional and morphological modifications (Fig. 1). The most encountered functional changes are the rapid dissipation of the osmotic gradient and a decrease in ultrafiltration capacity. Furthermore, some of the long-term PD patients also present with an impairment of free water transport. Especially in patients who develop EPS, free water transport appears to be the only peritoneal transport parameter, which can distinguish patients with this severe complication from patient with a long PD therapy duration (Lopes Barreto et al. 2014). The functionality of the peritoneal membrane can be monitored incessantly by peritoneal permeability tests (Coester et al. 2009), whereas the progression of morphological modifications remains unrevealed because no serial peritoneal biopsies can be performed. More importantly, the functionality of the peritoneal membrane is not inherent to the observed morphologic modifications. In

Table 2 Prerequisites for a peritoneal effluent biomarker

1.	Detectable in peritoneal effluent by means of omics technologies or other protein detection methods
2.	Computational assessment of local release or production within the peritoneal cavity
3.	Involvement in pathological pathway of the peritoneal membrane
4.	Good measures of diagnostic accuracy for PD-related outcomes (e.g., high sensitivity/specificity)

this perspective, more emphasis is placed on potential markers that are present in the peritoneal effluent and mirror the integrity of peritoneal tissues. Thus, promising peritoneal effluent substances have to meet several prerequisites before the acknowledgment of a peritoneal effluent biomarker is given to them (Table 2). Prior to the rise of suffix -omics technologies, effluent biomarker discovery in PD was merely based on hypothesis-driven research. Even though this is still valid within these translational scientific disciplines, at present the discovery of effluent biomarkers is evolving with a more global and exploratory perspective. The application of high-throughput laboratory techniques offers a great opportunity to gain insight in the peritoneal alterations that occur over time due to PD treatment and identify clinically relevant effluent substances that may serve as biomarkers. The vast majority of high-dimensional methodologies applied to the peritoneal effluent consist of genomics, metabolomics, and proteomics. Previous reviews have acknowledged the potential use of proteomics for effluent biomarker discovery and elucidation of pathophysiological processes of the peritoneal membrane (Brewis and Topley 2010; Thongboonkerd 2010). The present chapter highlights the evolving landscape of omics, in which the human peritoneal effluent is regarded as central specimen. Methodological considerations in effluent biomarker discovery are provided, and an overview is given to illustrate the application and progression of omics-conducted research within PD therapy.

Methodological Considerations

As a consequence of the large amount of data that is acquired by suffix -omics analyses, several methodological precautions have to be taken. These preventative measures include the optimization of analytical validity, well-designed study and selection of study population, as well as proper data analysis. This section describes these aforementioned considerations and provides examples in PD-conducted omics studies.

Analytical Validity

In biomarker discovery studies, three main phases are encountered on a laboratory technical level that might alter research findings and thwart the interpretation of the acquired data: pre-analytical, analytical, and post-analytical.

The pre-analytical phase evolves sample handling and has a direct influence on the accuracy and reproducibility. This is especially of importance when investigating the proteome, metabolites, or candidate effluent biomarkers, which are influenced by structural peritoneal membrane modifications over time. One of the bias-introducing factors could be the effect of storage on the assembled effluent biospecimens. DNA extraction and genotyping from frozen peritoneal effluent samples up to 7 years at −20 °C indicated no influence of storage duration (Gillerot et al. 2004). However, it is not known whether long-term storage of the effluent at lower temperatures is superior to temperatures of at least −20 °C. Moreover, the stability of metabolites and proteins present in peritoneal effluent and the effect of repeated freeze-thaw cycles have not been investigated yet. To preserve the quality of the effluent and reduce the amount of variability due to incorrect or unbalanced sample handling, standard operating procedures (SOPs) are needed. Included elements in a SOP for local or multicenter biorepositories should at least cover the amount and volume of aliquots, storage conditions, e.g., minimal temperature of −20 °C, mechanical freezer, or directly frozen by liquid nitrogen, and the necessity to document the number of freeze-thaw cycles. From the omics studies within the discipline of PD, only some of the articles provided detailed information on sample collection and archiving comprising momentum of effluent withdrawal, storage temperature, and time frame of sample processing. For comparability and assessment of study quality, reporting this information is essential.

Intra-analytical inaccuracies may contribute to random or systematic errors. These errors occur during assaying of effluent constituents, which could be influenced by sampling handling of the laboratory personnel, measurement apparatus, or reagents. By performing the experiments in dupli- or triplicate, the degree of precision can be assessed. Also, the range of standard reference curves should be wisely chosen in order to determine the appropriate detection limit of an assay. Typically acceptable coefficients of variation (CV) lie beneath 20 % for which one should strive for CVs of 5 %. Furthermore, in the validation phase of biomarker discovery by means of enzyme-linked immunosorbent assays (ELISAs) or Western blots, one could reduce intra- and inter-variability by even distribution of the study groups within and throughout batches. Preferably, the laboratory technician should be blinded for the outcome of interest as well. Recently, a methodological article proposed an optimal high-resolution effluent protein separation technique by two-dimensional gel electrophoresis (2D-DIGE) intended for proteomic analysis (Zhang et al. 2013). The authors investigated five precipitation methods followed by constraining abundant proteins of the peritoneal effluent. Such attempts are in favor of reducing sample preparation heterogeneity and enhance critical appraisal of the used high-throughput methodologies across studies analyzing the peritoneal effluent.

Incorrect post-analytical inferences may lead to differential misclassification bias. For example, if threshold values of the peritoneal effluent are inappropriately determined, the contrast between groups could be augmented or diminished. As a consequence, the results could indicate nonexisting differences between the group with the outcome of interest and those without. Eventually, this nonrandom measurement error may contribute to biased estimates of association.

Study Designs and Population

The majority of the omics studies with the peritoneal effluent follow a cross-sectional design. Generally, in these studies, the profiles of PD patients with the characteristic or clinical endpoint of interest are compared to PD patients with a stable condition. Omics studies have to be well designed, especially with regard to the study subjects where one should strive for a homogeneous population, as the peritoneal metabolome and proteome of PD patients are likely to be susceptive to posttranslational modifications due to patient-related and external factors. Therefore, the selection of patients as well as a priori specification of a validation subset is of similar or even of greater importance when compared to other epidemiologic studies. Unfortunately, often clinical and demographic data of the study population or independent validation sample is lacking in omics-conducted PD research. The sparse number of cohort and nested case-control studies is presumably the result of impracticable specimen collection or cost related. This is however a great loss, as the presence of a local biological bank with repetitive effluent specimens would enable trend analyses. In addition, the sample size is relatively small in the majority of the omics studies with PD therapy. Lastly, it is questionable whether the follow-up duration in a number of studies is sufficient to measure difference in peritoneal membrane alterations. Especially, since the factual peritoneal membrane dysfunction is usually observed after a therapy duration of at least 2 years.

Another important factor of the peritoneal effluent is its origin. As the peritoneal effluent can be obtained right after a regular PD exchange or after a standardized peritoneal function test, it follows from this that the biological variability increases concordantly. Therefore, the withdrawal of effluent samples in studies should be harmonized within or between centers. Regrettably, not all omics studies within PD report whether the peritoneal effluent is derived from a regular dwell of after a standardized peritoneal function test.

Omics Data Analysis

An effluent biomarker can be an outcome to monitor the progression of PD therapy as well as a prognostic factor of various PD-related complications. When a biomarker is intended as a diagnostic or prognostic instrument, receiver operating characteristic (ROC) curve and C-statistics are used in order to evaluate the discriminative power. Additionally, optimal threshold values can be estimated based on the sensitivity and specificity of a biomarker. However, these measures are highly dependent on the base study population, and misclassification may arise due to patient demographics, laboratory measurement errors, and the degree of the exposure to dialysis solutions. Hence, one has to be cautious with the definition and selection of a correct clinical endpoint in biomarker research. Nevertheless for that reason, the net reclassification index (NRI) was introduced suggesting a method to gain prognostic accuracy of a biomarker (Wilson et al. 2008). The interpretation of omics-derived findings usually requires the use bioinformatics or the application of molecular epidemiology. In general, the sample

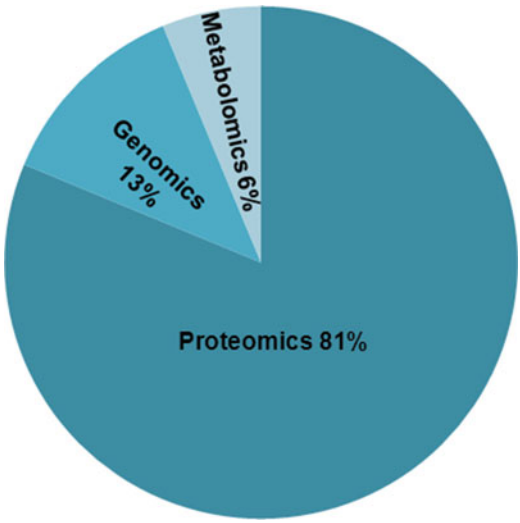

Fig. 2 Proportion of omics studies with the human peritoneal effluent as biospecimen. The number of studies applying omics technique with the human peritoneal effluent as central biospecimen is still limited. Proteomics attributed to 81 % of all studies followed by genomics (13 %) and metabolomics (6 %)

sizes of the current omics studies in PD are small. However, omics studies generate enormous amount of data that are subjective to false-positive or false-negative results when not handled as appropriate. Therefore, correction for multiple comparisons or adjustments with regard to p-value thresholds should be applied.

Current Developments

Proteomics is the main applied high-throughput technology followed by genomics and metabolomics for peritoneal effluent biomarker discovery (Fig. 2). These fields have the potential to elucidate underlying molecular mechanisms that are involved in the pathophysiology of the peritoneum. Moreover, they can empirically provide novel diagnostic and therapeutic biomarkers based on genome, metabolite, or protein profiles of PD patients. Nevertheless, the number of studies is still modest; a summary is given on the recent developments and main findings.

Proteomics

In nephrology, proteomics is merely applied on plasma, serum and urinary samples, or renal tissue. The number of proteomics studies with the peritoneal effluent as sample type is rising, and investigations have been executed from various perspectives other than the identification of novel biomarkers for PD (Fig. 3). The main obstacle for the discovery of effluent biomarkers includes the dynamic range in proteomic analyses.

Uremia in itself is suggested to alter the functional and structural organization of the peritoneal membrane. Therefore, the effect of a uremic environment was investigated

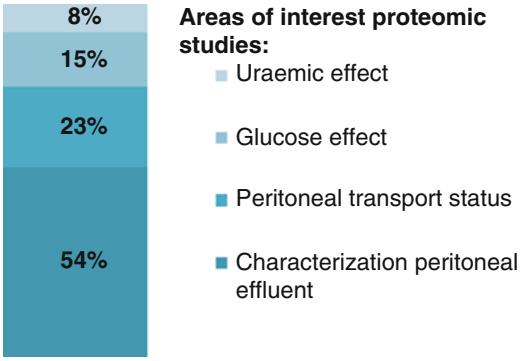

Fig. 3 Areas of interest within proteomics studies. The proof-of-principle studies applying proteomic analyses have been executed with interests in the effect of uremia (8 %) and glucose (15 %), peritoneal transport status (23 %), and characterization and profiling of the human peritoneal effluent (54 %)

by proteomic analysis in chronic kidney disease patients (stage five) versus patients with normal renal function (Wang et al. 2012). A number of protein alterations were found including elevated levels of vascular endothelial growth-A (VEGF-A).

The majority of proteomics research is aimed at the characterization and profiling of the peritoneal effluent in order to identify potential biomarkers. This discovery-based approach has been adapted in the peritoneal effluent of incident and prevalent adult PD patients (Wu et al. 2013; Cuccurullo et al. 2011). The study by Wu et al. included a number of ten incident PD patients for whom three patients were used for validation. The peritoneal effluent was assembled at start of PD therapy and once again after 1 year. Validation by Western blots showed elevated levels of immunoglobulin (Ig) μ chain, fibrinogen γ chain, and C-reactive protein (CRP) at baseline and Ig δ, α-1 antitrypsin, histidine-rich glycoprotein, apolipoprotein A1, and serum amyloid P-component after 1 year of PD therapy duration. The authors speculated that elevation in the protein levels that were measured after 1 year could indicate markers for early peritoneal membrane injury. The second study consisted of 15 prevalent PD patients with varying PD therapy duration ranging from 1 to 84 months. A subgroup was additionally defined to study the effect of glucose in PD solutions. Profiling and characterization of the peritoneal effluent has also been investigated in nine pediatric PD patients (Raaijmakers et al. 2008). A number of 88-shared proteins were identified. All of the abovementioned studies indicated that the effluent of PD patients is merely from systematic origin and reflects extracellular proteins. Characterization of the human effluent has furthermore been performed with regard to diabetic PD patients (Yang et al. 2013; Wang et al. 2010) and before and after a peritonitis episode (Lin et al. 2008; Tyan et al. 2013). Validation by Western blots was performed within the same study populations. The latter study identified up to 41 proteins with shared alterations in haptoglobin expression and revealed in an area under the receiver operating characteristic curve of 0.92.

Higher levels of glucose and osmolarity in PD solutions are known to induce a greater removal of excess fluid and toxic waste products. However, continuous exposure of these PD solutions high in glucose leads to damage to the peritoneal membrane. In this respect, proteomic analyses have been performed as well. An

increased appearance of advanced glycosylation end products was shown when patients were infused with higher percentages of glucose-based dialysis solutions (Pešić et al. 2011). Moreover, a number of nonredundant proteins including cystatin C, collagen, fibronectin, matrix metalloproteinase-2, plasminogen, and vitronectin were identified (Cuccurullo et al. 2011; Pešić et al. 2011). Additionally, an under-expression was found for α-1 antitrypsin, apolipoprotein A-IV, fibrinogen β-chain, and transthyretin in patients treated with the highest glucose concentration (4.25 %) of PD solutions when compared to those treated with 1.5 % or 2.5 % glucose-containing PD solutions (Cuccurullo et al. 2011).

Due to the large interindividual variation in peritoneal transport status at initiation of PD therapy, comparative analyses of the peritoneal effluent in PD patients with different peritoneal transport characteristics have been studied as well. In these studies, the authors found increased protein losses for PD patients with a fast transport status as compared to patients characterized by slow peritoneal transport rates (Wen et al. 2013; Sritippayawan et al. 2007). External validation by ELISA confirmed elevated levels of complement 4A and IgG in the fast transporters (Sritippayawan et al. 2007). Overall, the heterogeneity in study populations and practical laboratory techniques contributes to the variety of identified proteins and complexes inferences throughout studies.

Genomics and Metabolomics

Genomic biomarkers have not yet been identified, but intriguing single nucleotide polymorphisms (SNPs) have been found in the C/C genotype on the interleukin-6 (IL-6)-174G/C loci (Verduijn et al. 2012). The base population for this study originated from a Dutch multicenter cohort, also known as the Netherlands Cooperative Study on the Adequacy of Dialysis, archiving peritoneal effluent and serum of incident dialysis patients. Additionally, two external cohorts were used for independent data replication. A significant increased risk for mortality was associated with this IL-6 gene variant in adult patients who survived PD treatment over a period of at least 2 years. Albeit external cohorts were defined to authenticate these findings, further validation is necessary. Polymorphisms in the promoter region of VEGF have also been associated with an increased risk for mortality (Szeto et al. 2004). This was found in a prospective cohort study in 135 continuous ambulatory PD (CAPD) patients who had a follow-up duration of 1 year. The effluent was obtained within 2 months after start of PD and after 12 months. Furthermore, effluent levels of VEGF measured by ELISA showed a tendency toward lower levels in patients with the CC genotype when compared to those with an AA/AC genotype. In contrast, messenger RNA expression was significantly lower in PD patients with the CC genotype.

The GLOBAL Fluid Study group is a prospective longitudinal worldwide biobank within PD that serially collects peritoneal effluent alongside serum samples. The effluent samples within the cohort are all obtained at the end of a 4-h peritoneal

equilibration test in incident PD patients and repeated thereafter at predefined intervals. This group recently published a study in which an attempt was made to identify metabolic profiles specific for PD patients who developed EPS (Dunn et al. 2012). As no previous study investigated metabolites within the peritoneal effluent, this study secondly aimed to provide optimal strategies for analyzing the metabolome alongside the identification of differences in effluent composition. The authors found that the peritoneal effluent consists grossly of low molecular weight metabolites. Moreover, prior to the diagnosis of EPS, modifications in several amino and short-chain fatty acids and its derivates were present. The abovementioned studies demonstrate the capability and importance of longitudinal (multicenter) study cohorts containing effluent specimens.

Potential Applications to Prognosis, Other Diseases, or Conditions

Within the discipline of nephrology, suffix -omics technologies are widely applied. The earliest studies originate from 1997 with an increasing tendency in the number of published articles ever since. Genome-wide association studies (GWASs) have contributed greatly to the understanding of chronic kidney diseases (CKDs) as well as the main kidney failure diseases: renovascular disease, diabetic nephropathy (DN), and glomerulonephritis (GN) (Atzler et al. 2014; Kottgen 2010). The reviews on the recent developments of genomic and metabolomic analyses uncovered that over 98 % of the estimated heritability within nephrological diseases remains to be revealed. From this, it can be concluded that a majority of the pathophysiological mechanisms of kidney diseases and function still warrants elucidation. Proteomics have been applied widely as well with similar intentions such as biomarker discovery, to reveal causal pathways of kidney diseases and to indicate potential therapeutic targets (Bonomini et al. 2012). Proteomic studies within nephrology have focused on urinary biomarkers for DN, IgA nephropathy, lupus nephritis, and rejection of renal transplants (Papale et al. 2010; Rocchetti et al. 2008; Zhang et al. 2008; Metzger et al. 2011). However, harmonization of the analytical procedures is warranted. Another commonly mentioned issue encompasses the study designs, which are merely represented by transversal research rather than longitudinal cohorts investigating the course of events. Moreover, the sample sizes of some studies are relatively small. These tendencies are in line with the current advances observed in omics-conducted PD research.

The utilized biospecimens within nephrology range from blood and serum samples to urine and renal tissue. Within nephrology, these various specimens have been investigated extensively. However, the clinical application of potential biomarkers still fails to appear despite their promising results. One of the aspects that also should not be overseen is the collaboration between the clinicians, epidemiologists, (laboratory) scientists, and healthcare funding organizations to enable a more rapid integration and implementation of biomarkers.

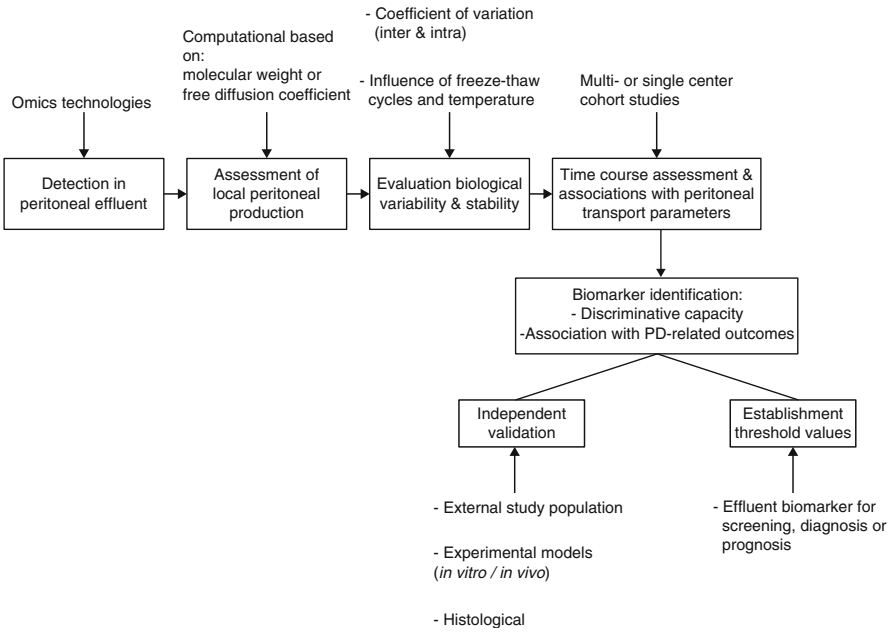

Fig. 4 Suggested flowchart for peritoneal effluent biomarker discovery. A flowchart is suggested for effluent biomarker discovery that are detected by means of omics technologies. Some of the suggested phases are based on the requirements of a peritoneal effluent biomarker. These comprise the detection of the substance in peritoneal effluent, which should be locally produced within the peritoneal cavity. Furthermore, the candidate peritoneal effluent marker is involved in the pathology of peritoneal membrane and related to peritoneal dialysis-related clinical outcomes

Future Directions

As blood, serum, and urine samples are not representative for intraperitoneal events, the central emphasis remains to be on the peritoneal effluent. PD treatment induces a complex and multifactorial pathogenesis of the peritoneal membrane. The deficiency of this non-defined common pathway contributes to the difficulties in effluent biomarker discovery. These challenges can possibly be overcome with the unbiased field of omics technologies, where associations between clinical and expression data from confirmatory investigations may lead to the perception of underlying biological processes preceding peritoneal injury and novel biomarker identification. A suggested flowchart for effluent biomarkers discovery is depicted in Fig. 4 in which their prerequisites are integrated. To our knowledge, unfortunately no peritoneal tissue of patients treated with PD has been investigated by means of omics technologies. The presence of serial peritoneal tissue alongside peritoneal effluents of PD patients would be of great additive value and a prerequisite for omics-conducted PD investigation. Nevertheless, it is doubtful that an individual peritoneal

effluent biomarker possesses the ability to mirror or predict all of these processes. More likely is that a synergism of effluent biomarkers will eventually be identified in order to predict clinically relevant PD outcomes. Thus, large multicenter biobanks with preferably longitudinal data would contribute significantly to the discovery of novel effluent biomarkers and their validation. The number of omics-based research in PD is still limited, and the early phase of high-throughput technologies warrants standardization and calibration. Essential in the conductance of omics studies is systematic collection and storage of peritoneal effluent. To date, the absence of longitudinal effluent biobanks including peritoneal tissue and a small sample size has prevented the analysis of modifications in proteomic profiles over time. However, longitudinal multicenter studies such as the GLOBAL Fluid Study or within center biobanks will hopefully bridge this gap. Furthermore, collaborations are necessary to facilitate independent replication and validation of candidate effluent biomarkers.

In summary, peritoneal effluent biomarker discovery is moving toward system biology where a holistic approach may eventually lead to personalized-guided medicine within PD. Nevertheless, the challenge remains, and the actual application and implementation of omics-discovered effluent biomarkers is a process that may encompass decades.

Summary Points

- This chapter focuses on the emerging field of omics technologies within peritoneal dialysis (PD) therapy for the discovery of peritoneal effluent biomarkers.
- The peritoneal effluent can be regarded as a noninvasive liquid biopsy within PD therapy that is easily acquired after a (standardized) predefined PD exchange.
- The peritoneal effluent contains clinically relevant proteins and substances such as lymphocytes, macrophages, and a variety of proteins that mirror intraperitoneal events.
- The peritoneal effluent is susceptible to posttranslational modifications.
- Methodological precautions on a laboratory level as well as computational techniques are essential for proper assessment and interpretation of omics-derived data.
- The number of studies that apply high-throughput laboratory techniques with the peritoneal effluent as biospecimen within PD therapy is still limited.
- Peritoneal effluent biomarker discovery is moving toward systems biology.

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