Effluent biomarkers in peritoneal dialysis: A captivating symphony from the peritoneal membrane

Lopes Barreto, D.

Publication date
2014

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
SUMMARY AND FUTURE PERSPECTIVES
Effluent biomarkers may be considered as powerful utensils, as they provide insight into anatomical intra-peritoneal processes in a non-invasive manner. Moreover, the functionality of the peritoneal membrane is not inherent to its morphology. When effluent biomarkers are used as complementary instruments for peritoneal integrity assessments, a more complete picture of the functional and morphological state of the peritoneal membrane will be gained.

The current thesis contributed to the field of effluent biomarker discovery in PD therapy through identification of candidate effluent biomarkers, characterization of the more established biomarkers and by providing insight into their clinical applications and validity. In this final section the presented effluent biomarkers are discussed and recommendations for future implementation strategies are proposed.

10.1 Part I Fundamentals
The aim of the first part of this thesis was to provide an overview on current developments within PD effluent biomarker discovery and to address the significance of novel and more established biomarkers.

Formerly, biomarker discovery was mainly hypothesis-driven. Since the induction of high-throughput technologies, the discovery of effluent biomarkers in PD is evolving with a more exploratory approach. In the review of chapter 2 genomic, metabolomic and proteomic studies with the peritoneal effluent as human specimen are discussed. Also, methodological considerations are addressed. It is possible to extract DNA from the peritoneal effluent, which could enable insight into potential genes that are at the foundation of the large variability observed between PD patients in terms of peritoneal transport after initiation of PD treatment. More interesting are the proteins and metabolites which function as mirrors and modify over time to intra-peritoneal events accordingly. Dissimilarities in these protein levels of locally produced peritoneal substances might indicate candidate effluent biomarkers for PD-related outcomes. Even though, the application of high-throughput technologies in peritoneal effluent is very promising, an immense challenge has yet to be overcome.

The peritoneal effluent is the most clinical relevant specimen in PD therapy containing a pool of substances which might be identified as effluent biomarkers. Therefore, chapter 3 covered the recent advances in effluent biomarker research and the potential use of emerging effluent biomarkers in monitoring PD patients. This was achieved by evaluating the advantages and limitations of each marker. Furthermore,
criteria were defined for the classification of effluent biomarkers. These requirements included the detection in peritoneal effluent, the presence of local peritoneal production, involvement in pathological mechanisms of the peritoneal membrane and preferably good estimates of sensitivity and specificity for PD-related clinical endpoints such as encapsulating peritoneal sclerosis (EPS). The inclusion of effluent biomarkers in combination with peritoneal function tests, in the clinical follow-up of PD patients, is an ambition for the near future.

10.2 Part II Identification and Characterization
The intention of Part II was to identify candidate effluent biomarkers based on the pre-specified criteria from chapter 3 and to characterize the most investigated effluent biomarkers within PD even further.

The biologic variability of peritoneal effluent biomarkers is an important issue, because it is indicative for the clinical utility and dispersion within PD patients. Nevertheless, to date investigations on the variability of effluent biomarkers are sparse. The more established effluent biomarkers in PD are cancer antigen 125 (CA125) and interleukin-6 (IL-6). However, their inter- and intra-individual variability when measured in non-standardized conditions is unknown. By means of the coefficient of variation, the biological variability of CA125 and IL-6 was determined in chapter 4. This study included seventeen short-term and thirteen long-term PD patients within our unit. Up to six consecutive dialysate specimens from the long day or overnight dwell were collected within a period of at least one year. Additionally, a random selection of eight PD patients, who underwent a standard permeability analysis (SPA), was employed for the establishment of effluent CA125 and IL-6 release patterns. Both effluent biomarkers a linear increase during the four-hour SPA was found. Hence, appearance rates (AR) could be calculated with the use of unstandardized dwell-times. A comparison between the two groups revealed low levels of effluent CA125 and elevated levels of effluent IL-6 in long-term PD patients. The overall intra-individual biological variability as the main outcome was 15% for AR of CA125 and 28% for IL-6 AR.

Over time vasculopathy arises within the peritoneal capillary bed due to PD therapy. Alterations within this core component of peritoneal transport may lead to a loss in peritoneal membrane function. Nowadays it is difficult to identify PD patients at risk for these vascular abnormalities. However, it is conceivable that endothelial dysfunction is the foremost preceding mechanism. In chapter 5 soluble E-Selection and vascular cell
adhesion molecule-1 (VCAM-1) were objectified as indicators of systemic and peritoneal endothelial dysfunction. Previous studies have reported on serum levels of these markers in PD patients, but no study has provided data concerning the effluent levels of E-Selectin and VCAM-1 and their value in the intra-peritoneal assessment of endothelial dysfunction. For this purpose, four patients groups were defined, characterized by extremes in terms of PD therapy duration and ultrafiltration capacity. This study showed the presence of soluble E-Selectin and VCAM-1 in peritoneal effluent. Additionally, the second criterion that a biomarker has to fulfil, comprising of local production within the peritoneal cavity, was evaluated by two methodologies: calculations based on molecular weight or free diffusion coefficient. Local production was found for soluble E-Selectin whilst the presence of VCAM-1 in peritoneal effluent was mainly due to systemic contributions.

Currently, no effluent biomarker has been identified being indicative for the degree of peritoneal fibrosis. Merely cross-sectional studies related the presence of matrix metalloproteinase-2 (MMP-2) in peritoneal effluent or plasminogen activator inhibitor-1 (PAI-1) in peritoneal tissues to peritoneal tissue remodelling and coagulation. In chapter 6 the presence and time course of MMP-2 and PAI-1 in incident PD patients treated solely with the more biocompatible dialysis solution Physioneal® was investigated. Primarily, the presence of local peritoneal production were assessed. A positive discrepancy was present between the measured effluent levels and the predicted levels. The effluent concentrations of MMP-2 and PAI-1 attributed to local production were respectively 90% and 74%. As this first requirement was met, the analyses were extended by exploring associations with parameters of peritoneal transport in a transversal way. AR of PAI-1 correlated strongly with free water transport (FWT), where high levels of PAI-1 AR were accompanied by low quantities of FWT. Both markers were also positively associated with peritoneal inflammation, as expressed by effluent IL-6 AR. The time-course assessment indicated a stable trend for MMP-2 AR, while an increasing trend was observed for PAI-1 AR. In addition, a rodent EPS-like model was used for in vivo verification by examining the relationship between peritoneal fibrosis score and the effluent levels of these potential biomarkers. Unfortunately, due to the absence of a biochemical PAI-1 assay suitable for the rat species, the verification was only possible for MMP-2. In vivo, high levels of effluent MMP-2 corresponded with an increased degree of fibrosis.
10.3 Part III Clinical Application

A final requirement for an effluent biomarker is to have a clear application for routine clinical practice in PD therapy. Before implementation, associations should be present with PD-related outcomes and evidence for clinical validity is essential. Part III was therefore focussed on the specific entities of the most promising effluent biomarkers discussed in this thesis. Their potential clinical application was concluded by making risk estimations and by appraisal of diagnostic accuracy measures.

PD-related technique failure due to membrane dysfunction is a phenomenon that is generally observed from two years onwards. The efficiency of ultrafiltration might indicate a decrease or reflect induction of more permanent anatomical modifications. The first component encountered and affected by PD solutions is the mesothelium. It is plausible that a dysfunctional mesothelium is the antecedent of PD technique failure due to malfunction of the peritoneal membrane as intracorporeal dialysis system. The association between levels of effluent CA125 and PD technique failure was thus explored in chapter 7. The effluent biobank of the Dutch prospective multicenter cohort NECOSAD (Netherlands Cooperative Study on the Adequacy of Peritoneal Dialysis) allowed retrospective determination of CA125 levels. Whereas, the prospectively measured CA125 levels, as routine patient care within our unit, enabled a five-year follow-up study including adult incident PD patients. Technique failure was defined as a permanent switch from PD to HD. To assess the risk of technique failure, association measures were calculated for both study populations based on continuous or low versus high effluent CA125 levels. These analyses were adjusted for age, gender, comorbidity and PD duration. Moreover, a competing risks analysis was executed for PD technique failure in the study population from our center as replication cohort. The point estimate from the case-control study nested in the longitudinal PD cohort from the NECOSAD (n=38) indicated a six-fold increased risk for PD technique failure in patients exhibiting CA125 levels below 14.0 kU/L. Even though the small number of patients results in wide confidence intervals, the point estimate of more than six does not designate a neglectable effect by means of unmeasured parameters. In the independent validation cohort (n=91) from our center the low event rate potentially prevented the statistical replication of the findings from the NECOSAD. Nonetheless, a hazard ratio of three was found for PD patients with CA125 levels below 12.0 kU/L, pointing towards a similar increased risk for PD technique failure after a minimum PD duration of two years.

As a consequence and in extension of chapter 6 where the body of evidence
suggests that MMP-2 and PAI-1 could be used as effluent biomarkers for intra-peritoneal alterations, the clinical validity of MMP-2 and PAI-1 in patients who developed EPS was evaluated in chapter 8. EPS is a rare, but detrimental complication of long-term PD therapy that is characterized by a dense cocoon of fibrous tissue covering the abdominal organs. Eleven patients have been diagnosed with EPS over an approximately twenty-year time period in our centre. The focus was to assemble a specific control subpopulation with a minimum PD therapy exposure of 57 months. In this manner an attempt was made to control for the potential confounding effect of PD duration and to increase the internal validity. Time-courses were established for both effluent markers with a lag time of maximum four years, adjusted for age and PD duration. Diagnostic accuracy measures were estimated by means of time-specific receiver operating characteristic (ROC) curves. As overall summary of diagnostic accuracy the area under the curve (AUC) was employed. The trend analysis demonstrated a considerable overlap in levels of MMP-2 AR between the cases and controls. In contrast, PAI-1 AR was elevated throughout the four years preceding EPS as compared to the long-term PD patients. The time-specific ROC-AUC analyses indicated a fair discriminative ability of PAI-1 AR from three years prior to diagnosis of EPS, whilst no distinction could be made between long-term PD patients and those who developed EPS for MMP-2 AR. Postulations for the absence of a different time trend of MMP-2 AR could include the biological function, because it is considered as a tissue remodeling stimulant. Furthermore, the rodent model as presented in chapter 6 in which an attempt was made to construct an EPS-like rat model was induced by either chlorhexidine solutions or a second hit. It is under debate whether the induction by chlorhexidine leads to severe inflammation and increase in the peritoneal permeability as reflected by the high peritoneal transport rate in these models rather than peritoneal fibrosis. Moreover, the correlation between de fibrosis score and levels of MMP-2 could also reflect the premature phase of fibrinogenesis, where MMP-2 promotes cell invasion and migration. Consequently, the focal point was solely on PAI-1 AR, where various threshold values were presented for PAI-1 AR based on the optimal balance between estimates of sensitivity and specificity or either high in sensitivity or specificity.

The detection of pre-clinical EPS could provide opportunities to intervene in early stages and potentially prevent the development of EPS. However, timely identification of EPS is hampered by the paucity on diagnostic instruments. The effect of long-term PD therapy on the peritoneal membrane is noticeable on a functional as well as anatomic level. The complementary nature of these alterations points to a methodology that
specifically encloses both parameters. For that reason, a diagnostic panel consisting of FWT and effluent AR of CA125, IL-6 or PAI-1 was constructed in chapter 9 for the early identification of EPS. FWT and perhaps the osmotic conductance are the only functional elements that indicate a different time-course between patients who develop EPS and the long-term PD patients. Morphologically, the observed dissimilarities comprise the mesothelium, degree of inflammation and fibrosis. To address these morphological factors, CA125, IL-6 and PAI-1 were used. First, time-specific ROC-AUC analyses were performed to assess the discriminative capacity of the individual parameters. Second, sensitivity and specificity were estimated based on pre-specified false positive and true positive rates. The corresponding level of each parameter was consequently utilized to indicate a test positive or test negative result. Finally, the diagnostic panel was arranged by combining FWT with either AR of CA125, IL-6 or PAI-1. From a lag time of three years and onwards, the discriminative capacity was most optimal for FWT followed by AR of PAI-1. As EPS is a rare disease in which the ideal treatment is thus far not established, the specificity was optimized in the composition of the diagnostic panel. For all panels the estimates of specificity was above 84%, with accompanied sensitivity estimates ranging from 63% to 100%. The most promising diagnostic panel for the early identification of EPS patients comprised FWT and PAI-1 AR.

10.4 Conclusions and Future Perspectives
A variety of candidate effluent biomarkers in PD have been addressed in this thesis. Their importance as reflectors of intra-peritoneal events, arranged by different pathophysiological pathways, has been discussed. Each one appeared a distinct and specifically representative of certain entities of the peritoneal membrane. Effluent biomarkers interact at different moments and take part in the greater whole of peritoneal membrane deterioration due to the unnatural exposure of dialysis solutions. Eventually, some will have more prominent roles and some of the effluent markers are only to orchestra intra-peritoneal events. However, the interplay of locally and non-locally produced substances are all necessary to maintain the harmony within the peritoneal membrane in order to serve long-term PD therapy. However, as every symphony is composed by different themes and cadences, the tone determines its disposition.
The conclusions that can be drawn from the current thesis are as follows:

1. Effluent biomarker discovery is moving towards systems biology.

2. A number of effluent biomarkers are available, yet nowadays their clinical implementation in the routine care of PD patients is marginal despite the promising properties.

3. The dispersion between PD patients in levels of effluent IL-6 is greater than CA125, which is favorable for discriminative ability. However, the large intra-individual coefficient of variation of IL-6, being 28%, hampers the interpretation of a single effluent measurement.

4. Local production of candidate effluent biomarkers should ideally be assessed by free diffusion coefficients as calculations based on molecular weight might represent an underestimation of the effluent levels attributed to local intra-peritoneal release.

5. Due to limited variation in the expression levels of effluent CA125 by mesothelial cells, it is difficult to identify EPS patients. Nonetheless, CA125 levels below 14.0 kU/L or 106.0 U/min are associated with an increased risk for PD technique failure.

6. Effluent MMP-2 is unsuitable as a diagnostic biomarker for EPS, but might be a risk factor for anatomical peritoneal membrane modifications.

7. Effluent PAI-1 has an increasing time-course with duration of PD treatment and is capable to distinguish long-term PD patients from those who develop EPS. Potential clinical applications for effluent PAI-1 comprise screening of long-term PD to appraise the degree of peritoneal fibrosis and diagnostic purposes for the early detection of EPS.

8. FWT, as only discriminative functional parameter, in alliance with either AR of CA125, IL-6 or PAI-1 can be regarded as a biomarker panel for the early identification of EPS patients.
Without invasive procedures, there is limited access to the visualization of the peritoneal membrane morphology. PD therapy offers the unique possibility to measure substances which mirror pathophysiological events occurred due to the therapy. The quantity of relevant substances offers a sea of information. However, due to intrinsic and extrinsic impediments these proteins are not always regarded as relevant. These include non-detectible dialysate levels due to absence or low sensitivity of biochemical measurements, nonappearance of local production, large intra-biological variability or insufficient diagnostic accuracy. Moreover, whilst the effluent is easy accessible, the absence of longitudinal effluent biorepositories limits the number of available studies reporting and validating emerging effluent biomarkers. This serial assembly of peritoneal effluent is of major importance to enable biomarker discovery in an unbiased fashion especially when the effluent is prospectively collected under standardized circumstances and with no ascertainment on clinical relevant outcomes in PD due to peritoneal membrane dysfunction on a morphologic level. In addition, the scarce amount of biomarkers used in clinical practice is mainly due to the lack of data on their clinical utility and external validation.

The translation from discovery to clinical application of effluent biomarkers may encompass decades. However, effluent biomarkers could be measured prospectively for monitoring and permit preventive care within PD therapy. Although no preventive strategy is available, case reports have suggested potential treatment by steroids or tamoxifen. Retrospectively, effluent biomarkers could be determined when there are clinical symptoms in order to confirm EPS diagnosis or to aid decisions when to discontinue PD therapy. A suggested continuum for monitoring PD patients is mentioned below:

1. Yearly peritoneal transport function assessments by standardized four-hour dwell with a 3.86% dialysis solution with temporary drainage, including FWT_{0-60} and dialysate specimen storage for future biomarker determinations

2. Prospective effluent CA125 determination from two years onwards

3. Retrospective measurements of effluent biomarkers in long-term PD patients of which currently PAI-1 seems to be the most promising effluent biomarker
4. Evaluation of individual time-courses of effluent biomarkers e.g. CA125, IL-6 or PAI-1 in combination with the magnitude of FWT_{0.60}

Future studies could aim at crystallizing the suggested panels with respect to improvement in related health outcomes such as a reduction in morbidity and mortality in the first years after the diagnosis of EPS. Caution is warranted with regard to lead-time bias in which an artificial survival benefit may arise due to the early detection of EPS. However, these analyses are only possible with a substantial number of EPS cases and when dialysate specimens are prospectively collected and archived. The focus of the European EPS registry is to expand the knowledge of pathophysiological processes related to EPS. However, often the diagnosis of EPS remains like a bolt of lightning out of heaven. Therefore, it would be of additive value to extend this registration by the inclusion and follow-up of PD patients with late ultrafiltration failure.

Incorporation of effluent biomarkers, in alliance with peritoneal transport function tests, into the clinical practice within PD therapy will lead to a more personalized medicine.