As long as there are patients suitable for,
and interested in home dialysis,
there will be an important role for peritoneal dialysis.

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SUMMARY

Damage of peritoneal membrane is the most serious event in PD, as it jeopardizes the organ on which the whole treatment modality is based. The main focus in this thesis is to explore causes of peritoneal remodeling evoked by installation of PD fluids from the early start of PD therapy as well as in prevalent PD patients with special attention to use of biocompatible PD fluids. Additionally, we studied a pharmacological intervention option using vitamin D to prevent peritoneal remodeling.

In **Chapter 1** we gave an overview of *ex vivo*, animal and human studies that have provided, over the past decades, more insight into the modifying processes of the peritoneal cavity upon instillation of PD fluid.

In **Chapter 2 and 3** we showed that the peritoneal membrane reacts immediately after insertion of a PD catheter with the production of pro-fibrotic factors and inflammatory cytokines. After initiation of installation of PD fluid the production of these factors is enhanced further and to the same extent in conventional and neutral-pH, bicarbonate/lactate-buffered PD fluid at this early stage in PD therapy. Furthermore, we showed a decrease in residual renal function after use of conventional fluids. Finally, higher hsCRP levels and lower D/P creatinine values in neutral-pH, bicarbonate/lactate-buffered PD fluid six weeks after catheter implantation.

In **Chapter 4** we studied a prevalent PD cohort comparing conversion to a neutral-pH, bicarbonate/lactate-buffered PD fluid with continuation on conventional PD fluid. After conversion to neutral-pH, bicarbonate/lactate-buffered PD fluid we found no effect on RRF during a follow-up of 2 years, but change in UF rate during PET was more modest. In addition, peritonitis incidence was lower. There was no difference in other outcomes such as, peritoneal membrane function and effluent markers of inflammation.

In **Chapter 5** an overview is given of various studies that have applied pharmacologic interventions aimed at regression of peritoneal damage and prolongation of PD treatment.

In **Chapter 6** we studied the effect of paricalcitol on UF, fibrosis and angiogenesis in a non-uremic, vitamin D sufficient, rat PD model. Loss of UF, increase in extracellular matrix thickness, angiogenesis and IL-5 levels due to PD fluid exposure were significantly attenuated by paricalcitol treatment.

In **Chapter 7** we performed a pilot study in PD patients investigating the effect of active vitamin D in PD patients. We found no specific benefit of active vitamin D_3 in vitamin D_3-sufficient PD patients. Additional studies in preferably incident patients, with adequate PTH suppression in the intervention groups and, during a longer period are required to test beneficial effects of active vitamin D_3 over no treatment as well as to investigate whether in 25(OH)D_3 deficient PD-patients the type of active vitamin D_3 does matter.

In **Chapter 8** we measured effluent vitamin D in PD patients. We found that vitamin D can be lost in the effluent of PD patients. However, in the majority of effluents this was not the case. Several patient characteristics were analysed for association with peritoneal vitamin D losses. An association was found with serum 25(OH)D3 and, with effluent volume.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Since the introduction of PD as a form of renal replacement therapy in patients with end stage renal disease, a lot of research has been performed that has led to improvement in our understanding of the pathobiology of structural and functional changes involved in this treatment and has resulted in many innovations. Nowadays this home treatment, has a similar and even better survival as hemodialysis (1). Nevertheless, this treatment still faces challenges that concern preservation of the peritoneal membrane.

Peritoneal dialysis; the first weeks of treatment

In chapters 2 and 3 we showed that that the inflammatory response of the peritoneal membrane is already initiated by the placement of the PD catheter and continues with the installation of PD fluids. Apparently, the catheter as a foreign body exerts an inflammatory response. This finding is in line with similar studies performed in rats (2). Peritoneal biopsies would have further completed our understanding of the situation in the peritoneal cavity in early PD. Unfortunately, successive biopsies are difficult to obtain in humans. The question remains whether development of other type of catheters using coating techniques (3) could result in a less inflammatory intraperitoneal milieu since insufficient attention has been given to this topic over the past two decades. Besides the inflammatory response the catheter exerts, later on in PD treatment a biofilm is created on PD catheters (4). Therefore, we tried to obtain more insight into the exact interplay between catheter, it's possible biofilm and PD fluids.

We found that in the first weeks of PD a cytokine storm is present in the peritoneal cavity. Most probably due to the presence of the catheter as first hit, and the introduction of PD fluids as second hit. We could not find differences in the reaction (measured by biomarkers and cytokines) of the peritoneal membrane between conventional and more biocompatible the study. This finding may suggest that Physioneal's more biocompatible profile (better) enhances ongoing tissue regeneration of the peritoneal membrane after injury due to surgery. However, but this could not be demonstrated in differences in biomarkers or cytokines.

Interestingly, a recent study by Sampaio *et al.* gives more insight in this topic (5). These investigators found that after culturing of removed PD catheters from patients with and without infection the microbial density in biofilms on PD catheters was respectively 87% and 90%. This suggests two things. First, the catheter with the formed biofilm is a possible source of bacteria that can lead to the occurrence of peritonitis which leads to peritoneal damage. Second, the presence of bacteria per se does not always directly lead to a clinical

infection. There seems to be a certain crucial transition point in the host's intra peritoneal defense mechanisms that either does or does not lead to a clinical manifestation of infection e.g. peritonitis. This could be related to characteristics of PD fluids themselves. The same study looked into the impact of PD fluids on the micro-organisms' biomass on the PD catheter and found no difference between conventional and biocompatible PD fluids. The investigators did find that both PD fluids had a detrimental effect on *Coagulase negative Staphylococus* but had less effect on *P. aeruginosa*. Also, biofilm masses were lower when using icodextrin. This suggests that the presence of glucose in the PD fluids is an important factor in the development of a biofilm on the PD catheter. Altogether, these results and the findings in chapter 2 and 3 indicate that the catheter itself should not be perceived as an indolent bystander in the inflammatory response that develops in the peritoneal cavity after starting PD treatment. Furthermore, at least at the early start of PD the different types of PD fluid do not seem to exert a different reaction (measured by biomarkers) in the peritoneal cavity.

Peritoneal dialysis and biocompatibility

Chapter 1 gives an overview of the effect of PD fluids and its components have on the peritoneal membrane. In animal studies conventional fluids seem to be more deleterious to the peritoneal membrane which led to the development of more biocompatible fluids. However, the topic of biocompatibility in PD fluids and its long term effects on clinically relevant end-points remain controversial. In chapter 4 we added evidence to this subject in a cohort of prevalent PD patients. Our main findings included no difference in the effect on RRF during a follow-up of 2 years, a modest change in UF rate during PET and a lower peritonitis incidence after conversion to neutral-pH, bicarbonate/lactate-buffered PD fluid. There was no difference in other outcomes such as, peritoneal membrane function and effluent markers of inflammation. Some of our findings are a confirmation of earlier studies. Less loss of UF after two years in prevalent patients was seen earlier in the Bicarbonate/ Lactate Study (6) and the study by Choi *et al.* (7) both performed in prevalent PD patients. A lower increase in peritoneal solute transfer rate six weeks after starting PD therapy with biocompatible PD fluids was also shown earlier (8,9). We found a lower incidence of peritonitis which was also reported in the balANZ study. However, the main findings of recent meta-analysis in incident PD patients (10,11) were higher urine output and better preservation of RRF if the use of biocompatible PD fluids exceeded 12 months. No significant effects were found on peritonitis, technique survival or patient survival. An explanation for the discrepancy between our findings and those of the meta-analysis could be the lack of existing studies exclusively performed in prevalent patients. Furthermore, it is important, for any future study looking for perceived benefits of more biocompatible PD fluids, to be aware of the heterogeneity of effects that may be measured among the various formulations of biocompatible PD fluids since they differ in pH, GDP content and buffer, as stated in chapter 1 of this thesis. It is important to realize that in the meta-analysis data

of studies which used different types of biocompatible fluids from different manufactures were pooled and therefore may have resulted in inconclusive or negative findings. Careful examination of PD fluids from different manufactures is necessary before pooling of data occurs. In order to do so, lager studies are needed.

Furthermore, in improving PD fluids, manufacturers have focused on changes in buffer (by replacing lactate by bicarbonate) and lowering the concentration of glucose degradation products (by developing multi-compartment bags). And yet, it's the peritoneal absorption of glucose during PD that has both cytotoxic and systemic metabolic effects as described in chapter 1, and, therefore, seems to be an important culprit of peritoneal damage, which highlights the need to explore another osmotic agent than glucose. Interesting in this respect are research results concerning hyperbranched glycerol in a PD rat model during long term PD that showed better preservation of the peritoneal membrane (less neutrophil infiltration and reduced thickness of the submesothelial compact zone) with better ultrafiltration than glucose (12). However, it seems that hyperbranched glycerol accumulates in the reticulo-endothelial system over time (13). Therefore, more studies are needed to explore the use of hyperbranched glycerol instead of glucose e.g. as daily single bag use.

In general, future investigators should consider one important limitation of our study and of many other PD studies, being the low number of patients included and finishing the studies, as well as the limited follow-up time of those studies. In order to achieve greater numbers of patients, the PD community should engage in more collaboration. Another advantage of larger studies could be to further analyze evidence that characteristics of the peritoneal membrane and clinical outcomes for PD patients have genetic determinants (14).

Peritoneal dialysis, pharmacological targeting and vitamin D

In the PD research community attention has been directed not only to the development of more biocompatible PD fluids and to the search for glucose alternatives, but also to the potential beneficial effects of pharmacologic agents.

In chapter 5 we reviewed various studies that have investigated pharmacologic interventions aimed at regression of peritoneal damage and prolongation of PD treatment. One immunomodulatory agent that was not very well investigated so far, is vitamin D. Evidence suggests that vitamin D_3 may play a direct role in the pathophysiology of inflammation, angiogenesis, and differentiation of many cell types. Vitamin D_3 has anti-inflammatory, anti-angiogenic and anti-proliferative effects (15). In chapter 6 we tested the rationale behind the use of vitamin D as an agent in preventing peritoneal remodeling in a rat model using paricalcitol. We showed that loss of ultrafiltration capacity, increase in extra cellular matrix thickness, angiogenesis, and rise in IL-5 levels due to exposure to PD fluids were significantly attenuated by paricalcitol treatment. Moreover a trend towards lower levels of HA, TGF-β, VEGF, IL-12, and IL-4 was also seen when using paricalcitol.

These results were the basis for the multi-center open label randomized clinical trial examining the effects of paricalcitol versus calcitriol on peritoneal remodeling in PD patients described in chapter 7. This study was set-up as a pilot study to obtain preliminary data on clinical outcome parameters to justify or not the set-up of a larger clinical trial.

We found no specific benefit of active vitamin D_3 in vitamin D_3 sufficient PD patients on peritoneal transport characteristics. In both groups no incidents of peritonitis were reported which could be seen as a confirmation of vitamin D_3's antimicrobial effects (16), although the observation period for incident episodes of peritonitis was short. Although this is a remarkable finding, this was not a pre-determined endpoint. However, our study had several limitations. The main one was the lack of a placebo or no treatment group. Furthermore, additional studies in preferably incident patients, with an adequate PTH suppression in the intervention groups and, during a longer period are required to test beneficial effects of active vitamin D_3 over no treatment and to investigate whether in 25(OH)D_3 deficient PD-patients the type of active vitamin D_3 does matter. Of interest would be the addition of vitamin K which also has anti-inflammatory properties and synergizes effects of vitamin D (17).

In chapter 8 we investigated whether the lack of effect of vitamin D on peritoneal transport parameters had to do with vitamin D loss via the peritoneal membrane.

Using a contemporary, standardized method, our study confirms that PD patients can lose 25(OH)D_3 in the effluent However, this phenomenon does not lead to systemic vitamin D deficiency. Compared to the only other study performed on this topic, our cohort had far lower losses of 25(OH)D_3 than those in the study by Sahin *et al*. (18). An important difference between the two studies was the method by which 25(OH)D_3 was measured. Maybe more importantly, the viability of the peritoneal membrane could have been even more compromised in Sahin's group because of a longer PD vintage compared to our patients. This could suggest that longer time on PD led to peritoneal damage and subsequently higher peritoneal 25(OH)D_3 losses due to peritoneal remodeling because of longer exposure to PD fluids. Finally, our patients were not vitamin D deficient which seems to be a beneficial consequence of the current, routine practice of vitamin D supplementation in PD patients.

Recently, attention has been drawn to aquaporins as a therapeutic target to increase ultrafiltration(19). Aquaporin are water channels in the peritoneal endothelial cells. Steroids seem to increase the expression of aquaporins and arylsulfonamide is the first pharmacologic agonist of aquaporin 1 and enhances water transport and net ultrafiltration in rodents (20). It could be a subject of interest to further investigate aquaporins as a target for pharmacological intervention.

Peritoneal dialysis and biomarkers

Other findings in this thesis concern the levels of effluent biomarkers (chapter 2, 4, 6,7). Effluent biomarkers are seen as representatives of the state that the peritoneal membrane

in the absence of peritoneal histology. Striking in our studies is that we found no significant differences in biomarkers between biocompatible and conventional PD fluids. Difficult in the interpretation is that many biomarkers are produced during acute inflammation (peritonitis) (21) but also during chronic stable PD treatment. Furthermore, changes in the levels of many biomarkers reflect the peritoneal morphological changes at a very late stage (22).

In case of the study in chapter 2 during the first weeks of PD treatment, one might argue that this period was too short and not stable due to the insertion of the PD catheter. Therefore, biomarkers are more difficult to interpret.

In chapter 7, we also found no differences during six months follow-up during stable PD treatment. Likewise, in the RCT in chapter 4 with a two year follow-up no differences were found either. The question arises what the value is of biomarkers in understanding peritoneal remodeling and if they have, did we measure the right ones? Interesting is the comparison between the study in chapter 4 and the recent study by Jung et al. who showed in incident patients lower CA125 and higher macrophage migration inhibitory factor levels, 6 months after a switch from a low glucose, low GDP regimen (PEN) to conventional PD fluids (23). In chapter 4, we did the opposite. We switched from conventional to biocompatible in prevalent patients and found no difference in CA125 or other biomarkers. This might suggest that with time, exposure to conventional PD fluids causes so much damage that healing of the peritoneal membrane (measured as increase in CA125) is not possible anymore and, therefore, an increase in CA125 was not seen in our study. However, the balANZ trial (24), a RCT in incident patients with a 2 year follow-up similar to our study in chapter 4, found increases in metalloprotease and it's inhibitors in relation to time on PD but no difference between conventional and biocompatible fluids. This in accordance with our findings. It suggests that with time a possible benefit of biocompatible PD fluids fades out. Furthermore, the interpretation of biomarkers tends to be difficult. In the study by Jung *et al*. lower levels of IL-6, a pro-inflammatory marker, were measured after the switch to conventional fluids. An increase in IL-6 and IL-8 was seen earlier with the use of the NEPP-regimen (25). Researchers have suggested that the finding of higher IL-6 levels when using low glucose and low GDP PD fluids actually indicates better preservation of the peritoneal membrane since in these studies higher levels of IL-6 were accompanied by higher levels of CA125, which is considered to be marker of viable mesothelial cell mass (26,27).This, by the way, implies another example of debate with regard to the interpretation of biomarkers: the understanding of CA-125. Since the study by Visser *et al.,* dialysate CA125 is considered to be a bulk marker for the mesothelial cell mass in stable PD patients and can thus provides data on the state of the peritoneal membrane in the follow-up of the individual patient (28). Still, it has been suggested that high levels of CA125 as seen during peritonitis, actually represent (subclinical) inflammation and mark mesothelial cell death (22). These differences in opinion indicate that we still do not have a clear view on the meaning and clinical value of individual biomarkers and their interactions. In this context, the Global Fluid study is a good example of an effort to obtain

more insight in these processes (29). This large study (n=959) with a follow-up of 8 years showed that systemic and local peritoneal inflammation are distinct processes and have different consequences in PD patients. Systemic inflammation independently predicts survival. In contrast, intraperitoneal inflammation is the most important predictor of loss of peritoneal small solute transport rate over time. Unfortunately, only 19% in incident and 16% in the prevalent cohort that were followed over the years used biocompatible PD fluids. This may be the reason for the lack of analysis in this subgroup to date. This study, did not lead to a specific tool or set of tools that can be used in clinical practice to predict outcome. Nevertheless, we can conclude that with the current knowledge the biomarkers we measured are significant ones, since they are well correlated to processes that lead to peritoneal remodeling (30).

It is clear that PD treatment induces a complex and multi-factorial pathogenesis of the peritoneal membrane in which many different cytokines and biomarkers play a role. But their individual clinical relevance and/or predictive value remains unclear. It is doubtful that one single biomarker will be able to properly reflect all the processes that occur during PD treatment. It is possible that a new approach in the understanding of biomarkers is needed. We might need to start looking more closely at patterns of different biomarkers measured over time, e.g. beginning at the start from PD therapy and have longitudinal follow-up of these markers. This should be done in large groups over a long period of time to be able to connect them to clinical endpoints. This is also the conclusion of a consensus paper on biomarker research written by the head investigators of the EuTRiPD network (31). Again, this will only be achievable if the PD community comes together to collaborate in research networks such as EuTRiPD and the Global Fluid Study group.