Functional and immunological studies in children with chronic renal failure: the effects of uremia and dialysis treatment
Bouts, A.H.M.

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Chapter 11

General Discussion and Summary
GENERAL DISCUSSION

Chronic renal failure and its treatment disrupt many aspects of children's normal life. Therefore, optimal treatment, and prevention of the complications caused by the disease or the treatment procedures, without impairment of the psychosocial development, needs to be pursued. Infections are major complications of chronic renal failure leading to significant morbidity and mortality. The use of peritoneal dialysis, which is the most popular dialysis technique in the Netherlands, is limited by progressive loss of the peritoneal permeability and by peritonitis episodes. In this thesis, several functional and immunological studies in children with chronic renal failure, either dialyzed or not, have been described, that might give a better understanding of the infectious susceptibility in these children.

Characteristics of peritoneal transport and membrane function.

The first published study on transperitoneal solute transport in children treated with peritoneal dialysis (PD) is that of Gruskin et al. in 1982 [1]. Since then, several studies have been described on this subject in PD children, in relation to age or peritonitis incidence, and in comparison with adult PD patients [2-7]. However, the results of these studies were conflicting. The belief that peritoneal transport is higher in children, is likely to be caused by differences in standardization of the test method. The assessment of peritoneal transport in PD children has mostly been done by the peritoneal equilibration test (PET), in which dialysate over plasma ratios (D/P) for urea and creatinine, or dialysate over dialysate ratios (D/D) for glucose are used. A modification and extension of this test, the standard peritoneal permeability analysis (SPA), has been described by our group in adult patients [8]. In this test, peritoneal transport of low molecular weight solutes is analyzed by mass transfer area coefficients (MTAC). Fluid transport is determined using intraperitoneally administered dextran, and peritoneal clearances of various serum proteins are calculated from which the restriction coefficient can be derived.

The results of the SPA in children were not essentially different from those obtained in adult patients. The MTAC and D/P ratios of low molecular weight solutes are dependent on the vascular surface area of the peritoneum [9]. Since the vascular surface area is closer related to the body surface area (BSA) than to body weight [10], the instilled test volume should be calculated in milliliters per square meter (mL/m$^2$) instead of mL per kilogram (mL/kg). The PET in children was mostly performed with a test volume in mL/kg. When this volume is expressed per m$^2$, especially in small children, a more rapid equilibration occurs resulting in higher D/P ratios. Therefore, it is likely that when the instilled volume is calculated according to BSA, children will not have enhanced peritoneal transport capacity compared to adults, as has been described previously [11-13]. It has been demonstrated in adult patients that the MTAC are less influenced by the instilled test volumes than D/P ratios [14]. Exceptionally low intraperitoneal volumes may result in low MTAC values because the peritoneal membrane surface area might not been used completely [15]. The results of the comparison between the two methods, MTAC and D/P ratios, in children were similar to
those of adults. In both groups, a good correlation was found between MTAC and D/P, but the D/P ratios overestimated the MTAC values in the lower ranges and underestimated them in the higher ranges.

The peritoneal clearances of β₂-microglobulin, albumin, IgG and α₂-macroglobulin were not significantly influenced by age in our study. Quan and Baum found an inverse correlation between BSA and peritoneal protein loss expressed in mg/m² per day [16]. These authors suggested that the greater amount of protein loss may result from both, a higher permeability and a greater peritoneal surface area in children. The protein clearances we measured reflect the functional state of the peritoneum. These results cannot be extrapolated to 24-hour loss of proteins, because it is dependent on the dwell time and the number of bag exchanges. The protein clearance is higher during the first hour of the dwell time [17]. This is probably caused by a vasodilatational effect of the dialysis solution and results in an increase of the vascular peritoneal surface. We cannot conclude that a higher peritoneal permeability in children exists, because the restriction coefficients were not different between children and adults. However, measurement of the protein content in a 24-hour collection of dialysate of our patients will definitely resolve this question.

Long-term PD treatment might result in a decrease of peritoneal membrane function. In our cross-sectional study, the MTAC and D/P ratios of the low molecular weight solutes showed no change with time on PD treatment. Again, a more extensive longitudinal follow-up will clarify individual changes according to duration of PD treatment. With regard to the macromolecules, an increase of the restriction coefficient was found, indicating an increased size selectivity or a reduced peritoneal permeability for higher molecular weight solutes. These results are in agreement with those found in adult PD patients [18], but have not been reported in children previously.

In this thesis, the peritoneal mesothelial cell mass has been analyzed by the dialysate cancer antigen 125 (dCA125) concentrations. It has been demonstrated in adult PD patients that CA125 is a good marker of mesothelial cell mass [19]. The dCA125 concentrations in children were not different compared to adults. dCA125 levels in children treated with PD decline with the duration of dialysis treatment, similar to that found in adult PD patients.

It has been reported that children treated with PD have a higher peritonitis incidence than adults [20,21]. No major differences were found in the function of the peritoneal membrane between children and adults when transport and tissue markers are studied. Consequently, these factors cannot explain why children have more peritonitis episodes than adults. Other factors, such as immunological disturbances are also involved in the susceptibility to infections.

**Characteristics of the immune system.**

In this thesis, various parts of the immune system have been investigated to analyze the effects of uremia and dialysis treatment modalities on the host defense and its
relation with the susceptibility to infections. The analysis of immunoglobulins was the main subject of the immunological part of this thesis. A deficiency of one or more IgG subclasses was present in forty percent of children with chronic renal failure, before the dialysis treatment had started. IgG₂ was the major subclass affected. These findings were in agreement with those of Kemper et al. [22], but in contrast to those of Schröder et al. who found normal Ig levels before the dialysis treatment had started which decreased after starting PD [23]. Nevertheless, we found that PD children had indeed the lowest serum Ig levels, probably caused by peritoneal loss. Children with an IgG₁ deficiency had a higher median peritoneal IgG clearance compared to children with a selective IgG₂ deficiency. This cannot be explained by the differences in IgG subclass clearances, since the IgG₁, IgG₂ and IgG₄ peritoneal clearances are similar, whereas the IgG₃ clearance is lower, probably because of its higher molecular weight. The IgG subclass deficiency might be caused by a specific reduction of Ig synthesis, due to an impairment of the B cell function, to a decreased help of T cells or both. IgG₂ and IgG₄ synthesis require more T cell help than that of IgG₁ and IgG₃ [24,25]. IgG₂ deficiency is also a common finding in diseases with a T cell defect such as Di-George syndrome and AIDS [24]. In adult PD patients, renal insufficiency has been associated with both B and T cell dysfunction [26-29]. It is not known if, and how the nutritional status plays a role in the occurrence of IgG or subclass deficiencies. The role of serum IgG deficiency in the pathogenesis of peritonitis during PD is questionable. Studies in adults could not establish a relationship between the peritonitis incidence and IgG or subclass deficiency [30]. On the contrary, Kuizon et al. found a relationship between the two in children [31]. This could not be confirmed by our study. However, the patients with a high number of peritonitis episodes were all found in the group with an Ig deficiency.

Also, no relationship between the dialysate IgG concentration and peritonitis incidence was found. But, it might be that the function of IgG in dialysate is altered, caused by non-enzymatic glycation of IgG in the presence of glucose. This glycated IgG modifies the function of IgG [32]. We found a positive correlation between the percentage of glycated IgG, glucose concentration, pH and incubation time under experimental conditions. Significantly increased percentages glycated IgG were found only after an incubation time of 24 hours. This does not reflect the normal, in vivo situation, where maximal dwell times are about 11 hours. It has been demonstrated that the peritoneal interstitium may constitute a third compartment between blood and dialysate, in which equilibrium with blood and dialysate takes place for serum proteins [33]. Therefore, we suggest that IgG glycation takes place in the peritoneal interstitium for periods longer than normal dwell times. Phagocytosis was not decreased in the presence of glycated IgG. But, glycated IgG did induce complement activation, which might influence the anti-infective and inflammatory mechanisms leading to peritoneal sclerosis.

The presence of low serum Ig levels does not necessarily imply that the specific antibody response to antigens is also reduced. Therefore, the antibody response after pneumococcal polysaccharide vaccination has been studied. We found that the anti-pneumococcal polysaccharide IgG and subclass antibody response was reduced in a
substantial number of children with chronic renal disease. These children are considered to be at increased risk for pneumococcal infections. Non-responders were equally present in children with or without low IgG or subclass levels. This is in contrast to a study performed in children with recurrent upper respiratory tract infections with or without reduced Ig levels [34]. No significant differences were found between children after renal transplantation or on dialysis treatment, except for the lower antibody titer increase for serotypes 9 and 23 in children after transplantation compared to PD children.

IgG (FcγR) and complement (CR) receptors are essential for the binding of antibody- or complement opsonized microorganisms to phagocytic cells and the subsequent ingestion. This is the first study that compared FcγR and CR in blood and dialysate in stable PD children and during periods of infections. We found that a lower proportion of macrophages and neutrophils in dialysate expressed FcγR and CR, combined with an increased receptor density (MFI) when compared to the cells in peripheral blood. It has been reported that peritoneal cells, especially macrophages are relatively immature cells with signs of activation [35-37]. However, it is our opinion that the FcγR- and CR- negative population does not reflect young immature cells related to the high turnover through frequent bag exchanges, because peritoneal cells are derived from the blood where the majority of the cells is FcγR- and CR- positive. Thus, other factors are likely to be involved, such as the migration process of cells from the blood circulation into the peritoneal cavity, or the toxicity of the dialysate affecting the expression of FcγR and CR. During a peritonitis episode the MFI of FcγR and CR increased on blood monocytes except for CD32 (FcγRII). The MFI of FcγR and CR on neutrophils in blood did not increase. We were not able to compare these results with studies in non-uremic children with infections. Therefore, a comparison was made with a study performed by Leino et al. [38]. These authors found a significant increase in the expression of CD64 (FcγRI), CD32 (FcγRII) and CD35 (CR1), but not of CD16 (FcγRIII), and CD11b (CR3) in "healthy" individuals during localized bacterial infections. The differences between the two studies might be partially explained by the fact that not all of our patients had fever during peritonitis. It is speculative whether the FcγR and CR function, especially on neutrophils and for CD32, is diminished in PD children. In dialysate, the percentage receptor positive cells increased whereas the MFI increased only on macrophages (CD16, CD64 and CD35). The FcγR and CR expression on phagocytes in blood and dialysate of children with a peritonitis incidence above 1 episode per year was not different from those with less than 1 peritonitis episode per year.

The comparison of FcγR and CR expression on white blood cells between children with chronic renal failure, either treated with dialysis (PD or HD) or not (CRF) and healthy children showed that the expression of CD32 was reduced in children with renal failure, whereas the CD35 expression was increased. We suggest that these alterations might be the result of increased plasma levels of granulocyte-macrophage-colony stimulating factor (GM-CSF), since similar alterations were found in children with solid tumors who have been treated with GM-CSF [39].
Low serum levels of Ig might be caused by reduced numbers of total B lymphocytes, of Ig producing plasma cells (or memory cells), or by disturbances in either T helper cell numbers or T cell function. We found that the total number of B lymphocytes in the blood was lower in uremic children compared to healthy children. Furthermore, the number of memory type B cells, defined as IgM/IgD double negative or CD27 positive B cells, was also lower in our patients. This might contribute to the lower Ig levels and the reduced specific antibody response found in children with chronic renal failure. Whether this is the result of a T-helper cell defect is not clear. The number of CD4 positive T helper cells was not different, but the CD4/CD8 ratio was increased because of lower CD8 positive T-cell numbers. We were not able to demonstrate a disturbed TH1/TH2 balance in these children.

In conclusion, several abnormalities of the immune system have been found in children with chronic renal failure. However, the exact mechanism leading to infections in children with chronic renal failure remains difficult to establish. This is because the defense against infections is a multifactorial process that could only be studied in a heterogeneous group of patients.

REFERENCES


SUMMARY

In this thesis several studies are described that might lead to a better understanding of the mechanisms favouring the occurrence of infections in children with chronic renal failure. In chapter 1, a theoretical background is given of the subjects that were investigated and described. This is followed by a cross-sectional analysis of the peritoneal transport characteristics in children treated with peritoneal dialysis (PD), in chapter 2. The study has been done with the standard peritoneal permeability analysis (SPA), in which the transport of low and high molecular weight solutes and of fluid were calculated. A comparison was made with results of the most commonly used peritoneal equilibration test (PET) in children, and with the SPA in adult PD patients. The results of the SPA in children were not essentially different from those obtained in adult PD patients when corrected for body surface area. The dialysate over plasma ratios (D/P) in children overestimated the mass transfer area coefficient (MTAC) values in the lower ranges and underestimated them in the higher ranges. Thus, MTAC is a better method to evaluate peritoneal transport in children, since the MTAC is independent from exchange volumes and the dialysate glucose concentration. The restriction coefficient for macromolecules increased with duration of PD treatment indicating an increased size selectivity or a reduced peritoneal permeability for higher molecular weight solutes. It is unlikely that a higher peritoneal permeability to macromolecules, such as immunoglobulins, is present in children compared to adults.

Peritoneal mesothelial cells are important for the local host defense and membrane integrity. CA125, is a good marker for the mesothelial cell mass. In chapter 3, the CA125 levels in peritoneal dialysis effluent (dCA125) of children treated with PD have been analyzed. No influence of age on dCA125 was found. Levels of dCA125 declined with the duration of PD treatment, reflecting a reduction of the mesothelial cell mass. No relation was found between the dCA125 levels and transport parameters of low molecular weight solutes, or the peritonitis incidence in stable PD patients.

In chapter 4, the effects of dialysis modalities on immunoglobulins in children with chronic renal failure have been studied, both in a cross-sectional and a longitudinal analysis. A deficiency of one or more IgG subclasses was present in forty percent of children with chronic renal failure, already before dialysis treatment had been started. HD treatment did not result in normalization of low Ig and subclass levels. The deficiencies were especially marked in children treated with PD, most likely caused by loss of these proteins in peritoneal dialysis effluent. Children with an IgG1 deficiency had a higher peritoneal IgG clearance compared with children with a selective IgG2 deficiency. During a follow-up period of one year on PD treatment, no significant changes of serum Ig concentrations were found. Also the peritoneal Ig clearances showed no change in this period. This implies that the peritoneal loss of these proteins is compensated by their synthesis creating a steady state in the absence of peritonitis. During a peritonitis episode IgG levels in peritoneal dialysis effluent increase, but this did not result in significantly lower serum IgG levels. Serum IgM was even higher in the acute phase of peritonitis than after recovery, probably reflecting an increase in the synthesis rate of immunoglobulins. IgG subclass deficiency was not
associated with more frequent peritonitis episodes. However, a high peritonitis incidence was related to reduced IgG subclass levels.

IgG in peritoneal dialysis effluent may have an important role in the protective mechanisms against peritonitis in patients treated with PD. Incubation of IgG with high concentrations of glucose leads to irreversible non-enzymatic glycation end products or early glycosylation end products which might influence the anti-infectious properties of Ig. This has been studied and described in chapter 5. A direct relationship between the percentage of glycated IgG, glucose concentration and incubation time has been found in vitro. Furthermore, the percentage of glycated IgG was directly related to the pH of the dialysis solution. Functional studies showed that glycated IgG did not suppress phagocytosis by polymorphonuclear leukocytes but that glycated IgG could induce complement activation. The latter might result in a reduction of complement factors available in dialysate for adequate anti-infective mechanisms.

Because IgG or subclass deficiencies occur frequently in children with chronic renal failure, these children might be more susceptible to infections with polysaccharide encapsulated bacteria such as Streptococcus Pneumoniae. In chapter 6, the antibody response to six serotypes of the unconjugated pneumococcal polysaccharide vaccine (Pneumovax) in children treated with dialysis or after renal transplantation have been investigated. A considerable number of these children showed a reduced IgG and subclass antibody response to vaccination. Transplanted patients showed the lowest IgG antibody response to serotype 9V and 23F. The number of non-responders was not different between children with normal or low serum IgG or subclass levels. No significant differences were found in the IgG1 or IgG2 response of children with normal or reduced serum IgG or subclass levels. When vaccination is performed, the administration of a pneumococcal conjugate vaccine, followed by Pneumovax might give better protection.

The receptors for IgG (FcγR) and complement (CR) on leukocytes serve as a bridge between the humoral and cellular immunity. Binding of IgG or complement opsonized particles to these receptors lead to phagocytosis of microorganism. Expression levels of these receptors have been studied in children with chronic renal failure, treated with dialysis or not. In chapter 7, the FcγR and CR on neutrophils and monocytes/macrophages, both in peripheral blood and peritoneal dialysis effluent of PD children have been investigated in a stable situation and during a peritonitis episode. In stable PD children, peritoneal cells showed a lower percentage FcγR and CR positive neutrophils and macrophages combined with an increased mean fluorescence intensity (MFI).

During peritonitis, an upregulation of FcγR and CR expression on blood monocytes, but not on neutrophils, could be demonstrated, with exception of the FcγRII (CD32). In dialysate, the percentage FcγR and CR positive neutrophils and macrophages increased. With regard to the MFI, only CR1 (CD35) and FcγRI (CD64) expression on macrophages increased. In chapter 8, the effects of uremia and dialysis modalities on the FcγR and CR expression on leukocytes in blood have been studied and compared to the results in healthy children. In children with chronic renal failure, either with or without dialysis treatment, lower
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expression levels of the FcγRII on lymphocytes, monocytes and neutrophils were found, combined with increased levels of CR3 (CD11b) expression.

In chapter 9, peripheral and peritoneal white blood cells of uremic and healthy children have been characterized by a white blood cell differentiation and lymphocyte subset analysis. No significant differences in total leukocyte, lymphocyte and neutrophil count were found among the groups. Lymphopenia was found in only a few children. The monocyte count was lower in children with chronic renal failure without dialysis treatment and in PD children compared to healthy controls. A deficiency of B cells occurred in about 10% of uremic children. The median B lymphocyte count was also lower in uremic patients compared to healthy controls. HD children showed the lowest B lymphocyte count. A deficiency of T cells was barely present in the patients, but the ratio between T-helper (CD4) and T-suppressor (CD8) lymphocytes was increased. The T-helper cell count was higher in PD children than the children who were not yet treated with dialysis. A deficiency of natural killer cells was present in 14% of uremic children. Also the median numbers of natural killer cells were lower in these children compared to healthy children. No direct relationship could be found between these abnormalities and the occurrence of peritonitis, but it might contribute to the increased susceptibility to viral or bacterial infections and malignancies in these children.

Both, lower numbers of B lymphocytes and lower levels of immunoglobulins have been demonstrated in children with chronic renal failure in the studied reported in the previous chapters. To assess whether the B cell differentiation in these children is disturbed, an analysis has been performed on the maturational stage of these B cells which could result in a lower Ig producing capacity (chapter 10). Furthermore, the balance between T-helper-1 cells (Th1) and T-helper-2 cells (Th2), and the in vitro production of immunoglobulins by lymphocytes were analyzed. The number of memory B cells, classified as either CD27 positive cells or IgM-IgD double negative cells, was lower in children with chronic renal failure compared to healthy controls. The CD5 positive, naive B cells, were present especially in the peritoneal cavity. However, the PD treatment did not lead to altered CD5 positive B lymphocyte numbers in peripheral blood. The lower number of memory B cells could not be explained by differences in the Th1/Th2 balance. In vitro studies could not demonstrate a lower Ig producing capacity by lymphocytes from uremic children.

It can be concluded that most immunological and host defense abnormalities found in the present studies were caused by uremia itself and not by dialysis treatment. The major abnormalities that we found were: low serum IgG subclass levels, low numbers of B cells and memory type B cells, reduced pneumococcal antibody response, reduced FcγRII (CD32) expression on lymphocytes, monocytes and neutrophils, a disbalance of T-helper/T-suppressor ratio (CD4/CD8) and low numbers of natural killer cells.