Albuminuria: more than a renal risk marker?

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Summary and future perspectives

Summary

Elevated levels of albuminuria are present in the general healthy population, and highly prevalent in patients with diabetes and hypertension. In all these populations, higher levels of albuminuria are associated with a higher cardiovascular and renal risk. Therefore, albuminuria is considered an important risk marker. Lowering of albuminuria by different intervention strategies is associated with reduction of cardiovascular and renal risk, rendering albuminuria an important target for therapy. However, the mechanism by which albuminuria results in renal disease is not completely understood. There is an ongoing debate in nephrology whether albuminuria represents a consequence of disease, or a causal factor in progression of disease, or both. Both experimental and clinical data support the hypothesis that albuminuria can cause kidney damage. The hypothesis that albuminuria is not only a consequence of kidney disease is also supported by the fact that increased levels of albuminuria can occur already at a young age, in absence of renal or cardiovascular disease, suggesting that albuminuria might be an inborn characteristic. The availability of a treatment target which is already present at birth is highly important, as it could facilitate a shift from intervention in disease to prevention of disease. However, there is a paucity of data on albuminuria in toddlers and young children, indicating the need for further research in this area. This thesis has investigated different aspects of albuminuria in children, including the prevalence, measurement, and treatment of albuminuria, in order to increase the knowledge on this subject, and to enable further research in this area.

In **Chapter 2**, the natural distribution and prevalence of (micro)albuminuria was investigated in approximately 1300 toddlers from the observational GECKO cohort study. To our knowledge, this was the first study that describes albuminuria levels in young children on an epidemiological scale. In these children, we found a wide range of albuminuria levels and, intriguingly, the distribution in albuminuria levels was similar to the adult population. In fact, increased levels of albuminuria, so-called microalbuminuria, were found with a prevalence of 7% in the toddler cohort, which is strikingly similar to the prevalence in young adults. Another important finding in this study was that the use of creatinine correction for albuminuria (urinary albumin:creatinine ratio; U_{ACR}) leads to an extremely high prevalence of microalbuminuria, when using the cutoff of U_{ACR}>30 mg/g, proposed for adults. Probably this is due to the relatively low levels of urine creatinine in children as compared to adults, due to the lower muscle mass in children. According to this finding, U_{ACR} might still be a useful measurement in toddlers, but there is a need for standardized cutoff values for different age categories and possibly gender. None of the assessed potential determinants were found to explain the presence of increased albuminuria in these children. In conclusion, the findings in this chapter show that elevated albuminuria levels are already present at a young age, to the same degree as in adults. This suggests that increased levels of albuminuria are not the result of a

disease in later life, but represent an inborn characteristic, possibly determining fate of the individual in later life. More research needs to be done to determine the cause(s) of increased albuminuria at a young age.

The collection of urine in the above cohort was rather cumbersome and performed with the use of pantyliners in diapers. To improve the quality of the collected data in future studies, better collection techniques are needed. **Chapter 3** compared the performance of different urine collection methods in young children, in order to identify the most reliable, practical and comfortable method. The pantyliner method, which was used in chapter 2, was tested against the newly developed PeeSpot method, and compared to a reference sample (the "standard" method), which was an unprocessed urine sample stored at 4°C. These three methods were compared using the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for method comparison. Wearing of the diaper and time for sending to the laboratory were simulated by storing the pantyliners and PeeSpots at different temperatures, to ensure that the results accurately represent the in vivo situation. The results show that the PeeSpot device has a very high analytical performance, with a small bias, high precision and high accuracy. The PeeSpot performed markedly better in measuring urinary albumin compared to the pantyliner method. Moreover, the PeeSpot had other advantages over the pantyliner for the use in clinical practice. It was easier to process at the laboratory, more hygienic in use and more convenient in use for children who were already continent for urine. Altogether, the PeeSpot was shown to be a very appropriate tool for the collection of urine for albuminuria assessment in young children.

In **Chapter 4**, the PeeSpot was used to evaluate different collection strategies for providing a guideline on albuminuria measurement in toddlers. Several strategies were tested and compared: single sample urine collection versus repeated urine sampling and first morning void urine sample versus random day sample. The most optimal method (with the lowest intra-individual variability of albuminuria) was three consecutive collections of first morning void urine samples, repeated over time. This is in line with earlier findings in adults. Interestingly, the use of albumin:creatinine ratio introduced additional variability in children, and therefore appears to be a less precise measurement than the use of albumin concentration alone. This larger variability could not be explained by a larger variability of urinary creatinine itself, nor could other explanations for this difference been found. Further studies into the creatinine measurement itself as well as into the influence of muscle mass on the use of urine creatinine corrections are needed to standardize albuminuria measurements and define cut-off values for albumin:creatinine ratio's in children. Taking the findings of this chapter into account, it should be advised to measure albumin concentration in multiple first morning void urine samples, repeated over time, in order to measure and monitor albuminuria in toddlers.

Very few data are available regarding the effect of albuminuria lowering on renal disease progression in children with chronic kidney disease. In **Chapter 5**, the initial proteinuria lowering effect of standardized ACE inhibition and its association with subsequent renal disease progression were evaluated in 280 children with CKD. In this post-hoc analysis of the ESCAPE study, it was shown that ramipril treatment with a dose of 6mg/m^2/day lowered proteinuria with a mean of 42%, with a large variability between individuals. Children with more initial proteinuria reduction were at lower risk for developing the composite renal endpoint (>50% eGFR decline or progression to end stage renal disease), which is in line with findings in adults. Moreover, higher levels of both residual proteinuria after attaining the full dose of ramipril, and the total exposure to proteinuria during follow-up, were associated with a higher risk of CKD progression. Importantly, these findings were independent of the underlying disease, baseline proteinuria and blood pressure control. Collectively, these data highlight the importance of proteinuria as a risk factor for renal disease progression in children with CKD and suggest that proteinuria lowering is an important target in the management of children with CKD to protect their kidney function.

Chapter 6 defines the effect of discontinuation of RAAS inhibition on important renal markers and renal disease progression in children with CKD. Using observational data from the 4C study, initial changes in blood pressure, albuminuria and potassium and the rate of eGFR decline were tested in 69 children who discontinued RAAS inhibition during observation. On the short term, discontinuation of RAAS inhibition was followed by an increase in albuminuria and decrease in serum potassium. The rate of eGFR decline became steeper after discontinuation of RAAS inhibition, suggesting that the use of RAAS inhibition was protective and that discontinuation accelerates the progression of renal disease progression. We also evaluated the most frequent clinical reasons for discontinuation of RAAS inhibition in this study. These were an increase in serum creatinine, symptomatic hypotension and hyperkalemia. This study has a unique design, using observational data before and after discontinuation in order to define the effect of discontinuation of RAAS inhibition on renal disease progression. Altogether, the results of this study indicate that, although there may be compelling clinical reasons to discontinue RAAS inhibition in children with CKD, this measure is associated with an acceleration of renal function decline. Therefore, stopping this therapy even for good clinical reasons should be weighed against the negative impact on long term renal function.

Future perspectives

Although this thesis has investigated several aspects regarding albuminuria in children, much more research should still be executed in order to learn more on the importance of albuminuria as a renal and cardiovascular risk marker and its treatment in children.

The optimal way to answer the question whether albuminuria is an inborn characteristic that is associated with renal and cardiovascular risk in later life, is to conduct a study that follows individuals from birth to the end of their life. Such a study should measure albuminuria regularly, to assess whether higher albuminuria levels at birth are associated with a changing phenotype with increased risk for renal and cardiovascular disease or death in later life. However, in clinical practice or even clinical trial conditions, the execution of such a study would be very difficult and almost impossible to accomplish. Alternatively, separate studies can be performed to answer several sub-questions. For example, in order to establish whether elevated levels of albuminuria in these children are consistent over time, the measurements of albuminuria in children from the GECKO cohort in Chapter 1 are currently repeated. Moreover, a longitudinal cohort study in the Bristol area in England (the Avon Longitudinal Study of Parents and Children (ALSPAC) study) has urine and blood samples collected at different ages (before, during and after puberty). These samples can help to answer the question whether elevated levels of albuminuria are associated with renal function decline over time.

In order to definitely proof that albuminuria is a valid treatment target for renal (and cardiovascular) protection, there is a need for prospective clinical trials that aim at lowering albuminuria, and have this as primary target. This to overcome the biases which are present in the post-hoc analyses of the currently available trials. An example of such an approach can be found in the Study of Diabetic Nephropathy with Atrasentan (SONAR). This study was recently terminated and results are expected to be available in the course of 2018. A positive result will strengthen the statement that lowering proteinuria is a very important target for treatment in adults. However, clinical trial data in children regarding this subject are still lacking. Although the results of chapter 5 are in line with earlier findings in adult populations, a prospective study in children, similar to the SONAR study, would be of great value, as the pediatric CKD population is very different from the adult CKD population (in terms of primary renal disease etiology, duration of disease, comorbidities, etc). Whether this study should be performed with an endothelin receptor antagonist as in the SONAR study, or a RAAS inhibitor, or even a new drug that targets albuminuria lowering in children specifically, is still open for discussion.

In general, it appears highly important to make greater use of the available data that have been collected in registries, observational studies, and randomized controlled trials, in order to start researching the many questions that are still left unanswered in children. Especially in vulnerable subjects like children, in which additional policies have been developed to protect them in clinical research, participation in clinical trials is limited. However, due to this lack of research in children, there is also a lack of data that

describes safety and efficacy of treatment in this population. With the use of pragmatic trials, research questions can be explored where clinical trial evidence is limited. The chapters in part 2 of this thesis are two examples of how a more pragmatic trial design can be executed.

In conclusion, more studies should be conducted to unravel the importance of albuminuria as a risk marker of disease, present already early in life. A combination of observational data from cohort studies and a randomized controlled trial should help us further in this quest.