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ORIGINAL ARTICLE

Achieving remission of proteinuria in childhood CKD

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Abstract
Background A multidrug treatment strategy that targets urinary proteins with an angiotensin-converting enzyme (ACE) inhibitor and angiotensin receptor blocker (ARB) up-titrated to the respective maximum tolerated dose combined with intensified blood pressure (BP) control has been found to prevent renal function loss in adults with proteinuric nephropathies. Herein, we investigated the effects of this treatment protocol in the pediatric patient population.
Methods From May 2002 to September 2014 we included in this observational, longitudinal, cohort study 20 consecutive children with chronic nephropathies and 24-h proteinuria of >200 mg who had received ramipril and losartan up-titrated to the respective maximum approved and tolerated doses [mean (standard deviation) dose:2.48 (1.37) mg/m^2 and 0.61 (0.46) mg/kg daily, respectively]. The primary efficacy endpoint was a >50 % reduction in 24-h proteinuria to <200 mg (remission). Secondary outcomes included changes in proteinuria, serum albumin, BP, and glomerular filtration rate (GFR).
Results Mean (± standard deviation) patient age at inclusion was 13.8 ± 2.8 years, and the median [interquartile range (IQR)] serum creatinine level and proteinuria were 0.7 (0.6–1.0) mg/dl and 690 (379–1270) mg/24 h or 435 (252–711) mg/m^2/24 h, respectively. Proteinuria significantly decreased by month 6 of follow-up, and serum albumin levels increased over a median follow-up period of 78 (IQR 39–105) months. In the nine children who achieved remission, proteinuria reduction persisted throughout the whole follow-up without rebounds. The GFR improved in those children who achieved remission and worsened in those who did not. The mean GFR slopes differed significantly between these two groups ($p < 0.05$), being positive in those children with remission and negative in those without remission (+0.023 ± 0.15 vs.−0.014 ± 0.23 ml/min/1.73 m^2/month, respectively), whereas BP control was similar between the two groups. Hyperkalemia was observed in two children.
Conclusions Combination therapy with maximum approved doses of ACE inhibitors and ARBs is a safe strategy which may achieve proteinuria remission with kidney function stabilization or even improvement in a substantial proportion of children with proteinuric nephropathies.

Keywords ACE inhibitor · Angiotensin receptor blocker · Children · Chronic nephropathy · Proteinuria

Piero Ruggenenti, Paolo Cravedi and Antonietta Chianca contributed equally to this work.

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Introduction

Most adults and children with chronic kidney disease (CKD) tend to progress to end-stage kidney disease (ESKD). Hypertension and proteinuria are the two major causes of progressive renal damage and function loss [1]. In particular, experimental and human data converge to indicate that there is a continuous relationship, without thresholds, between proteinuria [2], including residual proteinuria while on angiotensin-converting-enzyme (ACE) inhibition therapy [3], and renal disease progression. Moreover, proteinuria reduction is, independently of treatment, renoprotective in adults [4] as well as in children [5] and may even result in the regression of renal lesions [6] and regeneration of kidney vasculature [7]. Effective reduction of blood pressure (BP) slows CKD progression in adults [8] and, among different antihypertensive agents, those that inhibit the renin–angiotensin–aldosterone-system (RAAS), namely, ACE inhibitors and angiotensin receptor blockers (ARBs), are the most renoprotective owing to their specific antiproteinuric effect [9–11].

According to the U.S. Renal Data System 2015 Annual Data Report (available at: https://www.usrds.org/adr.aspx), children account for 1.5 % of the whole patient population with ESKD. The mortality rate for these children while on chronic renal replacement therapy is approximately 30-fold higher than that for their healthier peers [12]. Small-scale studies have reported a decrease in proteinuria and a slowing of renal function loss in children with CKD who receive ACE inhibitor or ARB therapy [13–15]. A larger randomized study showed that both losartan and enalapril decreased proteinuria in 268 children with normal or high BP [16]. The ESCAPE trial [17] found that a 5-year-long intensified management of BP in 385 children with CKD who received a fixed dose of an ACE inhibitor delayed the time to 50% reduction in glomerular filtration rate (GFR) or progression to ESKD more effectively than did conventional management of BP. In this study, proteinuria decreased by more than 50% during the first 6 months, but, unexpectedly, gradually increased thereafter towards baseline values despite good BP control in both treatment groups. According to the authors [17], this rebound was most likely explained by a progressive activation of enzymes other than ACE, such as chymases, resulting in increased angiotensin II production and secondary enhanced adrenal aldosterone release and aldosterone "escape" [18]. Given that proteinuria plays a central role in the progression of CKD [19], this rebound might limit the long-term renoprotective effect of ACE inhibitor therapy [20].

Since ARBs also block the activity of the angiotensin II produced through non-ACE pathways, add-on ARB treatment may theoretically prevent angiotensin II-mediated aldosterone breakthrough and rebound proteinuria in patients on ACE inhibitor therapy. Combination therapy with ACE inhibitors and ARBs has consistently been shown to reduce proteinuria in adults with CKD more effectively than ACE inhibitor monotherapy [21]—in particular when the treatment is titrated to urinary proteins [22]. The Remission Clinic, a multidrug treatment strategy that targets urinary proteins with an ACE inhibitor and ARB up-titrated to maximum tolerated doses in combination with intensified BP control, significantly slowed renal function loss and reduced the risk for terminal kidney failure by 8.5-fold in 56 adults with proteinuric nephropathies as compared to matched controls treated with an ACE inhibitor titrated to the BP [23]. This proof-of-concept study intentionally included patients at high risk of accelerated renal function loss due to heavy proteinuria. A subsequent study, however, found that the Remission Clinic program was also renoprotective in adult patients with Alport syndrome and micro- or macro- albuminuria [24]. On the basis of these findings, this multidrug intervention was extended to all patients with CKD and urinary protein excretion exceeding 0.5 g per 24 h ([25]; available at: http://clinicalweb.marionegri.it/international-remission/remission.php). In the study reported here, our aim was to assess whether this therapeutic option could also be offered to children at increased risk of progression to ESKD due to proteinuric CKD. We therefore investigated the benefits, risks and feasibility of the Remission Clinic program in patients aged <18 years who had proteinuria persistently exceeding 200 mg per day who were monitored and treated in the context of a Pediatric Nephrology outpatient clinic.

Materials and methods

All consecutive patients aged <18 years with 24-h proteinuria of >200 mg for at least 6 months and no specific indication for immunosuppressive therapy nor contraindications to renin–angiotensin system (RAS) inhibitor therapy who had been referred to the Pediatric Nephrology Outpatient Remission Clinic of the Ospedale Papa Giovanni XXIII of Bergamo (Italy) were eligible for enrolment in this longitudinal, observational, cohort study. Children with ortostatic proteinuria or urological abnormalities, including congenital anomalies of the kidney and urinary tract (CAKUT) were excluded (Fig. 1). Legal tutors provided written informed consent to the registration and use of data with preservation of each patient's anonymity and privacy. Data were recorded and reported according to the "Strengthening the Reporting of OBservational studies in Epidemiology (STROBE)" guidelines for reporting observational studies [Electronic Supplementary Material (ESM) Table S1].

Children were advised to avoid salt-rich foods, but no specific restriction to dietary protein intake was enforced. At baseline, we recorded the medical history, anthropometric values and clinical and laboratory data, including BP,

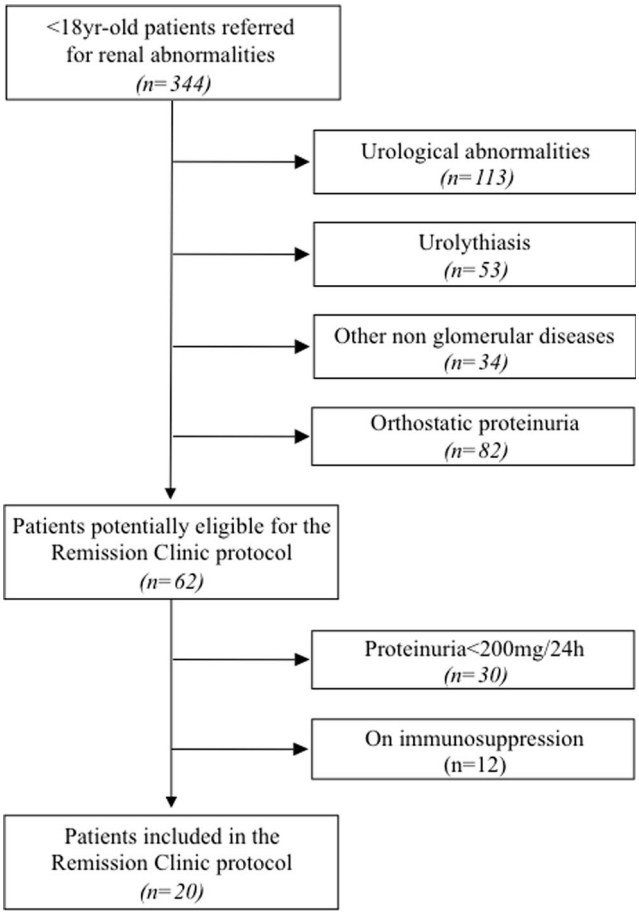

Fig. 1 Flow diagram of patient enrolment in the study

proteinuria and estimated GFR (eGFR). The children then entered a pre-defined, standardized Remission Clinic protocol [23] that included treatment with an ACE inhibitor and an ARB together with other antihypertensive agents that were progressively titrated to 24-h proteinuria <200 mg (remission, primary endpoint). Each patient was started on a low (1.5 mg/m^2) daily dose of ramipril that was progressively titrated up to a maximum dose of 5 mg/m^2. If remission was not achieved, losartan was added to the therapeutic regimen at a starting daily dose of 0.35 mg/kg that could be titrated up to 1.4 mg/kg. No child received doses of ramipril or losartan above the reported limits. Timing for dose escalation (or reduction) was response-driven and individually tailored. Thus, each treatment step had to be implemented until remission was achieved or the protocol had to be stopped because of safety or tolerability reasons. More specifically, whenever treatment up-titration was associated with symptomatic hypotension, serum potassium increases to ≥5.5 mEq/L despite concomitant diuretic therapy and correction of metabolic acidosis and/or an increase in serum creatinine by ≥25 % versus baseline, the dose of ramipril and/or losartan was down-titrated until full recovery of any clinical or laboratory abnormality was achieved. If the abnormalities persisted, losartan (first) and then ramipril were withdrawn, but the patient was maintained on active follow-up. Treatment could also be transiently back-titrated or withdrawn when the patient experienced adverse events, such as vomiting and diarrhea.

At any time, a diuretic (1 mg/kg/day of hydrochlorothiazide or 0.5 mg/kg/twice daily of furosemide if the GFR was above or below 40 ml/min/1.73 m^2, respectively) could be added to the treatment regimen and up-titrated to control edema or hyperkalemia and maximize the antiproteinuric effect of RAAS inhibition (ESM Fig. S1).

Study parameters were recorded in an ad hoc database. BP was the mean of three values taken 2 min apart by a standard sphygmomanometer. Throughout the whole study period the serum creatinine level was always measured by the same enzymatic assay [26], a reference method that does not require standardization, and the GFR was estimated by the 2009 revised Schwartz formula [27] in all study children. Percentiles and standard deviation score (SDS) based on the fourth report on Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (available at: https://www.nhlbi.nih.gov/files/docs/resources/heart/hbp_ped.pdf) and the U.S. Centers for Disease Control and Prevention Growth Reference Year 2000 [28] were used to evaluate BP and to assess anthropometric measures to help evaluate children's growth, respectively.

Sample size estimation

Because of the observational nature of the study, the sample size was not calculated a priori on the basis of a predefined treatment effect. However, on the basis of previous evidence in adult patients with non-diabetic proteinuric CKD [23], we expected that a study of 20 children would be able to detect the antiproteinuric effect of the Remission Clinic program in this context.

Statistical analyses

The primary endpoint was the reduction of 24-h proteinuria to <200 mg with >50 % reduction over baseline. Secondary outcomes included changes in proteinuria, serum albumin, BP, and eGFR. The primary outcome was analyzed on a time-to-event basis by means of Cox proportional-hazard survival analysis to assess the effects of potential risk factors.

The occurrence of missing follow-up data on 24-h proteinuria, eGFR, systolic and diastolic BP, serum potassium levels and hemoglobin concentration was addressed by multiple imputation using multi-level random-effects models on repeated measurements [29]. We generated ten multiple imputed datasets based on the linear mixed effects models of the longitudinal variable, including the baseline and time-visit, and the median value of the imputed estimates over the time-visit were considered.

Longitudinal changes in proteinuria were evaluated with the use of repeated-measure analysis. The linear mixed effects models of 24-h proteinuria (log transformed) included baseline 24-h proteinuria and time-visit and were adjusted for eGFR or systolic or diastolic BP SDS or gender to test the robustness of the results. Sensitivity analyses were performed by evaluating the outcome 24-h proteinuria adjusted for body surface area (BSA). The BSA was calculated by the Dubois formula [30]. eGFR slopes (ΔeGFR) were estimated using linear mixed effects models (by month 3) that included the baseline eGFR, binary primary endpoint and time-visit.

Groups were compared by using the paired *T* test, Wilcoxon signed-ranks test, analysis of covariance or quantile regression, as appropriate. Normality for continuous variables was assessed by means of the Q-Q plot. The data on the baseline characteristics were presented as numbers and percentages, means and standard deviations or medians and interquartile ranges (IQR), as appropriate. All *p* values were two-sided. The analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC) and Stata version 13 (StataCorp LP, College Station, TX) software programs. Realcom (Realcomp II Ltd., Farmington Hills, MI) was used for the missing imputation.

Results

Twenty children (n = 10 males) who fulfilled the selection criteria for the Remission Clinic protocol were identified among the 344 patients referred to our Pediatric Nephropathy outpatient clinic (Fig. 1). These children were enrolled in the study from May 2002 to September 2014 and followed-up to May 2015. At inclusion all patients were proteinuric, with normal or moderately reduced kidney function and BP values in the recommended target range. Mean (±SD) patient age at inclusion was 13.8 ± 2.8 years, median (IQR) serum creatinine levels and proteinuria were 0.7 (0.6–1.0) mg/dl and 690 (379–1270) mg/24 h [or 435 (252–711) mg/m^2/24 h], respectively. Mean systolic and diastolic BP were 108.2 ± 14.6 and 64.4 ± 9.4 mmHg, respectively, with a SDS of −0.22 ± 1.21 and 0.17 ± 0.84, respectively. Sixteen children had a biopsy-proven chronic glomerular disease (Table 1). One child had a history of shigatoxin-associated hemolytic uremic syndrome (HUS) without identification of genetic abnormalities predisposing to the disease, and a second child had a history of atypical HUS with a heterozygous mutation in the factor H gene. None of the enrolled children showed any evidence of active microangiopathy or was on ongoing treatment with plasma or complement inhibitors at the time of inclusion.

Main outcomes

Over a median follow-up of 78 (IQR 39–105) months, nine children achieved remission of proteinuria. The endpoint was reached at a median (IQR) of 11.6 (10.8–14.1) months after inclusion. Baseline characteristics of children who achieved or did not achieve remission were similar (Table 1). Remission was predicted by lower baseline proteinuria at univariable analysis and by male gender and lower proteinuria at multivariable analyses (Table 2). The outcome of children with VACTERL syndrome, neonatal sepsis, autosomal recessive polycystic kidney disease (ARPKD) or CKD from unknown causes was similar to the outcome of the other study children, and no association was observed between underlying etiology and progression to remission. The eGFR tended to increase in patients who achieved remission and to decrease in those who did not (Fig. 2a). The mean eGFR slope was positive in children with remission and negative in those without, and it significantly differed between the two groups (+0.023 ± 0.15 vs. −0.014 ± 0.23 ml/min/1.73 m^2/month; $p < 0.05$; Fig. 2b).

Overall, proteinuria had decreased significantly at 6 months of follow-up and was persistently lower than that at baseline throughout the whole observation period. The reduction was greater in patients who achieved remission than in those who did not, and in those with remission it persisted throughout the whole follow-up without rebounds (Fig. 3a). Linear mixed effects models showed that the proteinuria reduction was statistically significant [Coefficient −0.010 (95 % confidence interval −0.016 to –0.004); $p < 0.001$] and that it retained its significance even after adjustment for gender, eGFR, and systolic or diastolic BP SDS (ESM Table S2). Similar findings were obtained when the statistical analyses considered proteinuria normalized by BSA (ESM Table S3).

The reduction in proteinuria was associated with a significant ($p < 0.01$) increase in serum albumin at the last available follow-up visit (from 4.04 ± 0.43 g/dl at inclusion to 4.30 ± 0.34 g/dl; Fig. 4) that was largely driven by increases (from 3.68 ± 0.21 to 4.01 ± 0.24 g/dl; $p < 0.01$) in children with albumin levels below the median at inclusion (Fig. 4b, c).

Treatment and blood pressure control

At study end, 12 participants were on ACE inhibitor + ARB combination therapy and eight were on ACE inhibitor monotherapy. Six and two participants could not be maintained on combination therapy due to hypotension and hyperkalemia, respectively (Table 3). Final doses of ramipril and losartan averaged 2.48 ± 1.37 mg/m^2 and 0.61 ± 0.46 mg/kg daily, respectively. BP was persistently in the target range and did not differ between patients who achieved remission during the observation period and those who did not (data not shown).

Table 1 Baseline demographic and clinical characteristics of the study group considered as a whole and in subsets according to remission status

Patient characteristics	Entire patient study population ($n = 20$)	Remission—YES ($n = 9$)	Remission—NO ($n = 11$)
Age (years)	13.84 ± 2.78	13.10 ± 2.60	14.43 ± 2.89
Male gender	10 (50.0 %)	6 (66.7 %)	4 (36.4 %)
Underlying renal disorder			
Immunoglobulin A nephropathy	7 (35.0 %)	4 (44.4 %)	3 (27.3 %)
Alport syndrome	3 (15.0 %)	1 (11.1 %)	2 (18.2 %)
Hemolytic uremic syndrome	2 (10.0 %)	1 (11.1 %)	1 (9.1 %)
Focal segmental glomerulosclerosis	1 (5.0 %)	0 (0 %)	1 (9.1 %)
VACTERL syndrome	1 (5.0 %)	0 (0 %)	1 (9.1 %)
Chronic kidney disease from neonatal sepsis	1 (5.0 %)	0 (0 %)	1 (9.1 %)
Autosomal recessive polycystic kidney disease with hepatic fibrosis	1 (5.0 %)	1 (11.1 %)	0 (0 %)
Unknown[a]	4 (20.0 %)	2 (22.2 %)	2 (18.2 %)
Systolic BP (mmHg)	108.2 ± 14.6	107.2 ± 19.6	108.9 ± 9.8
SDS	−0.22 ± 1.21	−0.18 ± 1.32	−0.26 ± 1.18
>95th percentile	2 (10 %)	1 (9 %)	1 (11 %)
Diastolic BP (mmHg)	64.4 ± 9.4	63.9 ± 12.0	64.8 ± 7.2
SDS	0.17 ± 0.84	0.05 ± 0.96	−0.01 ± 0.78
>95th percentile	0 (0 %)	0 (0 %)	0 (0 %)
Serum creatinine (mg/dl)	0.70 [0.58–0.97]	0.69 [0.54–1.00]	0.80 [0.58–0.94]
Serum albumin (g/dl)	4.04 ± 0.43	4.17 ± 0.48	3.94 ± 0.38
Proteinuria (mg/24 h)	690 [379–1270]	460 [270–890]	700 [540–2000]
Proteinuria/BSA (mg/m^2/24 h)	435 [252–711]	436 [205–595]	433 [308–1182]
eGFR (ml/min/1.73 m^2)	90.7 ± 26.5	87.2 ± 28.7	93.3 ± 27.0

Data in table are presented as the mean ± standard deviation (SD), median with the interquartile range (IQR) in square brackets or as the absolute number with the percentage in parenthesis, as appropriate

There was no significant statistical difference in any of the variables between the Remission—yes and Remission—no patient subsets

BP, Blood pressure; SDS, standard deviation score; BSA, body surface area; eGFR, estimated glomerular filtration rate

[a] In 3 cases kidney biopsy was not informative due to limited material

Safety

Serum potassium levels and hemoglobin concentration were relatively stable during the follow-up. No appreciable differences in serum potassium levels were observed between children who achieved or did not achieve remission (Fig. 3b). In both of these groups hemoglobin concentration significantly decreased at month 6 compared to baseline; thereafter, it

Table 2 Univariate and multivariate Cox analysis showing the association between baseline factors and the outcome remission

Variables	Univariate Hazard Ratio (95% CI)	p value	Multivariate Hazard Ratio (95% CI)	p value
Age (years)	0.85 (0.67–1.10)	0.215		
Male gender (n %)	2.65 (0.66–10.67)	0.169	13.51 (1.92–95.09)	0.009
eGFR (ml/min/1.73 m^2)	0.99 (0.97–1.02)	0.509		
Proteinuria (mg/24 h)[a]	0.30 (0.11–0.84)	0.022	0.10 (0.02–0.45)	0.002
Proteinuria/BSA (mg/m^2/24 h)[a]	0.35 (0.13–0.98)	0.045		
Systolic BP SDS	1.06 (0.59–1.89)	0.85		
Diastolic BP SDS	0.97 (0.41–2.29)	0.951		

Data for univariate and multivariate analyses are presented as the hazard ratio with the 95 % confidence interval (CI) in parenthesis

[a] log transformed

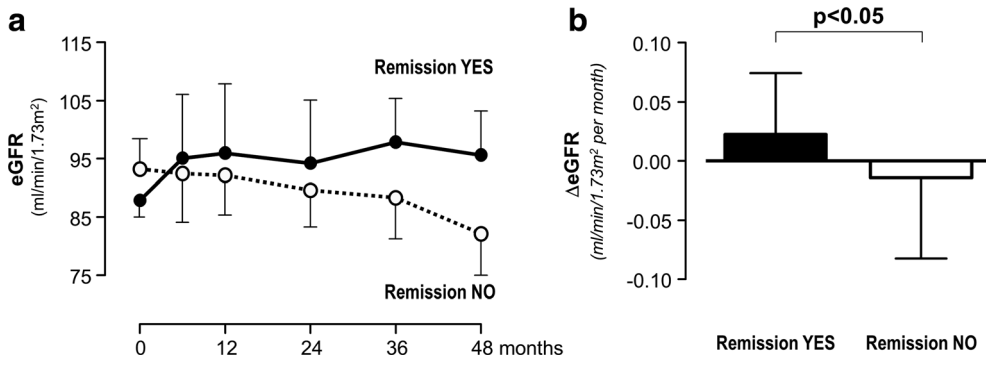

Fig. 2 **a**, **b** Course of estimated glomerular filtration rate (*eGFR*) (**a**) changes in eGFR over time (Δ*eGFR*) (**b**) considered separately in the two subgroups of patients: those achieving remission of proteinuria (*Remission YES*) and those not achieving remission (*Remission NO*). Data are presented as the mean ± standard error of the mean

progressively increased in children who achieved remission, whereas it was persistently lower as compared to baseline in those who did not (Fig. 3c).

Overall, adverse events were more frequent in children who did not achieve remission than in those who did. Three infectious events that were considered serious were observed in children without remission (Table 3). No patient had acute renal function deterioration, severe refractory hyperkalemia, anemia or any other serious adverse event possibly related to treatment. Eight non-serious events prevented up-titration to dual RAAS blockade or required back-titration from dual RAS blockade to ACE inhibitor monotherapy. These included six cases of symptomatic hypotension and two cases of persistent hyperkalemia that were equally distributed among children with or without remission (Table 3).

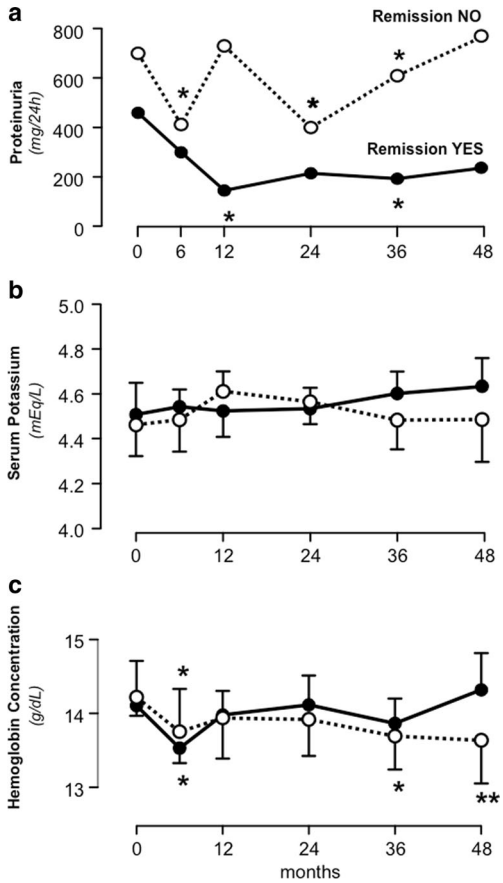

Fig. 3 Course of 24-h urinary protein excretion **a**), serum potassium (**b**) and hemoglobin concentrations (**c**) considered separately in the two subgroups of patients: those achieving remission of proteinuria (*Remission YES*) and those not achieving remission (*Remission NO*). Data are presented as the mean ± SEM. $*p < 0.05$, $**p < 0.01$ vs. baseline (time 0)

Discussion

In this longitudinal, observational study, intensified BP control with a Remission Clinic treatment regimen which targets urinary proteins through the up-titration of an ACE inhibitor and an ARB to maximum tolerated doses achieved complete remission of proteinuria in nine of 20 children with chronic renal parenchymal disease. Consistent with previous evidence in adult patient populations, proteinuria reduction translated into significant protection against renal function loss and amelioration of hypoalbuminemia. In children achieving remission, the reduction was sustained over time, and no rebound proteinuria was observed over a median follow-up period of >6 years. Lower proteinuria at inclusion and male gender predicted a higher probability of remission, whereas the outcome was not associated with underlying etiology and BP control, with BP within the recommended target range in all children. The treatment was well tolerated, and no child had to stop the program because of acute renal function deterioration, severe, refractory hyperkalemia and/or anemia. In children achieving remission the progressive improvement in kidney function was also associated with a parallel increase in hemoglobin concentration.

The results of an earlier study demonstrated that the Remission Clinic protocol may achieve persistent remission of proteinuria and halt renal disease progression in approximately 50% of adults with proteinuric chronic nephropathies [23]. The findings of the present study show that this protocol is also efficacious in children with proteinuric CKD. Our finding that proteinuria remission prevented renal function loss over time provides convincing evidence that, independently of BP control, residual proteinuria, even in the sub-nephrotic range [3], is a major risk factor for renal disease progression

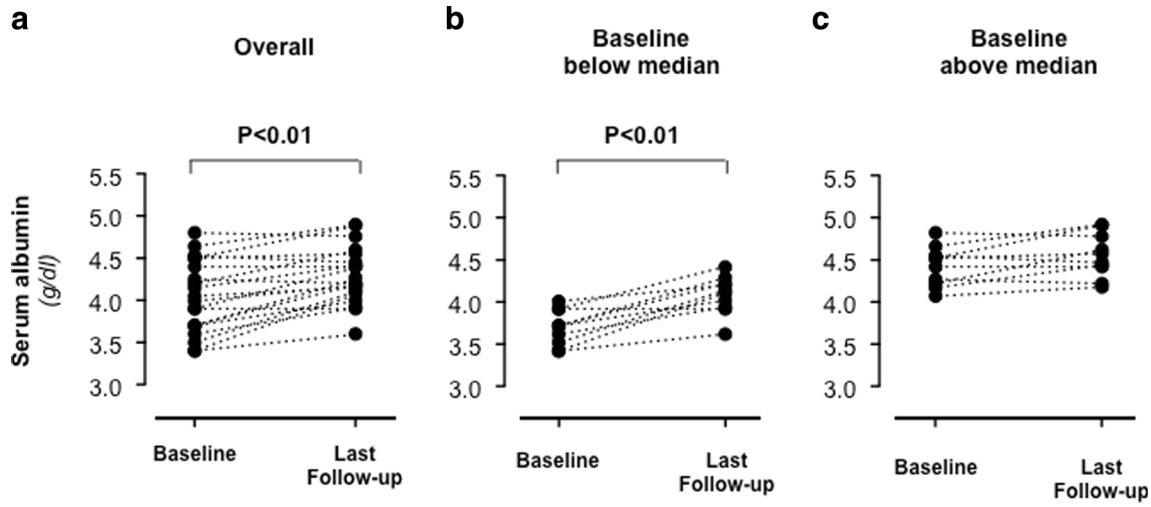

Fig. 4 Single patient serum albumin levels at baseline and at last available follow-up visit in the study group considered as a whole (**a**) and in the two subgroups with baseline serum levels below (**b**) or above (**c**) the median

also in children. This finding may have clinical implications since a sustained reduction in proteinuria is expected to translate into effective protection against progression to ESKD [3]. The concomitant amelioration of hypoalbuminemia may also be beneficial.

It is notable that persistent remission of proteinuria could not be achieved in the ESCAPE trial despite good BP control [17]. Within the limitations of comparative analyses between different studies that included patients with different characteristics, these findings suggest that, unlike single drug blockade of the RAAS with a fixed dose of an ACE inhibitor [17], combination therapy with an ARB may possibly prevent aldosterone "escape" [18] and proteinuria rebound. Since proteinuria plays a central role in the progression of CKD [19], prevention of this rebound by a treatment titrated to urinary proteins may be instrumental to maximization of the long-term renoprotective effect of RAAS inhibitor therapy also in children with proteinuric chronic nephropathies [20]. In this context it is interesting to note that the Supra Maximal Atacand Renal Trial (SMART) found that supramaximal dosages of candesartan lead to greater reductions of proteinuria compared with the highest approved antihypertensive dosage of candesartan (16 mg/day) in patients with primary glomerular diseases, diabetes or hypertensive glomerulosclerosis and

Table 3 Serious and non-serious adverse events in the study group considered as a whole and in subsets according to remission status

Event	Entire patient study population ($n = 20$)	Remission—YES ($n = 9$)	Remission—NO ($n = 11$)
Any adverse event	16	5	11
Any serious adverse event	3	0	3
Pyelonephritis	1	0	1
Pneumonia	1	0	1
Appendicitis	1	0	1
Any non-serious adverse event	13	5	8
Requiring treatment back-titration	8	4	4
Persistent symptomatic hypotension	6	3	3
Refractory hyperkalemia	2	1	1
Not requiring treatment back-titration	5	1	4
Hypoacusia	1	0	1
Erythema nodosum	1	0	1
Urinary tract infection	1	0	1
Transient transaminase increase	1	0	1
Mononucleosis	1	1	0

Therapy was temporarily withdrawn in 1 patient in the Remission—no group because of pregnancy

persistent proteinuria of ≥1 g/day, despite treatment with approved doses of candesartan [31]. These data confirm that intensified RAAS inhibition is an effective approach to reduce proteinuria in patients with CKD. However, high-dose ACE inhibitors are at least as effective as high-dose ARBs and are less expensive [32]. Thus, the cost/effectiveness of ARB uptitration to maximum tolerated doses is—at the very least—questionable. Moreover, in patients with non-diabetic CKD ACE inhibitor + ARB combination therapy reduced proteinuria more effectively than each agent alone, even at high doses (reviewed in [33]). Thus, as previously reported in adults [23, 24], dual RAAS blockade appears to be the most efficient approach to reduce proteinuria in non-diabetic patients with CKD.

In the present study, proteinuria decreased due to an effect that appeared to be largely independent of BP control, and BP was similar in children who achieved remission and those who did not. Our finding that the proportion of children on ACE inhibitor monotherapy was small and similar in the two patient groups suggests that failure to achieve remission was unlikely explained by ineffective RAAS inhibition. Thus, intrinsic, possibly genetically determined mechanisms [34] may explain the response of different individuals to the same treatment protocol.

The etiology of renal disease in our study patients was heterogeneous. However, they shared a unifying characteristic: all were at risk of progressive renal function loss due to a common pathogenic pathway mediated by protein traffic [1]. The Remission Clinic approach, a multidrug intervention designed to specifically target this common target by maximized RAAS inhibition, consistently achieved remission of proteinuria independently of the underlying renal disease. Our finding that the BP was already in recommended target range at study inclusion and that it did not change appreciably throughout the whole observation period can be taken to suggest that the reduction in proteinuria we achieved with the Remission Clinic approach can be reasonably attributed to more effective RAAS inhibition rather than to improved BP control.

Safety

Pediatric patients are a medically fragile patient population, and the potential risks of ACE inhibitor + ARB combination therapy must be carefully evaluated in this context, in particular in children with more advanced CKD. Independent of this general consideration, however, the results of trials such as the Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial (ONTARGET), which questioned the risk/benefit profile of dual RAAS blockade in adults with cardiovascular disease but no evidence of overt proteinuric nephropathy [35], should not be generalized to the average population of patients (including children) with proteinuric kidney disease [36]. Indeed, ONTARGET showed that ramipril + telmisartan combination therapy prevents microalbuminuria but facilitates transient renal function impairment in non-proteinuric patients with atherosclerotic vascular disease or diabetes. However, only a very small proportion of ONTARGET patients were proteinuric, whereas most of them were at high risk for ischemic kidney disease and other clinical conditions predisposing to acute renal function deterioration and/or hyperkalemia in the case of dehydration from any cause or for intercurrent events such as sepsis, acute myocardial infarction with heart failure, bleeding, major trauma or surgery. Thus, these patients had no cogent indication to receive dual RAAS blockade and were at the same time at excess risk of complications related to unnecessarily maximized inhibition of the RAAS. This does not apply to the children evaluated in our study since all of them were proteinuric and none had atherosclerotic vascular disease. Two other studies, the VA NEPHRON D [37] and the ALTITUDE [38] trials, found that dual RAAS blockade with an ACE inhibitor [37] or a renin inhibitor [38] as add-on therapy to ARBs failed to improve nephroprotection as compared to ARB monotherapy in type 2 diabetes patients with nephropathy and was associated with more side effects. Both studies were stopped prematurely for safety and futility reasons, leading regulatory authorities, such as the European Medicines Agency's (EMA) Committee for Medical Products for Human Use (CHMP), to endorse restrictions for dual RAAS inhibition therapy in patients with diabetes or moderate to severe renal impairment (GFR <60 ml/min/1.73 m^2; http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2014/05/WC500167421.pdf). When the VA NEPHRON D study stopped, dual RAAS inhibition had already reduced ESKD events by 34 % compared with ARB monotherapy, a treatment effect never achieved earlier in type 2 diabetes patients and one which approximated nominal significance ($p = 0.07$) over just 2.2 years of follow-up. Conceivably, the opportunity to demonstrate clinically relevant nephroprotection over the planned 5-year follow-up was missed because of premature study closure dictated by adverse events, such as hypotension, hyperkalemia and acute kidney injury, which could have been prevented by avoiding the up-titration of lisinopril (up to 40 mg daily in patients with an estimated GFR as low as 30 ml/min/1.73 m^2) on top of full-dose losartan. The excess risk of adverse events observed with dual RAS blockade was therefore largely explained by treatment-related and potentially reversible hemodynamic effects which could be expected since type 2 diabetes patients with overt nephropathy are in most cases older patients who, similar to the ONTARGET patients, are at

increased risk of ischemic kidney disease or nephroangiosclerosis. Independent of these considerations, none of the children in our study were diabetic, which may explain why in our pediatric population the Remission Clinic protocol effectively reduced proteinuria and was safe and well tolerated, as previously observed in adults with non-diabetic proteinuric CKD [23]. Thus, we fully agree with the EMA recommendation that in cases where combined use of an ARB and ACE inhibitor is considered absolutely essential—such as in adults and children with non-diabetic proteinuric CKD—"it must be carried out under specialist supervision with close monitoring of renal function, electrolytes and blood pressure". Whether this approach can be safely extended to children with a GFR of <60 ml/min/1.73 m^2 is worth investigating.

Limitations and strengths

The lack of a parallel control group and the small sample size were conditional to the rarity of the disease entity under evaluation here. We included only children without evidence of urogenital abnormalities to avoid the risk that factors other than proteinuria and BP control, such as obstruction, vesicoureteral reflux, intercurrent urinary tract infections or chronic nephrolithiasis, could affect the considered outcomes and therefore confound data interpretation. Thus, our findings are specifically applicable to children with proteinuria and chronic renal parenchymal disease who share a common pathogenic pathway mediated by protein traffic [1]. The treatment effect was consistently similar between the seven patients with VACTERL syndrome, previous sepsis, ARPKD or unknown disease and the rest of the cohort.

The long follow-up is a strength, and the fact that the study was conducted in the context of an outpatient clinic allows data generalizability to the average population of children referred to pediatricians or nephrologists in everyday clinical practice. Avoidance of fixed, pre-defined standard doses and the use of a flexible treatment protocol that can be modulated according to patient response, in combination with close clinical monitoring, most likely explains why treatment was effective in reducing proteinuria and at the same time was safe and well tolerated. This approach, which reflects everyday clinical practice, further enhances data generalizability.

Conclusions

Our results support the use of combined ACE inhibitor and ARB therapy to achieve remission of proteinuria and stabilize or even improve kidney function in children with chronic renal parenchymal disease. Provided children are closely monitored and treatment is cautiously tailored to individual response, this approach can be safely applied in day-to-day hospital practice.

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Compliance with ethical standards The research ethics boards of all participating hospitals approved the study. Written informed consent was obtained from the parents and/or the patients.

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Conflict of interest All authors declare that they have no conflicts of interest regarding this work.

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