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The next generation: Urinary epidermal growth factor is associated with an early decline in kidney function in children and adolescents with type 1 diabetes mellitus

Kristien J. Ledeganck [a,*], *Marieke den Brinker* [a,c], *Emma Peeters* [a], *Aline Verschueren* [a], *Benedicte Y. De Winter* [a], *Annick France* [c], *Hilde Dotremont* [c], *Dominique Trouet* [a,b]

[a] *Laboratory of Experimental Medicine and Paedciatrics and member of the Infla-Med Centre of Excellence, University of Antwerp, Antwerp, Belgium*

[b] *Department of Paediatric Nephrology, Antwerp University Hospital, Antwerp, Belgium*

[c] *Department of Paediatric Endocrinology, Antwerp University Hospital, Antwerp, Belgium*

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ABSTRACT

Aims: Micro-albuminuria is considered an early clinical sign of diabetes nephropathy, however, early decrease of glomerular filtration can be present years before the presence of microalbuminuria. In this study, we explored whether urinary epidermal growth factor (uEGF) might serve as an early marker of diabetes nephropathy compared to microalbuminuria in children and adolescents.

Methods: Children with type 1 diabetes mellitus (n = 158) and healthy controls (n = 40) were included in this study. Serum and urine samples were collected three times with an interval of at least one month to determine creatinine (serum and urine), epidermal growth factor and albumin (urine). Demographic data and routine lab values were extracted out of the electronic patient files.

Results: uEGF was significantly lower in children with T1DM compared to healthy controls ($p = 0.032$). A relatively lower glomerular filtration rate (eGFR) was associated with a decreased uEGF ($p < 0.001$). uEGF was independently associated with eGFR in a multivariate analysis.

Conclusion: This study provides evidence that uEGF can serve as an early marker of diabetes nephropathy in children and adolescents.

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1. Introduction

Type 1 diabetes mellitus is an auto-immune metabolic disease with chronic hyperglycaemia. For many patients with type 1 diabetes (T1DM), it is challenging to maintain near-normal glucose blood levels and to reduce the risk of both acute (hypoglycaemia, ketoacidosis) and chronic microvascular (retinopathy, neuropathy and nephropathy) and macrovascular complications [1,2].

In adults, albuminuria is considered the first sign of diabetes nephropathy followed by a decline in kidney function

* Corresponding author at: University of Antwerp, Universiteitsplein 1, T3.34, 2610 Antwerp, Belgium.
E-mail address: kristien.ledeganck@uantwerpen.be (K.J. Ledeganck).

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and eventually leading to end-stage renal disease [3]. In addition to glomerular injury, renal tubulointerstitial changes are a major feature of diabetes nephropathy as well, representing an important predictor of renal dysfunction [4]. Diabetes nephropathy is rarely encountered in childhood; however, structural and functional subclinical abnormalities can already be detected within a few years after the clinical diagnosis of diabetes [5]. The first step in the screening and diagnosing of diabetes nephropathy is to measure albumin in a spot urine sample, collected either as the first urine in the morning or at random. Currently, microalbuminuria cut-off values of 17 – 20 mg/dl [6,7] or > 30 mg/g creatinine [8] are withheld to diagnose diabetes nephropathy. However, although microalbuminuria is widely used as a predictor for diabetes nephropathy, only 30% of the patients with microalbuminuria progress to overt proteinuria. On the other hand, some patients without microalbuminuria display advanced renal pathological changes, indicating that microalbuminuria may not be an optimal marker for early detection of diabetes kidney disease [9]. Therefore, there is a need to identify more sensitive and specific biomarkers than microalbuminuria to early detect diabetes kidney disease.

Epidermal growth factor (EGF) stimulates cell growth, proliferation and differentiation by binding to its receptor EGFR [10]. It has been proven to be a potent mitogen by stimulating mRNA, DNA and protein synthesis of epithelial cells [11]. EGF is locally produced in several tissues, such as Henle's loop and the distal convoluted tubule in the kidney, salivary glands and duodenum [12]. In the kidney, EGF is involved in the repairing process of kidney structures [11,13]. EGF is synthetised along the kidney tubules and excreted into the urine, making urinary EGF a potential hallmark for regenerative functional capacity of the tubulointerstitial compartment of the kidney. Lower urinary EGF values in children with T1DM might thus point to a reduced regenerative capacity of the kidney, making urinary EGF values a potential biomarker of early diabetes nephropathy. In children with Alport syndrome, a rare hereditary kidney disease, the uEGF/creatinine ratio decreased with age with a significantly faster rate compared to healthy children with the same age and in addition, uEGF/creatinine was significantly associated with the glomerular filtration rate in a multivariate model [14]. In contrast to microalbuminuria, which is a marker of glomerular damage, a disturbance in the renal EGF production will rather reflect tubulointerstitial kidney damage.

In this prospective single centre study, we aimed to explore whether the urinary EGF concentration might serve as an early marker of diabetes nephropathy compared to microalbuminuria and kidney function.

2. Subjects

The study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. The study protocol was approved by the Ethics Committee of the Antwerp University Hospital (file numbers 16/22/228 (children with T1DM) and 9/44/231 (healthy children)). All patients and healthy children and their parents or legal guardians gave a written informed consent.

Paediatric patients with T1DM were included at the Antwerp University Hospital (n = 158) between October 2016 and April 2018 in this observational, monocentric, prospective cohort study. At 3 time points with an interval of at least 1 month, blood and urine samples were collected from each patient to determine creatinine, magnesium, albumin and EGF (urine).

Inclusion criteria: the patients included into the study needed to meet the following inclusion criteria: 1) age $\leq$ 18 - years, 2) type 1 diabetes was diagnosed for at least one year and 3) minimum dose of insulin treatment $\geq$ 0.5E/kg/d (thereby excluding patients with a significant autonomous insulin production).

Exclusion criteria: patients using drugs related to hypomagnesaemia (such as diuretics, calcineurin inhibitors and cisplatin) and patients with known genetic syndromes were excluded from the study.

Healthy controls were recruited voluntary by email advertisement in our faculty (n = 40). All patients and the parents or legal guardians of the children below 18 years old gave a written informed consent. Patients with a chronic medical condition or on any chronic medication were excluded. Both serum and urine samples were collected during a single visit.

3. Materials and methods

3.1. Clinical data

From the T1DM patients, age, sex, medical history and medication, dose of insulin (units/kg/d), time of diagnosis of diabetes type 1, height and weight were extracted out of the electronic patient files. In the healthy control group, the children's parents completed a questionnaire on medical history and biometrics.

Body surface area (BSA): In children, the body surface area was calculated from height and weight as defined by Mosteller [15]: BSA (m^2) = (height (cm) $\times$ weight (kg)/3600)^{½}. *Body mass index (BMI):* The body mass index was calculated as follows: weight (kg)/height (m^2). *Z-scores:* For children, Z-scores were calculated for BMI, height and weight. All anthropometrics were measured (for weight and height) or calculated (for BSA, BMI and z-scores) at each time point.

Autoimmune disease: Evidence for autoimmune disease was defined as the clinical presence of celiac disease and/or thyroiditis. Besides, serum antibodies against thyroid peroxidase and transglutaminase were once determined. The presence of at least one antigen was scored as positive for autoimmune disease.

3.2. Laboratory measurements

Serum and urine creatinine, urine total protein and microalbuminuria were analysed with the Dimension Vista system (Siemens Healthcare Diagnostics, Deerfield, MA, USA).

Creatinine clearance was calculated using the Bedside Schwartz equation, which is the recommended equation to estimate the GFR (eGFR) in children [16].

Urinary EGF was measured using an EGF human Elisa kit® (Invitrogen, California, USA), according to the manufacturer's

guidelines. The detection limit of this assay was 3.9 pg/ml. For the comparative analysis in children and adolescents, we needed to correct the values accounting for growth. Therefore urinary EGF was logarithmically transformed and corrected for kidney function (urinary creatinine) and body surface area (BSA) as previously published [17].

3.3. Statistical analysis

All data were analysed using SPSS (version 27.0). Statistical significance was predetermined as a p-value < 0.05. Normality was tested with the Kolmogorov-Smirnov test. Parametric data are expressed as mean ± standard deviation (SD) and non-parametric data are expressed as median (minimum–maximum). Urinary EGF was log transformed to normalise the data.

Baseline differences between the study and control population were calculated with Student's t-tests for parametric variables and with Mann-Whitney-U tests for skewed variables. The correlation analysis between variables was tested with a Pearson or Spearman correlation test for parametric or skewed data respectively. Several blood and urine samples were collected from each patient (1–3 samples per patient); therefore blood and urine samples could not be considered independent but instead 'nested' per patient. As such, we assumed that the n_j blood samples recruited from the j^{th} patient might share some proportion of variance in characteristics (e.g. serum and urine values) attributable to the patient. Generalized Estimating Equations (GEE) were used to analyse the data collected per visit since the groups had unequal sample sizes. Moreover, the number of visits per patient varied from 1 to 3. GEEs were used to calculate the estimated means of variables and to compare them between groups. Models were constructed to study the time-dependency of the variables and to determine the relationship between outcome variables, amongst others kidney function and urinary EGF excretion.

4. Results

4.1. Population demographics

One hundred and fifty-eight patients with T1DM and 40 healthy children were included in the data analysis. From 76% (n = 120) of the patients with T1DM, three consecutive samples were collected, from 17% (n = 27) of the patients only two samples were collected and from 7% (n = 11) of the patients a single blood and urine sample was collected.

The children with T1DM in the study population were older compared to the healthy control group. Therefore, the control group was matched for age with the T1DM group, thereby excluding 9 children younger than 5 years old. Urinary EGF was significantly lower in T1DM compared to healthy children as presented in Table 1. In children with T1DM, the mean duration of diabetes was 5.49 ± 3.29 years with a mean HbA1c of 7.7 ± 1.0% (60 ± 11 mmol/mol) and a mean insulin need of 0.87 ± 0.20 units per kg per day. Forty-four children (27.8%) were younger than 6 years when T1DM was diagnosed.

4.2. Urinary EGF as a potential marker of diabetes nephropathy

Age (p < 0.001) and the eGFR (p < 0.001) were associated with the urinary EGF/creatinine while the duration of diabetes (p = 0.393), the insulin dose/kg/day (p = 0.108), microalbuminuria (p = 0.661) and HbA1c (p = 0.115) were not. In a multivariate GEE model, age (p < 0.001) and eGFR (p = 0.003) remained independently associated with uEGF/creatinine, indicating that urinary EGF is related to kidney function in patients with T1DM independent from age.

4.3. Data analysis according to the kidney function

In analogy with Di Bonito *et al.* [18], the population was subdivided according to kidney function. Since age significantly differed between the 3 groups according to the kidney function with an inverse relation between age and kidney function, an additional step was included in the statistical analysis. First, it was investigated whether a specific variable was related to age, which was true for triglycerides, HDL, the daily dose of insulin, uric acid, the neutrophil/lymphocyte ratio, HbA1c, the duration of diabetes and uEGF. Next, the difference between the 3 groups according to their kidney function was calculated using GEE and for each of the age-dependent variables, the calculated value was corrected for age by including age in the model, as displayed in Table 2.

Strikingly, a significant difference in urinary EGF was noticed between the three groups of estimated GFR, thereby observing a clearly lower urinary EGF in parallel to progressive decrease in renal function. None of the patients with an eGFR < 90 ml/min/1.73 m^2 had microalbuminuria > 30 mg/g creatinine while 9.5% and 10.1% of the patients with an eGFR 90–110 and > 110 ml/min/1.73 m^2 respectively did have microalbuminuria > 30 mg/g creatinine (p = 0.466).

In contrast to what would be expected from the current literature, relatively low estimated GFR in our population is associated with very low microalbuminuria. When focusing on the non-microalbuminuric subpopulation, an early decrease in renal function in our patients was associated with a microalbuminuria lower than 8.5 mg/g (5.7–9.5 mg/g). Extrapolating the cut-off line of 90 ml/min/1.73 m^2, this very low microalbuminuria appeared systematically accompanied by a urinary EGF/creatinine value lower than 30 ng/mg (see Fig. 1).

In a multivariate GEE analysis, log uEGF/creatinine (B = 0.189; p = 0.013) remained independently associated with kidney function (eGFR) after correction for age.

5. Discussion

This clinical study provides evidence for new insights in early kidney damage in children and adolescents with T1DM. Urinary EGF/creatinine was significantly lower in T1DM when compared to age-matched healthy controls. Urinary EGF/creatinine corrected for age was significantly lower in children with T1DM who had a relatively lower eGFR, an observation that was confirmed in a multivariate analysis. A decreased urinary EGF/creatinine appears to be an early sign of kidney

Table 1 – Baseline group characteristics.

	T1DM (n = 158)	Healthy Controls (n = 31)	p-value
Age (Y)	13.28 ± 3.10	12.90 ± 3.77	0.551
Male sex (%)	44	42	0.808
Height Z-score	0.01 ± 1.07	0.34 ± 1.03	0.114
Weight Z-score	0.37 (-1.84 – 2.61)	0.40 (-1.53 – 3.14)	0.690
BMI Z-score	0.38 ± 0.96	0.41 ± 1.27	0.595
Urinary EGF (ng/ml)	46.5 (2.51 – 128.66)	86.3 (17.9 – 129.6)	**<0.001**
Urinary EGF/creatinine/BSA (ng/mg/1.73 m^2)	36.5 (7.3–250.1)	53.8 (20.9–212.9)	**0.010**
Urinary EGF/creatinine (ng/mg)	32.8 (6.2–96.3)	46.0 (23.1–121.0)	**0.001**
eGFR (ml/min/m^2)	111 ± 18	105 ± 16	0.125

Normally distributed variables are presented as mean ± SD; skewed data are presented as median (minimum–maximum). T1DM: type 1 diabetes mellitus; BMI: body mass index; EGF: epidermal growth factor; BSA: body surface area; eGFR: estimated glomerular filtration rate

damage in children and adolescents with T1DM. Its screening potential for diabetes nephropathy should further be explored in a long term follow-up study.

Diabetes nephropathy still remains the leading cause of death in patients with T1DM. Early DN is characterized by an increased intraglomerular pressure resulting in renal hyperfiltration leading to glomerular injury. This process remains frequently beneath any diagnostic radar due to lack of clinical symptoms. Eventually, there will be a progressive GFR decline, with microalbuminuria being considered as the earliest sign of DN [19]. However, despite its recognition as the golden standard method to diagnose early DN, microalbuminuria has drawbacks as a biomarker. As already mentioned in the introduction, on the one hand, some patients with advanced renal pathological changes do not develop microalbuminuria while on the other hand, only 30% of patients with microalbuminuria progress to overt proteinuria. Besides, the presence of orthostatic microalbuminuria might also impede proper interpretation of urine samples. This illustrates that it remains highly important to keep on searching for alternative or additional markers that not only allow for the early diagnosis but also reflect the underlying pathogenic mechanism of the diabetes nephropathy. Not only glomerular damage but also tubulointerstitial injury are major features of renal decline in T1DM [4].

Although no overt microalbuminuria was seen in our clinical study, a paradoxically lower microalbuminuria was observed in children and adolescents with a lower limit eGFR. This could implicate that glomerular hyperfiltration precedes and even triggers early tubulointerstitial kidney damage and that the combination of low microalbuminuria plus low urinary EGF is indicative of early decline in renal function due to superposition of tubulointerstitial fibrosis upon glomerular injury.

In adults, urinary EGF has been identified as a predictor of CKD progression in patients with glomerular disease[20]. In children with Alport syndrome, urinary EGF has recently been highlighted as a prognostic biomarker for tubulointerstitial fibrosis leading to renal decline [21]. In view of the similarities in this underlying pathogenic mechanism in diabetes, also children with T1DM might exhibit a disturbed renal EGF production indicative of insidious kidney disease. Here, it is of utmost importance to realize that the urinary EGF concentration decreases with age as demonstrated earlier[17]. Therefore, age should always be taken into account when investigating EGF concentration during childhood. In this prospective study, urinary EGF/creatinine was associated with the eGFR independent from age in a multivariate model. Our results are in agreement with a cross-sectional study in children and adults executed in 1990 where Lev-Ran *et al.* described a positive correlation between urinary EGF/creatinine and the creatinine clearance [22]. Urinary EGF has been suggested as a potential biomarker of progression of kidney disease in adults with T2DM as well [23].

In comparison to an age-matched healthy control group, urinary EGF/creatinine was lower in T1DM in the present study. This was also the case in children with Alport syndrome when compared to healthy subjects[21]. The level of urinary EGF/creatinine in both control groups from the Li-study and the present study are comparable taking age into account. The decrease in urinary EGF/creatinine in patients with Alport syndrome depending on the slope of decline of renal function, fluctuated between 10.59 ng/mg and 27.83 ng/mg [21]. In our study, the urinary EGF/creatinine level was 32.8 ng/mg which approximates the urinary EGF/creatinine concentration in the earliest phase of renal decline in Alport syndrome. The pathophysiology of kidney failure in children with Alport syndrome and T1DM is similar, however, in Alport syndrome, the kidney failure progresses more rapidly compared to T1DM nephropathy. The observed decreased values of urinary EGF/creatinine in our study are in line with the reported additive value of decreased urinary EGF/creatinine according to stage of kidney failure in children with Alport syndrome. Compilating these data, we have arguments to complete the current algorithm of early prevention of renal decline in T1DM children and adolescents, by taking into account both the paradoxically low microalbuminuria, as well as the 'next generation' biomarker urinary EGF. According to the KDOQI guidelines, normotensive patients with diabetes and macroalbuminuria should be treated with ACE inhibition or ARB, while it is also recommended in patients with diabetes and microalbuminuria. From our results, we believe to have enough arguments to start ACE inhibition also in patients with urinary EGF/creatinine lower than 30 ng/mg similarly to the classically microalbuminuric patients as proposed in the flow chart underneath (see Fig. 2).

The following step should focus on the economic benefit to the use of urinary EGF/creatinine as an add-on biomarker.

Table 2 – overview of demographic and laboratory values according to the kidney function (# corrected for age where appropriate).

	eGFR < 90 ml/min/ 1.73 m^2 (n = 17)	eGFR 90–110 ml/min/ 1.73 m^2 (n = 67)	eGFR > 110 ml/ min/1.73 m^2 (n = 74)	p-value
Demographic data				
Age (years)	16.4 ± 0.27	14.8 ± 0.28	11.9 ± 0.35	**<0.001**
Sex (% male)	52.9	41.8	44.6	0.709
Autoimmune disease				
Autoimmune diseases (%) based on clinical decision	5.9	6.0	5.4	0.989
Autoimmune diseases based on ≥ 1 positive antigen detection (%)	5.9	10.4	15.3	0.481
Metabolic features				
BMI Z-score	0.59 ± 0.18	0.52 ± 0.12	0.24 ± 0.10	0.097
Waist Z-score	0.48 ± 0.23	0.31 ± 0.17	0.32 ± 0.12	0.816
Triglycerides #	78.55 ± 11.23	82.44 ± 5.80	83.14 ± 5.64	0.941
HDL (mg/dl) #	74.14 ± 6.27	70.93 ± 2.38	66.20 ± 2.11	0.327
Daily dose of insulin (IU/kg/d) #	0.72 ± 0.05	0.86 ± 0.02	0.92 ± 0.02	**0.002**
Features of low grade inflammation				
Uric acid (mg/dl) #	4.16 ± 0.28	3.89 ± 0.12	3.80 ± 0.10	0.501
Neutrophil/Lymphocyte ratio #	1.47 ± 0.22	1.83 ± 0.14	1.73 ± 0.12	0.393
hsCRP (mg/l)	1.06 ± 0.18	2.35 ± 1.08	1.64 ± 0.52	0.307
Features of diabetes mellitus				
HbA1c (%)	7.2 ± 0.2*	7.6 ± 0.1	7.9 ± 0.1	0.055
HbA1c (mmol/mol) #	55 ± 3*	60 ± 1	62 ± 1	0.062
Duration of diabetes (y)	4.34 ± 0.73	5.53 ± 0.42	5.91 ± 0.38	0.208
Features of impaired kidney function				
eGFR (Bedside Swartz)	86.6 ± 1.3	100.6 ± 0.79	125.6 ± 1.5	**<0.001**
Microalbuminuria/creatinine (mg/g)	7.3 ± 0.8	13.2 ± 1.5	21.9 ± 3.9	**<0.001**
Urinary EGF/creatinine (ng/mg) #	33.89 ± 2.53	32.72 ± 1.87*	39.44 ± 1.92	**0.070**
Urinary EGF/creatinine/BSA (ng/mg/1.73 m^2)	23.42 ± 2.25	31.98 ± 3.10	60.68 ± 5.06	**<0.001**

The T1DM population was subdivided into 3 subgroups according to the kidney function determined by eGFR. Statistics were performed using generalized estimated equation (GEE) analysis taking all visits per patient into account and expressed as mean ± SE or as % for categorical variables. *P < 0.05 compared to eGFR > 110. # GEE was performed corrected for age. BMI: body mass index; HDL: high density lipoprotein; hsCRP: high sensitive C-reactive protein; eGFR: estimated glomerular filtration rate; BSA: body surface area.

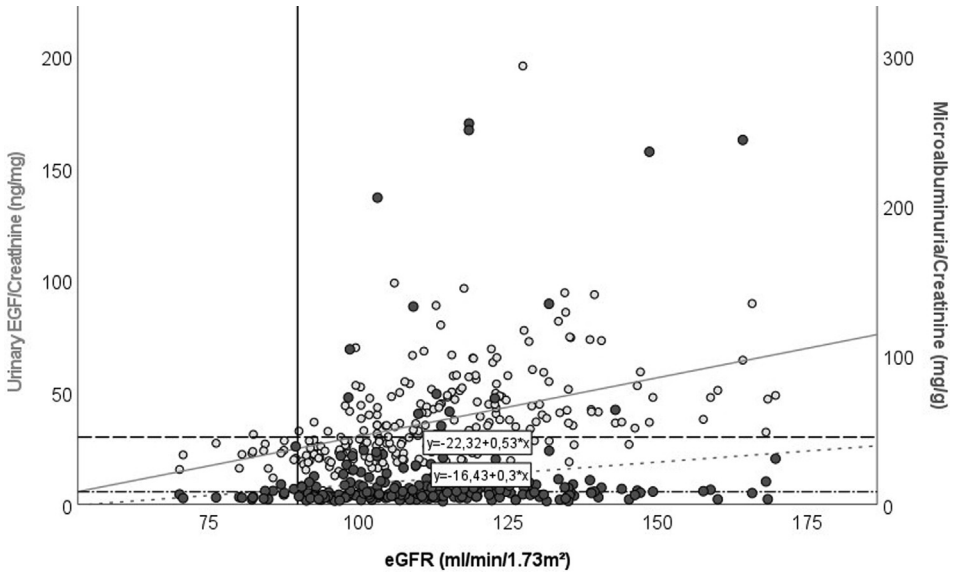

Fig. 1 – urinary EGF/creatinine (open dots) and microalbuminuria/creatinine (black dots) in relation to the kidney function. In this figure, the measurements of each visit (1–3 per patient) are displayed. A vertical reference line represents the eGFR cut-off line of 90 ml/min/1.73 m^2 while the upper dashed horizontal line represents the urinary EGF/creatinine cut-off line of 30 ng/mg and the lower dash-dotted horizontal line represents a microalbuminuria/creatinine of 8.5 mg/g. An early decrease in renal function was associated with a microalbuminuria lower than 8.5 mg/g and this very low microalbuminuria appeared systematically accompanied by a urinary EGF/creatinine value lower than 30 ng/mg. EGF: epidermal growth factor; eGFR: estimated glomerular filtration rate.

With increasing overall healthcare costs in times that resources are limited, one will more and more rely on this information, while making decisions about reimbursement in clinical practice. The majority of studies examine the economic benefits of more expensive treatments and interventions compared to laboratory tests, most probably due to the fact that only a small number of randomized control trials (RCT) investigate biomarkers[24]. Nevertheless, it can be expected that more and more laboratory tests will be subjected to cost studies in future. The specific economic benefit of adding urinary EGF/creatinine to the standard of care regimen in patients with T1DM will most probably be experienced at the long term. The earlier diabetes nephropathy can be diagnosed, the sooner preventive therapy can be initiated to slow down the evolution to chronic kidney disease. However, as stated before, RCTs are needed to confirm these hypotheses.

In order to study the impact of autoimmunity on the above described process of renal decline, we verified the same panel of conditions as proposed by Di Bonito *et al.* more specifically an increased uric acid, the presence of autoimmunity and low-grade inflammation[18]. In the present study, higher serum uric acid were observed in the children and adolescents with a lower eGFR, however, after correction for age, this was no longer the case. In our population, there was no relation between the eGFR and the presence of autoimmunity or low-grade inflammation. In a multivariate analysis, only urinary EGF remained an independently associated with the kidney function.

The current findings seem to create a wake-up call for paediatric diabetologists, since early signs of diabetes nephropa-

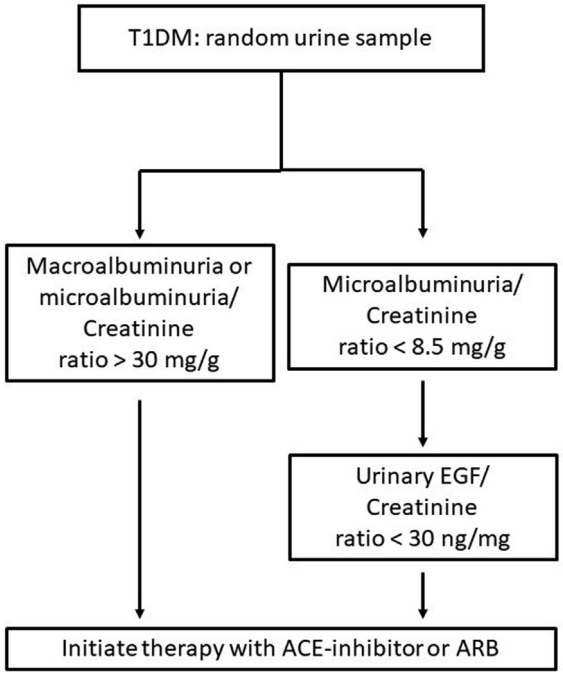

Fig. 2 – Flow chart: current KDOQI guidelines recommend in T1DM patients to start ACE inhibition or treatment with ARB macroalbuminuria or microalbuminuria is observed (left flow). We believe to have enough arguments to start ACE inhibition also in patients with paradoxically low microalbuminuria/creatinine accompanied with urinary EGF/creatinine lower than 30 ng/mg (right flow). EGF: epidermal growth factor; ACE: angiotensin converting enzyme; ARB: angiotensin receptor blocker.

thy in this paediatric study population are present, irrespective of the duration of diabetes. Even with a relatively recent diagnosis of T1DM, children and adolescents can thus already be prone to renal damage. Moreover, the patients with the highest HBA1C presented with the highest eGFR, indicative of hyperfiltration rather than of better kidney function. Hyperfiltration in paediatric diabetes patients should thus be considered a red flag, since it can initiate early renal interstitial deterioration at short term.

6. Study strengths and limitations

Strengths: this study had several strengths that led to new insights in the detection of early kidney damage in children and adolescents with T1DM. The study design with multiple measurements per patient with a low dropout rate, the low prevalence of comorbidities in this paediatric population and the inclusion of a healthy control group enabled us to draw sound conclusions. Even in this population with a limited duration of diabetes (~ 5 year), the very early observed signs of renal decline can be considered as a supplementary strength. *Limitations:* the sample size was rather small in this study with the inclusion of 159 patients with T1DM. Therefore, several samples (1–3) were collected per patient. With this repeated measures study design, the study was more efficient, allowing for smaller than usual subject groups. The fact that random urine samples were collected can be considered as a limitation of the study, since orthostatic microalbuminuria can therefore not be excluded in our population. It would have been beneficial to evaluate the nutritional status of the patients and future studies should definitely consider to include this additional information in order to provide a complete image of the study groups.

7. Future perspectives

Based on the knowledge that was gathered in this study, future RCTs are needed to confirm that the flow chart as proposed in Fig. 2 indeed slows down the evolution of early diabetes nephropathy to progressive chronic kidney disease in comparison to the existing standard of care regimen. It is recommended that future studies also gather additional information in patients with T1DM such as nutritional status and that the research population is expanded to children and adolescents with different insulin needs (ranging from very low to high daily doses) which depend, amongst others, on age, BMI, insulin resistance and puberty stage.

8. Conclusion

uEGF is a promising 'next generation' biomarker associated with early signs of diabetes nephropathy in children and adolescents and worth further investigation as a single biomarker or as one of a multi-biomarker panel. It would be interesting to see if the implementation of our proposed algorithm (starting ACE-inhibition at low urinary EGF level) would be able to prevent further renal decline or even achieve stabilisation of kidney function at later age.

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