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Proenkephalin A as a marker for glomerular filtration rate in critically ill children: validation against gold standard iohexol GFR measurements

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Abstract

Objectives: Accurate determination of glomerular filtration rate (GFR) is important. Several endogenous biomarkers exist for estimating GFR, yet, they have limited accuracy, especially in the paediatric population. Proenkephalin A 119–159 (PENK) is a novel and promising GFR marker, but its relation with age in children remains unknown. Also, the value of PENK has never been validated against measured GFR (mGFR) in children when compared to traditional GFR markers including serum creatinine (SCr), SCr-based estimated GFR (eGFR) and cystatin C (cysC).

Methods: Critically ill children and term-born neonates were included in this single-centre, prospective study. Iohexol-based mGFR, SCr, and cysC were determined in each patient. eGFR was calculated using the bedside Schwartz equation, incorporating SCr and height. Spearman correlation coefficients were calculated to determine the correlation between mGFR and PENK, SCr, cysC and eGFR.

Results: For 97 patients (56 children and 41 neonates), mGFR, SCr, cysC and PENK levels were available. PENK levels were higher in young children and decreased to adult PENK reference values around two years of age. PENK levels were highly correlated with mGFR ($\rho=-0.88$, $p<0.001$), and similar to mGFR–eGFR correlation ($\rho=-0.87$, $p<0.001$). For cysC and SCr the correlation with mGFR was lower ($\rho=-0.77$ and $\rho=-0.46$, respectively. Both $p<0.001$).

Conclusions: The correlation of PENK with mGFR was as good as SCr-based eGFR-mGFR correlation. To determine the added value of PENK in paediatric clinical care and prior to implementation, PENK reference values are needed and the development and validation of a paediatric PENK-based eGFR equation is necessary.

Keywords: acute kidney injury; glomerular filtration rate; proenkephalin.

Introduction

Acute kidney injury (AKI), resulting in a diminished glomerular filtration rate (GFR), is a frequent and serious condition in intensive care patients. As AKI and decreased GFR affect fluid and electrolyte management, as well as drug clearance, accurate determination of GFR is important. In daily clinical care, GFR is currently estimated using endogenous markers, most frequently serum creatinine (SCr). Similarly, AKI is often diagnosed using a combination of SCr, whether or not combined with urine output. Accurate estimation of GFR is, however, challenging in children. Neonatal SCr values reflect maternal SCr values in the first days of life [1] and GFR increases with age [2]. Furthermore, tubular secretion of creatinine varies, leading to imprecise estimation of GFR. Other markers, such as cystatin C (cysC), are available. Yet, cystatin C levels are influenced by situations of increased metabolism including infections, hyperthyroidism and the use of corticosteroids [3]. Therefore, the value of cysC over SCr as a marker for GFR in critically ill patients remains debatable. In addition to estimation of GFR using endogenous biomarkers, GFR can be directly measured using exogenous

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substances. As serum concentrations of these markers are not influenced by other factors than GFR, their use is considered gold standard for GFR determination [4]. As these GFR measurements are more cumbersome than estimation of GFR with SCr and/or cysC, the latter remains standard of care.

Due to the drawbacks of existing endogenous markers and the complexity of measured GFR (mGFR) determination, the search for accurate GFR biomarkers is ongoing. One of the more recently discovered biomarkers is proenkephalin A 119–159 (PENK). PENK is a stable opioid peptide cleaved from preproenkephalin alongside other enkephalins. Enkephalins are one group of endogenous opioid peptides that bind to the μ-, κ- and δ-opioid receptor. Via neural signalling, they can affect respiratory depression, antinociception and physical dependence.

PENK is a small molecule (size 4.5 kDa) and is freely filtered through the glomerulus without active clearance in the kidney or elsewhere [5]. PENK reference values were established in healthy adults [6] and PENK was strongly correlated with GFR in adult sepsis patients [7]. Interestingly, in healthy children under one year of age, PENK reference values were on average five-fold higher than adult PENK concentrations [8]. However, the exact trajectory of PENK levels between one and 18 years of age remains unknown. Age-appropriate reference values for the whole paediatric age range are needed to determine physiological values and appropriate cut off values for the diagnosis of AKI. Additionally, in infants below one year of age, PENK clearly discriminated between infants with and without AKI [8]. Yet, in this study, sensitivity and specificity were calculated using SCr-based AKI diagnosis as a reference, preventing comparison between PENK and 'true' GFR. Hence, there is a need to compare the value of PENK with mGFR as gold standard in order to define its value as a marker for GFR across the entire paediatric age range. Therefore, in a cohort of critically ill children, 0–18 years of age, we aimed to determine both iohexol-based mGFR and PENK levels in order to a) study the relationship of age with PENK levels in critically ill children and b) validate the value of PENK against mGFR as compared to SCr, SCr-based eGFR and cysC.

Materials and methods

The methods of this single-centre, prospective study were previously described in detail [9]. The Iohexol for Measuring Renal Function (HERO) study was registered on clinicaltrials.gov (registration number NCT03946345) before start of the study. The HERO study protocol was approved by the Medical Ethics Review Board (CMO Arnhem-Nijmegen, NL68547.091.18, 2018–5025).

Patient selection

Patients were eligible for inclusion when they were below 18 years of age, term-born (≥37 weeks of gestation, if <1 year of age), had a bodyweight of more than 2,500 g and at least one failing organ as defined by a Paediatric Logistic Organ Dysfunction II (PELOD-II, ranging from 0 to 33) score of 1 or higher [10]. They also needed to have an indwelling central venous or arterial line already in place for clinical purposes. Exclusion criteria were a known medical history of allergic reaction to injection of iodinated contrast material, receiving kidney replacement therapy or extra corporeal membrane oxygenation, and language or cognitive inability of parents/caregivers to understand written and/or oral information. Informed consent needed to be provided by parents or other legal representatives if the child was below 16 years of age. Consent of the child was needed if aged above 12 years of age and medical and cognitive state permitted.

mGFR and eGFR determination

After inclusion, iohexol (Omnipaque® 300 mg/mL, GE Healthcare, Chicago, Illinois, USA) was administered as a single bolus dose adapted to bodyweight as follows: <10 kg, 1 mL; 10–20 kg, 2 mL; 20–30 kg, 3 mL; 30–40 kg, 4 mL; ≥40 kg, 5 mL [11]. To determine mGFR, blood samples were drawn for analysis of iohexol concentrations at 2, 5 and 7 h after administration. Two-point blood sampling at 2 and 5 h after administration is a validated method for mGFR determination in children [11]. To enhance accuracy as low GFR values were expected, we added another sampling point at 7 h after administration for neonates with a bodyweight of at least 3.5 kg and children older than 28 days of age. SCr, cysC and PENK levels were determined 2 h after iohexol infusion for eGFR determination to reflect the clinical situation in which biomarkers are measured at point of care and to correspond to the first blood withdrawal point needed for mGFR, preventing an extra blood withdrawal.

SCr and cysC levels were determined as previously described [8]. PENK was measured in EDTA plasma samples using the immunoluminometric assay sphingotest® penKid® (SphingoTec GmbH, Hennigsdorf, Germany) as described previously [6]. Iohexol plasma concentrations were determined at the Leiden University Medical Centre, Leiden, the Netherlands, using a validated high-performance liquid chromatography diode array detection assay [12]. The assay was validated according to the European Medicines Agency bioanalytical method validation guidelines [13]. The laboratories performing the PENK and iohexol measurements were blinded to clinical and demographic data of the patients.

Iohexol-based mGFR and eGFR were determined in each patient at a standardized timepoint early at admission, regardless of clinical status in order to reflect GFR for the entire critically ill population. Calculations to determine mGFR in children using iohexol were previously published [11]. mGFR based on iohexol plasma clearance was calculated based on the ratio between the administered iohexol dose and the area under the plasma concentration time curve. A slope-intercept method, using the Jødal and Brøchner–Mortensen formula with early normalization to 1.73 m^2 body surface area (BSA) was employed as this method was previously validated in children with

chronic kidney disease (CKD) [11, 14]. The Haycock-formula was used to calculate BSA [15, 16].

eGFR based on SCr was estimated using one equation including two different age-specific coefficients (k):

$$\text{eGFR}\ (\text{mL/min/1.73 m}^2) = k * \text{height}\,(\text{m})/\text{SCr}\,(\text{mg/dL})$$

This frequently used Schwartz equation was used with fixed coefficients reported for children below (k=44) and above one year of age (k=41.3) (eGFR-bedside) [17, 18].

AKI diagnosis

AKI was diagnosed using SCr, urine output, and a combination of SCr and urine output with the Kidney Disease Improving Global Outcomes (KDIGO) criteria. The KDIGO AKI definition was defined using median age specific reference values of enzymatic SCr for neonates [19] and children from 28 days of age [20] to circumvent the lack of baseline SCr values due to unplanned admissions. AKI was defined as >150% of median age specific reference values for SCr, corresponding to stage 1 AKI, as this approach was previously described by Zwiers et al. to diagnose AKI in critically ill infants [21]. Patients were also diagnosed with AKI when their urine output was below 0.5 mL/kg/h for at least 6 h [22].

Statistical analysis

All demographic data were analysed for the entire study cohort as well as separately for neonates (≤28 days of postnatal age) and children (>28 days of postnatal age). For continuous variables, data were expressed as median values with interquartile ranges (IQR) or ranges whereas for categoric variables numbers and percentages were used.

To assess the relationship of age with PENK levels, PENK was plotted against age, as well as against mGFR, and AKI status was visualized. Because our cohort consisted of critically ill children, definition of age-dependent PENK reference values was not possible. To further examine the relation between PENK and mGFR, PENK levels were visualized per GFR category according to the classification of the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative [23]. This includes the following five GFR categories: (1: ≥90 mL/min/1.73 m^2, 2: 60–89 mL/min/1.73 m^2, 3: 30–59 mL/min/1.73 m^2, 4: 15–29 mL/min/1.73 m^2 & 5: <15 mL/min/1.73 m^2). Differences in PENK levels between categories were assessed by using a Kruskal Wallis test for independent samples. To assess the influence of AKI on PENK levels, differences between AKI and non-AKI patients were assessed by using a Mann Whitney-U test.

The relation between PENK and mGFR was visually displayed after log transformation. Because SCr levels are age-dependent, a correlation between SCr alone and mGFR will lead to an underestimation of the value of SCr in estimating GFR. Therefore, we used the well-known bedside-equation from Schwartz, also incorporating height, to calculate eGFR [18]. Next, Spearman correlation coefficients (ρ), including 95% confidence intervals (CI), were calculated to determine the correlation between PENK and mGFR. Again, data were analysed for the entire study cohort as well as separately for neonates and children. Coefficients were also calculated for SCr vs. mGFR, eGFR vs. mGFR, and cysC vs. mGFR in order to enable comparison between methods. Data were analysed by using SPSS version 25.0.0.1 for Windows (SPSS, Chicago, IL, USA).

Results

For 97 patients, mGFR, SCr, cysC and PENK levels were available. The patient characteristics were presented for neonates (n=41) and children (n=56) (Table 1). At inclusion, median age was 2 days (range 0–27 days) for neonates and 5.4 years (range 0.1–17.2 years) for children. Iohexol was administered after a median duration of 27 (IQR 17–52) hours after admission. According to KDIGO criteria, 26 patients were diagnosed with AKI (8 neonates and 18 children).

In this critically ill population, PENK levels were higher in young children and decreased to adult PENK reference values around two years of age (Figure 1A). Also, mGFR levels were dependent on age and reached adult GFR values around a similar age (Figure 1B).

PENK levels were different between the different GFR categories (p=0.006) with PENK levels increasing while GFR was decreasing (Figure 2). Additionally, PENK levels were higher in AKI than in non-AKI patients in both neonates and children (Figure 3). For neonates, non-AKI patients (n=33) had lower median PENK levels than AKI patients (n=8) 632 (IQR 490–891) pmol/L vs. 1,088 (IQR 731–2020) pmol/L (p=0.005). Also in children, PENK levels were lower in non-AKI patients (n=38) compared to AKI patients (n=18) 92 (IQR 54–211) pmol/L vs. 523 (IQR 87–1,106) pmol/L (p=0.005) (Figure 3).

Furthermore, PENK levels were highly correlated with mGFR (ρ=−0.88, p<0.001), which was also the case for eGFR (Schwartz formula) (ρ=−0.87, p<0.001 (Figure 4A, D)). For cysC and SCr this correlation was lower (ρ=−0.77 and ρ=−0.46, p<0.001 respectively). When looking at neonates and children separately, distinct differences with regards to correlation with mGFR were observed (Table 2). Correlation of PENK and cysC with mGFR was higher in children than in neonates, whereas correlation of SCr and eGFR bedside with mGFR was higher in neonates.

Discussion

In conclusion, there is a clear age-related variation in PENK levels. To the best of our knowledge, we are the first to report these levels for the entire paediatric age range and to describe an age-related effect. Our results suggest that PENK levels in children reach adult PENK values around two years of age. This is interesting as GFR, when corrected for BSA, increases between birth and two years of age but remains stable thereafter [2], which could explain the high correlation between PENK and mGFR, especially in children older than one month of age.

Table 1: Patient characteristics. Diagnosis of AKI was defined based on adapted KDIGO criteria as described in the text.

	Total	Neonates (<28 days of age)	Children (>28 days of age)
Number of patients	97	41	56
Number of AKI patients[c]	26 (27%)	8 (20%)	18 (32%)
Male sex[c]	61 (63%)	24 (59%)	37 (66%)
Age			
– Years[b]	0.2 (0.0–17.2)		5.4 (0.1–17.2)
– Days[b]	78 (0–6,274)	2 (0–27)	
Weight, kg[a]	5.8 (3.5–27.3)	3.5 (2.9–3.8)	18.5 (7.6–39.3)
Height, cm[a]	61 (51–131)	51 (49–52)	109 (68–150)
Primary diagnosis[c]			
– Shock	4 (4%)	0 (0%)	4 (7%)
– Cardiovascular problems	19 (20%)	7 (17%)	12 (21%)
– Respiratory problems	44 (45%)	24 (59%)	20 (36%)
– Surgical problems or trauma	16 (17%)	8 (20%)	8 (14%)
– Central nervous system problems	13 (13%)	2 (5%)	11 (20%)
– Sedation or pain management	1 (1%)	0 (0%)	1 (2%)
Total PELOD-II score[b]	5 (1–18)	5 (2–16)	5 (1–18)
PRISM-III score[b]	–	–	6 (0–26)
PIM2 probability score[b]	–	–	0.039 (0.016–0.98)
SNAP-II-score[b]	–	0 (0–68)	–
Patients using vasoactive drugs[c]	51 (53%)	14 (34%)	37 (66%)
Patients using nephrotoxic drugs[c]	47 (48%)	15 (37%)	32 (57%)
Time between admission & start of study, h[a]	27 (17–52)	29 (17–46)	26 (17–55)
Duration of total stay at ICU, h[a]	181 (98–300)	181 (98–285)	183 (98–311)

PELOD-II score, paediatric logistic organ dysfunction version 2 score; PRISM-III score, paediatric risk of mortality score, third version; PIM2 score, paediatric index of mortality score, second version; SNAP-II score: score for neonatal acute physiology, second version. [a]Median (IQR), [b]median (range), [c]n (%).

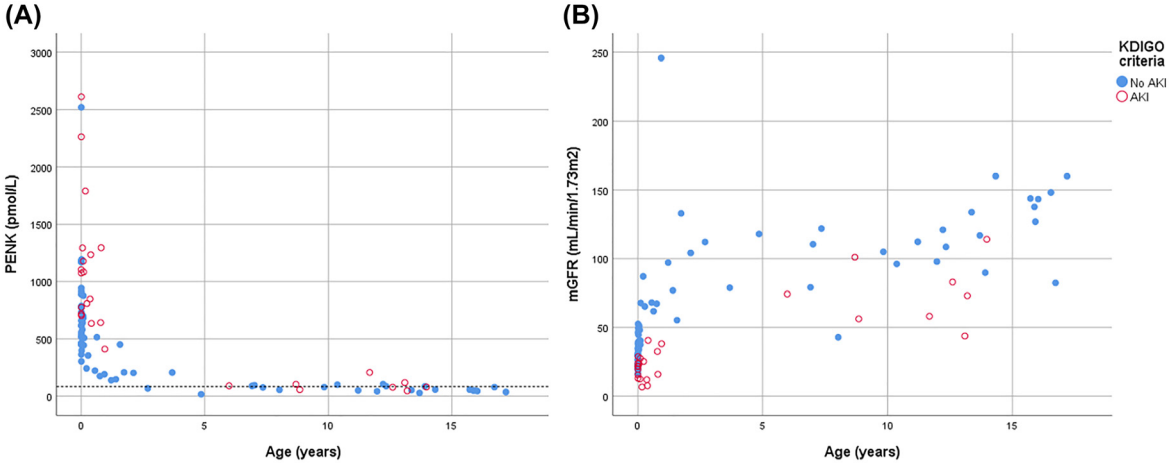

Figure 1: PENK and mGFR levels in critically ill children. (A) PENK levels are age-dependent up to around two years of age in critically ill children. The dashed line represents the upper normal reference value for PENK in adults at 89 pmol/L [6]. (B) Age dependency of mGFR levels in critically ill children. Red dots represent AKI patients, blue dots represent non-AKI patients. AKI, acute kidney injury; mGFR, measured glomerular filtration rate; KDIGO, kidney disease improving global outcomes; PENK, proenkephalin A 119–159.

Our results demonstrate higher PENK levels with lower GFR. Further analysis showed that PENK levels were higher in AKI patients compared to non-AKI patients, which is in line with results previously obtained in critically ill infants [8]. Most importantly, despite the age dependency, PENK outperforms SCr and cysC with regards to correlation

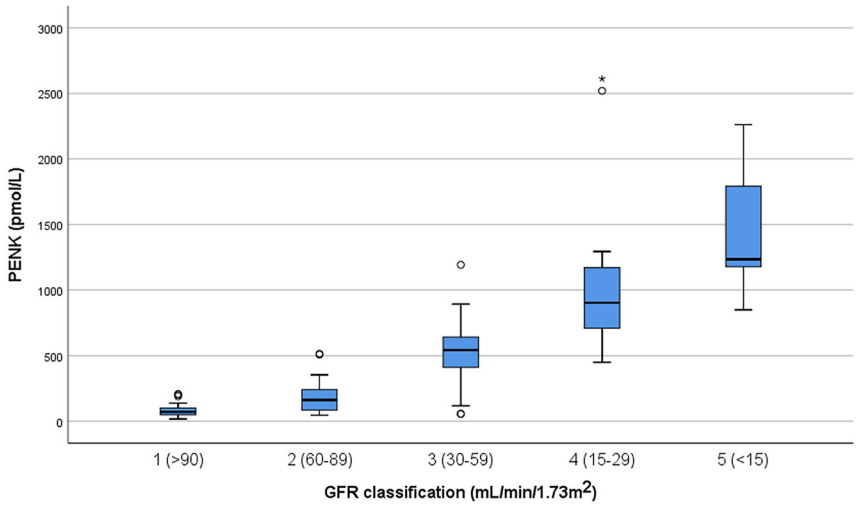

Figure 2: PENK levels were different across the different GFR categories (p=0.006). Categories according to the classification of the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative. Glomerular filtration rate; PENK, proenkephalin A 119–159. Open circles represent outliers (3rd quartile + 1.5*IQR), asterisks represent extreme outliers (3rd quartile + 3*IQR).

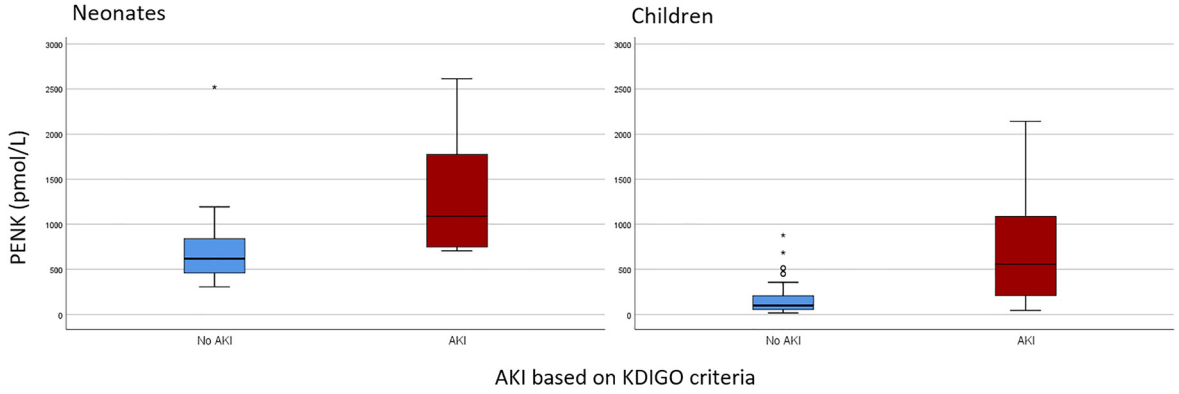

Figure 3: PENK levels were different between AKI and non-AKI in neonates (Left panel, p=0.005) and children (right panel, p=0.005). PENK, proenkephalin A 119–159; GFR, glomerular filtration rate. Open circles represent outliers (3rd quartile + 1.5*IQR), asterisks represent extreme outliers (3rd quartile + 3*IQR).

with mGFR. This high correlation of PENK and mGFR confirmed previous results obtained in adults [6, 7]. Because the correlation of creatinine-based eGFR with mGFR was similar to PENK-mGFR correlation, at this point in time, it remains to be determined whether a paediatric PENK-based eGFR equation leads to a higher eGFR-mGFR correlation compared to creatinine-based eGFR–mGFR equation. Furthermore, we indicated a different PENK-mGFR correlation between neonates and children. Yet, when calculating correlation coefficients in small subgroups, the full measurement range might not be covered, thereby decreasing the correlation when compared to the total cohort. Because the majority of our neonatal cohort did not have AKI, the full GFR range is not equally covered, which could thus explain the lower correlation coefficients observed.

Surprisingly, the correlation between cysC and mGFR was poor. Differences in performance between neonates and children need to be investigated further, as the current cohort was too small to draw firm conclusions about cysC performance. Although the use of cysC as a marker for GFR is considered promising in neonates [24], only one other study investigated the correlation between cysC and mGFR, albeit in preterms between 4 and 7 days of postnatal age, finding moderate correlation [25]. Therefore, to elucidate the value of cysC as a marker for neonatal GFR warrants further studies. Additionally, differences in mGFR correlation between neonates and children are of interest. However, because cohorts are relatively small, interpretation needs caution.

Our results clearly demonstrate that the correlation between SCr and mGFR increases when corrected for height. We believe this difference in correlation between SCr and mGFR on one hand and SCr-based eGFR and mGFR on the other can be well explained by the fact that height is included in the eGFR equation and thus addresses the

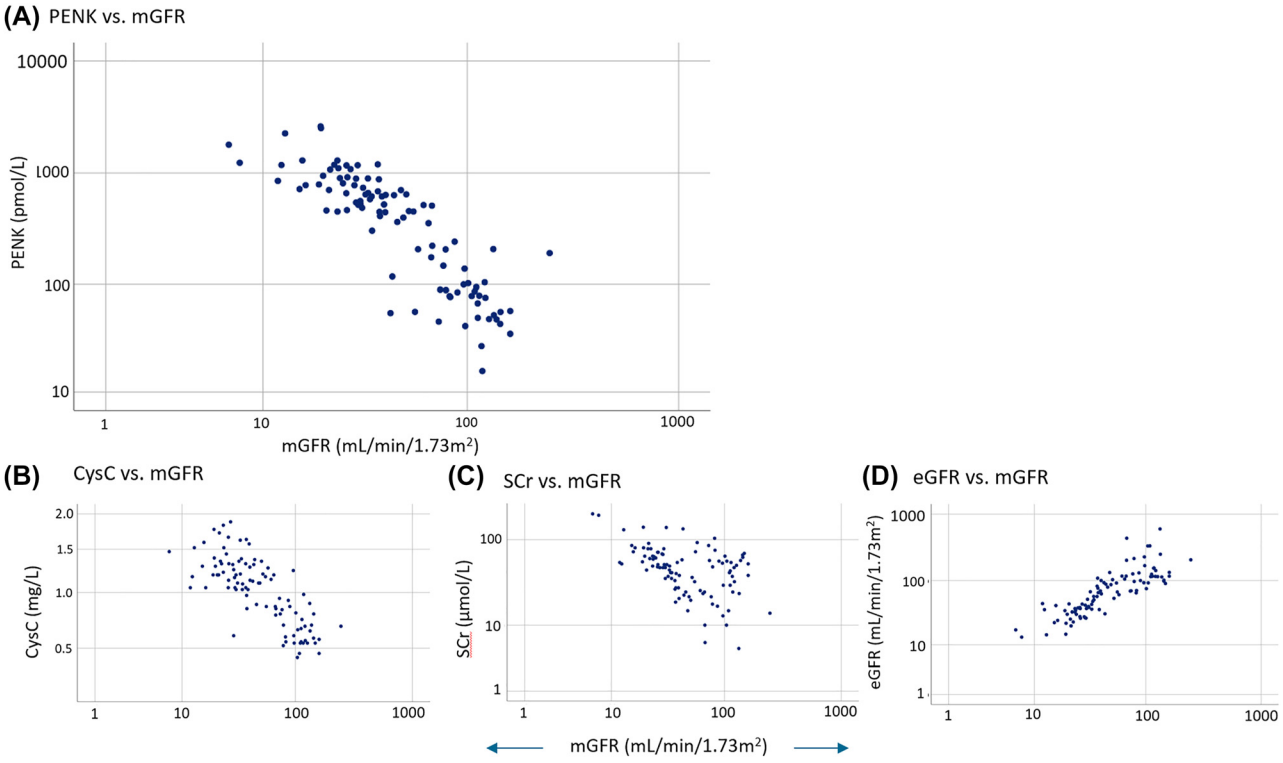

Figure 4: mGFR correlation plots. (A) The correlation between PENK and mGFR in critically ill neonates and children (n=97) is higher (ρ=−0.88, p<0.001) compared to correlation between (B) cysC vs. mGFR (ρ=−0.77, p<0.001) and (C) SCr vs. mGFR (ρ=−0.46, p<0.001). The correlation between (D) eGFR (Schwartz equation) and mGFR is similar (ρ=−0.87, p<0.001) compared to the correlation between PENK and mGFR. Variables are plotted on a log-log-scale. PENK, proenkephalin A 119–159; SCr, serum creatinine; CysC, cystatin C; eGFR, estimated glomerular filtration rate; mGFR, measured glomerular filtration rate.

Table 2: Spearman correlation coefficients (95% confidence interval) show differences in mGFR correlation between the different biomarkers and eGFR.

	PENK	CysC	SCr	eGFR bedside
Neonates (n=41)				
mGFR	−0.56 (−0.73; −0.29)	−0.32 (−0.55; 0.00)[b]	−0.85 (−0.94; −0.70)[a]	0.85 (0.7; 0.93)[a]
Children (n=56)				
mGFR	−0.76 (−0.90; 0.62)[a]	−0.77 (−0.86; −0.62)[a]	−0.13 (−0.42; 0.08)	0.67 (0.46; 0.79)[a]
Total (n=97)				
mGFR	−0.88 (−0.91; −0.83)[a]	−0.77 (−0.83; −0.68)[a]	−0.46 (−0.61; 0.30)[a]	0.87 (0.83; 0.90)[a]

PENK, proenkephalin A 119–159; SCr, serum creatinine; CysC, cystatin C; mGFR, measured glomerular filtration rate; eGFR, estimated glomerular filtration rate. [a]Correlation is significant at the 0.01 level (2-tailed). [b]Correlation is significant at the 0.05 level (2-tailed).

dependence of SCr on muscle mass. Whether the correlation between PENK and mGFR would also increase when accounted for growth using eGFR equations, remains to be investigated. Up to now, no paediatric PENK-eGFR equation exist, which is a major limitation. Future perspectives are, however, promising as a new equation based on PENK, SCr and age (PENK-SCr eGFR) in critically ill adults demonstrated higher accuracy and precision than SCr-based eGFR equations (Beunders et al., unpublished data). The lack of possibilities to use PENK for eGFR determination also prevented a clear comparison between PENK-based eGFR and mGFR by calculating median biases and visualization in a Bland-Altman plot. Until such a PENK-eGFR equation is available, it remains unknown whether PENK-based eGFR is of added value in paediatric clinical care. On a final note, excellent correlation does not

equal the absence of bias between two methods as significant and consistent over or underestimation might occur, as was previously shown for the eGFR equation in the same cohort (median bias of 11.0 (IQR 0.69–36.7) mL/min/1.73 m^2) [9]. This further highlights the need for a PENK-based eGFR equation.

Future studies will be necessary to determine the value of PENK as a biomarker to diagnose AKI in children of all ages. Our cohort included critically ill children only, therefore, we were unable to define age-specific, physiological reference values for PENK By including AKI patients in our analysis, PENK levels could have been elevated and establishing paediatric PENK reference values was therefore not possible. The extent in which this has affected our results is difficult to determine, and to extensively discuss the drawbacks of KDIGO-based AKI diagnosis in (critically ill) neonates and children falls outside the scope of this manuscript. Yet, as underdiagnosis of AKI by these criteria occurs and accurate diagnosis of AKI remains complex [9], the exact effect of AKI on PENK levels remains to be established. In order to implement PENK as a biomarker for the diagnosis of AKI in critically ill neonates and children, several steps need to be taken first. To start with, reference values need to be established in healthy children to define physiological and age-specific values. Sample size was too limited to establish solid reference values for narrow age intervals and to study the relationship between AKI and PENK levels, especially in the children >2 years of age. In order to implement PENK in paediatric clinical care, reference values are needed to determine cut-off values for the diagnosis of AKI. Last, the development of a paediatric PENK-based eGFR equation is necessary to offer PENK-based GFR adapted drug dosing for drugs cleared by the kidneys.

In conclusion, our results demonstrate that there is a clear age-related variation in PENK levels and suggest that PENK levels in children reach adult PENK values around two years of age. Additionally, we showed higher PENK levels with lower GFR and indicate that PENK has a high correlation with mGFR, especially in children older than 28 days of age. Also, because PENK levels were higher in AKI patients compared to non-AKI patients, we believe PENK is a promising marker for paediatric GFR determination and the diagnosis of AKI.

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Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Oliver Hartmann and Janin Schulte are employed at SphingoTec GmbH, the company that holds patent rights related to the PENK assay. All other authors do not have a conflict of interest.

Informed consent: Informed consent needed to be provided by parents or other legal representatives if the child was below 16 years of age. Consent of the child was needed if aged above 12 years of age and medical and cognitive state permitted.

Ethical approval: Details of the Iohexol for Measuring Renal Function (HERO) study were registered on clinicaltrials.gov (registration number NCT03946345) before start of the study. The HERO study protocol was approved by the Medical Ethics Review Board (CMO Arnhem-Nijmegen, NL68547.091.18, 2018–5025).

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