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Proenkephalin as a new biomarker for pediatric acute kidney injury – reference values and performance in children under one year of age

Abstract

Objectives: Acute kidney injury (AKI) is common in critically ill children, but current biomarkers are suboptimal. Proenkephalin A 119–159 (PENK) is a promising new biomarker for AKI in adults, but pediatric data is lacking. We determined PENK reference intervals for healthy children, crucial for clinical implementation, and explored concentrations in critically ill infants aged under 1 year.

Methods: Observational cohort study in healthy infants and critically ill children aged 0–1 years. Reference values were determined using generalized additive models. Plasma PENK concentrations between healthy children and critically ill children with and without AKI, were compared using linear mixed modelling. The performance of PENK as AKI biomarker was compared to cystatin C (CysC) and β-trace protein (BTP) using receiver-operating-characteristic (ROC) analysis.

Results: PENK concentrations in 100 healthy infants were stable during the first year of life (median 517.3 pmol/L). Median PENK concentrations in 91 critically ill children, were significantly higher in those with AKI (n=40) (KDIGO Stage 1 507.9 pmol/L, Stage 2 704.0 pmol/L, Stage 3 930.5 pmol/L) than non-AKI patients (n=51, 432.2 pmol/L) (p < 0.001). PENK appeared to relate better to AKI diagnosis than CysC and BTP (AUROC PENK 0.858, CysC 0.770 and BTP 0.711) in the first 24 h after recruitment.

Conclusions: PENK reference values are much higher in young infants than adults, but clearly discriminate between children with and without AKI, with comparable or better performance than CysC and BTP. Our results illustrate the importance of establishing age-normalized reference values and indicate PENK as a promising pediatric AKI biomarker.

Keywords: acute kidney injury; biomarkers; pediatrics; proenkephalin; reference values.

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Introduction

Acute kidney injury (AKI) is common in hospitalized children, with a prevalence in the pediatric ward and pediatric intensive care unit (PICU) ranging from 5 to 51% [1, 2]. AKI causes an accumulation of possibly toxic solutes, including renally excreted drugs, and is independently associated with morbidity and mortality [3, 4]. Timely AKI detection can drive clinicians to alter the dose or dose interval to prevent drug-induced organ toxicity. Additionally, a conservative fluid management strategy can be applied to prevent fluid overload, which is also associated with mortality [5]. While the search for accurate AKI biomarkers has seen promising candidates, they all have important limitations.

AKI is most often diagnosed using the serum creatinine concentration (SCr) as a surrogate marker for glomerular filtration rate (GFR). However, SCr is a suboptimal biomarker for estimating GFR both in adults and (young) children: its increase following AKI is delayed, it may overestimate GFR due to tubular secretion, its production depends on muscle mass and it reflects maternal GFR in the first days of life due to placental transfer [6–9]. Other biomarkers, such as cystatin C (CysC) and β-trace protein (BTP), can be used to estimate GFR as functional markers, but also show several limitations [10–12]. CysC detects AKI two days earlier than SCr [13], but its concentrations are influenced by inflammation and age [14, 15]. BTP is less influenced by age, but also less accurate than other biomarkers [10]. Urinary biomarkers of tubular damage, like neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), have been proposed for rapid AKI detection, but both are influenced by several pathophysiological factors [13, 14, 16]. Hence, these biomarkers all have their limitations for diagnosing AKI.

Proenkephalin A 119–159 (PENK) is an endogenous, monomeric peptide cleaved from preproenkephalin A, together with enkephalin peptides [17]. Enkephalins bind to opioid receptors and are produced in the central nervous system, kidney, muscles, lung, intestine and heart [18]. Since the density of opioid receptors is highest in the kidneys, enkephalins are implicated in kidney function regulation [19]. PENK possesses several characteristics for an ideal GFR biomarker: it is endogenous, freely filtered by glomeruli due to its small molecular size (4.5 kDa), has no known tubular handling or extra-renal clearance, is not bound to plasma proteins and is stably produced in various disease states, independent of inflammation and other non-renal factors [20].

Research in healthy and diseased adults supports PENK as an early indicator of AKI and independent predictor of impaired kidney function. Moreover, PENK was strongly correlated with estimated and measured GFR after cardiac surgery and in septic patients [20–29].

PENK data in children, however, are currently lacking. As children's renal function s rapidly changing, especially in the first year of life [8], pediatric biomarker research should first focus on identifying age-adjusted reference values [30]. We aimed to determine reference values for PENK in healthy children under 1 year of age. Subsequently, we focused on obtaining diagnostic properties of PENK during AKI in critically ill children, and compare its performance with other serum biomarkers.

Materials and methods

Overview

Prospective cohort study as part of a larger project on AKI biomarkers in critically ill children (Sophia Foundation for Scientific Research, Grant No. 633) [31–34]. The main aim of this larger project is to establish reference values for AKI biomarkers in healthy children under 1 year of age and identify their predictive capabilities for AKI in critically ill children.

Setting

Healthy, full-term born children under 1 year of age were included from the general pediatric or surgical wards in the Erasmus University Medical Center Rotterdam (Erasmus MC) and the Albert Schweitzer Hospital Dordrecht, The Netherlands. Their healthy status was confirmed by the attending physician, based on medical history and physical examination. The following exclusion criteria applied: concurrent viral or bacterial infection, congenital abnormalities of the kidney or urinary tract, prerenal pathologies like dehydration, sepsis or shock; skeletal muscle disease, received nephrotoxic (e. g., gentamicin or cytostatic drugs) or glucocorticoid drugs.

Critically ill children under 1 year of age on mechanical ventilation were recruited from the level III PICU of Erasmus MC. Those with pre-existing kidney disease, congenital kidney or urinary tract defects, rapidly expected death or ECMO treatment were excluded. Details on inclusion and exclusion criteria, sample collection and analysis of both cohorts were published previously [31–34]. The study was approved by the Erasmus MC medical ethics review board and deferred informed consent was obtained from parents and/or caregivers of all study subjects, in concordance with the declaration of Helsinki.

Clinical data

Demographic parameters (gender, diagnosis, postnatal age, ethnicity, weight) were collected for each subject. In addition, the following data were collected for critically ill children: gestational age, birth weight, disease severity scores (Pediatric Risk of Mortality [PRISM-III] and Pediatric Index of Mortality [PIM-II]), mechanical ventilation, vasopressor treatment, length of stay and mortality.

Sample collection and analysis

Blood samples were obtained from an indwelling arterial line or by capillary or venous puncture. In healthy children, at least one sample was obtained before any medical procedure or surgery. In critically ill children, multiple blood samples were obtained in consecutive time frames after intubation (0–6, 6–12, 12–24, 24–36, 36–48 h) and once daily afterward up to 7 days after inclusion.

Serum samples were analysed for SCr (enzymatic assay), CysC (particle-enhanced immunoturbidimetric assay) and BTP (protein assay) in previous studies [31–33]. PENK was measured with a commercially available, double monoclonal sandwich immunoassay (SphingoTec GmbH, Hennigsdorf, Germany) [35].

AKI diagnosis

Healthy children were regarded as not having AKI, verified by age-adjusted SCr z-scores, adapted from literature [8]. SCr z-scores between −2 and +2 were considered normal; children with z-scores outside this range were excluded.

Critically ill children were categorized into "AKI" and "non-AKI" subgroups according to their highest attained KDIGO stage [36] within 48 h of intubation, based on age-adjusted SCr reference values [8]. This approach was used since baseline SCr values were often lacking in patients under 1 year of age. Non-AKI was defined as SCr of <150% relative to the age-adjusted reference value; KDIGO stage 1, 2 and 3 were defined as a 150–200, 200–300 and >300% increase, respectively [37]. An acute rise in creatinine of >26.5 μmol/L within 48 h of a previously measured SCr was also defined as stage 1, and patients on renal replacement therapy were categorized as KDIGO stage 3. The KDIGO criteria of eGFR <35 mL/min/1.73 m^2 was not used since eGFR formulas are not validated in children under 1 year of age. Urine output criteria were not used for diagnosing AKI, as this would be confounded by diuretic treatment frequently used in critically ill children. Patients were excluded when insufficient serum was available for PENK analysis within 48 h of intubation.

Statistical analysis

Continuous data are presented as mean or median values with 95% confidence intervals (CI) or interquartile ranges (IQR), depending on their distribution pattern. Categorical variables are presented as fractions with percentages (%).

Reference values of PENK were determined in healthy children using the Generalized Additive Models for Location, Scale and Shape (GAMLSS) package in R [38]. Akaike Information Criterion (AIC) values were used to determine the optimal model for PENK reference values and to identify age-related patterns. Reference values were converted to z-scores that follow a normal distribution, which were subsequently used for mixed model analyses in the critically ill cohort.

For critically ill children, baseline and clinical characteristics were compared between AKI and non-AKI subgroups using Mann–Whitney U tests for continuous variables and Pearson's chi-square tests for categorical variables.

Mixed model analysis

The number of measured PENK samples during the study period varied among the critically ill subjects. A linear mixed model analysis was used to account for repeated measurements and independent variables. Additionally, because renal function is dynamic during admission, mixed model analysis was performed using the KDIGO stage at each blood sampling moment (as a time-dependent variable).

This analysis served to determine whether PENK differed significantly between critically ill children with different AKI severity. The dependent variable (PENK concentration) was transformed to z-scores to ensure an approximately normal distribution of model residuals. The independent variables in this model were KDIGO stage (as a time-dependent variable), gender, postnatal age, time after intubation (as a continuous variable), vasopressor use, and diagnosis categories based on their potential influence on PENK concentrations. A random intercept was included to account for within-subject correlations. This resulted in estimated marginal means (EMMs), which are the predicted values of the outcome (PENK z-score) adjusted for repeated measurements and effects of independent variables. Using the inverse of the z-score formula these EMMs are back-transformed to an estimated prediction of median PENK values and its 95% CI. To compare PENK concentrations between critically ill and healthy children, predicted z-scores (EMMs of the mixed model) were compared to the population mean for healthy children (PENK z-score 0) using Wald's t-test.

Exploratory analysis – association of PENK and other biomarkers with AKI diagnosis

Receiver-operating-characteristic (ROC) curves were used to evaluate the association of biomarker concentrations with having a KDIGO stage >0 at the blood sampling moment in two different time frames (0–24 and 24–48 h after intubation). The area under the ROC curve (AUROC) and its 95% CI were determined for PENK, CysC and BTP. Since AKI diagnosis was based on SCr, this biomarker was not included. To correct for repeated sampling and missing data, only the first sample per patient within each time frame in which all three biomarkers were measured was included. As this was an exploratory analysis, the statistical significance of differences in AUROC was not tested.

Statistical analyses were performed using SPSS statistics version 25.0, GraphPad Prism version 5.03, and R version 3.5.1. A two-sided p-value of 0.05 was considered the limit of statistical significance.

Results

Healthy children

16 of the 116 healthy children enrolled in the original trial [32] were excluded because insufficient serum was available for PENK measurement or SCr z-scores were outside the predefined range. Characteristics of the 100 remaining children are presented in Table 1.

For these 100 children, there was no clear association between PENK and age during the first year. The GAMLSS models including age performed only marginally better than those without, indicated by low AIC differences (0.952). Therefore, we used a GAMLSS model that assumes PENK concentrations were normally distributed after a Box–Cox transformation during the first year. PENK z-scores were calculated with a formula derived from the GAMLSS model:

$$PENK\ z-score = \frac{\left(\frac{PENK}{517.34}\right)^{-0.01748}-1}{-0.01748*e^{-1.074}}$$

In 145 samples, the median PENK concentration was 517.3 pmol/L (95% CI 488.9–547.4 pmol/L, 2.5th and 97.5th percentile at 265.2 and 1017.1 pmol/L, respectively, Figure 1).

Table 1: Patient characteristics for healthy and critically ill cohorts, including subgroup analysis between AKI and non-AKI subgroups.

Characteristic	Healthy children (n=100)	Critically ill All patients (n=91)	Critically ill Non-AKI[b] (n=51)	Critically ill AKI[b] (n=40)	p-Value Non-AKI vs. AKI
Baseline characteristics					
Gender: male, n	66/100 (66%)	59/91 (65%)	33/51 (65%)	26/40 (65%)	0.977
Gestational age: weeks; median (IQR)	[a]	39.0 (37.6–40.0) Missing: 2	39.1 (38.0–40.0)	38.9 (37.4–40.0) Missing: 2	0.454
Birth weight, kg; median (IQR)	[a]	3.1 (2.8–3.6) Missing: 6	3.2 (2.9–3.6) Missing: 2	3.1 (2.8–3.6) Missing: 4	0.666
Ethnicity: Caucasian, n	81/100 (81%)	64/91 (70%)	33/51 (65%)	31/40 (78%)	0.185
Clinical characteristics at admission					
Postnatal age, days; median (IQR)	89.1 (57.4–165.9)	25.8 (0.9–76.6)	31.1 (0.9–61.6)	8.0 (0.7–99.0)	0.631
Weight, kg; median (IQR)	5.0 (4.0–6.8) Missing: 3	3.8 (3.1–5.3)	3.7 (3.4–5.2)	3.8 (3.0–5.8)	0.795
Diagnosis healthy cohort, n					
Inguinal hernia repair	47/98 (48%)				–
Orthopaedic surgery	13/98 (13%)				
Bronchoscopy	9/98 (9%)				
Hyperbilirubinemia	7/98 (7%)				
Sleep apnea test	6/98 (6%)				
Other	16/98 (16%) Missing: 2				
Diagnosis critically ill cohort, n					
Respiratory failure		32/91(35%)	19/51 (37%)	13/40 (32%)	0.441
CDH		23/91 (25%)	12/51 (24%)	11/40 (27%)	
Cardiac failure		14/91 (15%)	5/51 (10%)	9/40 (23%)	
Sepsis		14/91 (15%)	9/51 (18%)	5/40 (13%)	
Other critical illness		8/91 (9%)	6/51 (12%)	2/40 (5%)	
Severity of illness					
PIM-II predicted mortality, %; median (IQR)	[a]	10.0 (3.7–19.5) Missing: 6	7.8 (2.3–16.9) Missing: 2	12.6 (4.1–30.0) Missing: 4	0.054
PRISM-III score, points; median (IQR)	[a]	34.5 (15.7–64.6) Missing: 6	33.8 (11.0–54.6) Missing: 2	41.8 (19.5–75.0) Missing: 4	0.107
Outcomes					
Duration of mechanical ventilation, days; median (IQR)	[a]	5.8 (3.1–11.0)	5.3 (3.1–9.1)	6.8 (3.1–18.2)	0.095
Length of ICU stay, days; median (IQR)	[a]	9.7 (6.2–22.2)	8.4 (5.9–15.6)	16.2 (7.0–31.9)	0.077
Hospital mortality, n	[a]	17/91 (19%)	8/51(16%)	9/40 (23%)	0.408
ICU mortality, n	[a]	14/91 (15%)	6/51 (12%)	8/40 (20%)	0.534
Initial biomarker values					
Initial PENK concentration, pmol/L; median (IQR)[c]	584.0 (444.4–700.5)	502.4 (367.7–754.1)	416.5 (316.4–557.6)	669.4 (434.3–982.3)	**<0.001**
Initial SCr z-score for age, median (IQR)[c]	−0.08 (−0.71 to 0.64)	0.31 (−0.58 to 1.70)	−0.42 (−1.0 to 0.21)	1.83 (0.92 to 3.1)	**<0.001**

AKI, acute kidney injury; CDH, congenital diaphragmatic hernia; ICU, intensive care unit; IQR, interquartile range; PENK, proenkephalin A 119–159; PIM, pediatric index of mortality score; PRISM, pediatric risk of mortality score; SCr, serum creatinine concentration. p-Values represent the differences between non-AKI and AKI subgroups in the critically ill cohort. [a]Not collected in healthy cohort. [b]Critically ill children were stratified in AKI and non-AKI subgroups according to the highest attained KDIGO stage within 48 h after intubation. [c]Initial values represent the first sample after intubation that was available for analysis.
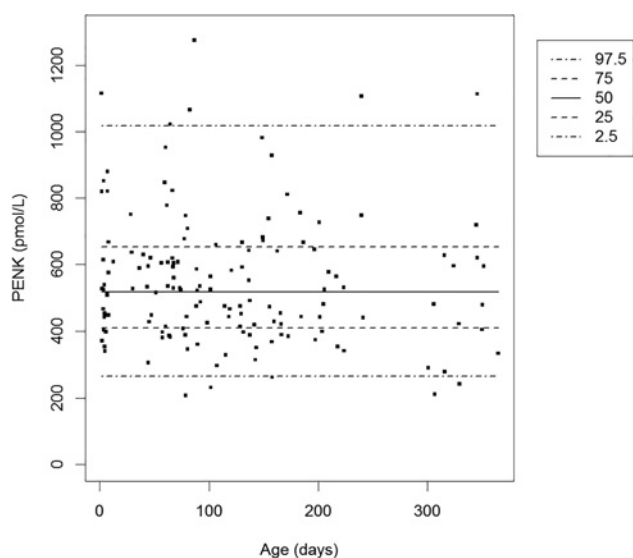
Figure 1: Reference percentiles for proenkephalin A 119–159 (PENK) as a linear function of postnatal age in healthy children. Squares represent all measured PENK concentrations (145 samples) of all healthy patients (n=100). Reference percentiles for PENK are indicated at the 2.5th (dash-dotted, bottom), 25th (dashed, bottom), 50th (solid), 75th (dashed, top) and 97.5th (dash-dotted, top) percentiles.

Critically ill children – patient characteristics

Ten of the 101 critically ill children included in previous studies were excluded: six had insufficient serum for PENK analysis, four had no samples within 48 h after intubation, leaving 91 (Table 1).

Within 48 h after intubation, 40 patients (44%) were categorized in the AKI subgroup; 20 with KDIGO stage 1, 11 with stage 2, and 9 with stage 3 as their highest KDIGO stage score. There were no significant differences in gender, age, ethnicity and weight between non-AKI and AKI subgroups. Disease severity, length of stay and mortality were higher in the AKI subgroup, but differences were not statistically significant (Table 1).

Critically ill children – PENK concentrations

PENK concentrations were determined in 578 serum samples of critically ill children. Median (IQR) initial PENK concentration in the non-AKI subgroup was 416.5 (316.4–557.6) pmol/L vs. 669.4 (434.3–982.3) pmol/L in the AKI subgroup (p < 0.001). For the time frames up to 48 h after intubation, PENK concentrations were significantly higher in the AKI subgroup (p < 0.01 for every time frame except 36–48 h after intubation (p=0.123)) (Figure 2).
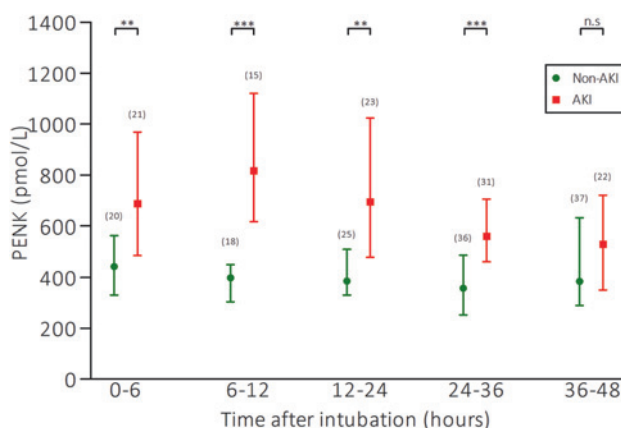

Figure 2: PENK concentrations in critically ill children with AKI compared to non-AKI in the first 48 h after intubation.
Serum PENK levels presented as median and interquartile range, for five time frames following intubation. Outcomes are stratified for 'Non-AKI' (green, circles) or 'AKI' (red, squares) within 48 h after intubation, one sample per patient per time frame. Numbers in brackets above each time frame represent the number of patients in each category. **p < 0.01, ***p < 0.001. n.s: not statistically significant (p > 0.05).

Critically ill children – mixed model analysis

Of the included covariates in the mixed model, only age was significantly correlated with PENK concentrations. An overview of estimates of fixed effects for this model are presented in (Supplemental Table 1).

Median PENK concentrations in critically ill children with KDIGO Stage 1 (507.9 pmol/L [95% CI 454.3–567.9]) were significantly higher than those in the non-AKI group (432.2 pmol/L [95% CI 398.2–469.2], p < 0.001) (Figure 3). Those with KDIGO stage 2 or 3 had significantly higher median PENK concentrations than both healthy children and children in the non-AKI group (stage 2 704.0 pmol/L [595.8–832.3] and Stage 3 930.5 pmol/L [763.2–1135.2], p < 0.01 between all subgroups) (Figure 3). PENK concentrations in the non-AKI group were significantly lower than those in healthy children (517.3 pmol/L [95% CI 488.9–547.4], p < 0.001,). This pattern was also observed for other serum biomarkers like CysC, BTP and Scr (Supplemental Table 2).

Critically ill children – correlation with other biomarkers (exploratory analysis)

The AUROC for AKI by PENK was the highest in the first 24 h after intubation (0.858), with CysC having the second highest association with AKI (0.770) (Figure 4). In the 24–
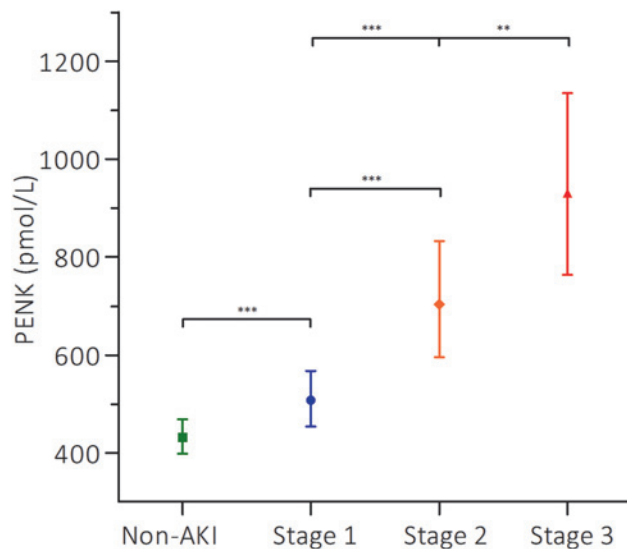
Figure 3: PENK concentrations in critically ill children with different levels of AKI.
Overview of median PENK concentrations with 95% CI in 91 critically ill children with different degrees of AKI at the moment of blood sampling based on KDIGO stages (561 total samples: 422 non-AKI (green, square), 86 Stage 1 (blue, circle), 28 Stage 2 (orange, diamond), 25 Stage 3 (red, upward facing triangle). 17 PENK samples unused due to missing corresponding KDIGO stages. Statistical significance based on results of estimated marginal means (EMMs) from the linear mixed model analysis, corrected for repeated sampling and covariates (gender, postnatal age, time after intubation, vasopressor use and diagnosis categories). **p < 0.01, ***p < 0.001.

48 h timeframe, the AUROC for PENK (0.667) was slightly lower than that for CysC (0.685), but higher than that for BTP (0.622) (Table 2).

Discussion

We present the first data for PENK as an AKI biomarker in children. We established PENK reference values in healthy children under 1 year of age, and showed that levels are much higher than in adults. Additionally, we found that PENK levels, even while its reference values are much higher than in adults, clearly discriminates between critically ill children with and without AKI and that its performance might be comparable to or even better than other AKI biomarkers.

The availability of age-related reference values is crucial for the clinical implementation of biomarkers in children, as ignoring age-dependent changes in normal values may lead to inaccurate performance in children [30]. Our results underline this importance, as we found more than tenfold higher reference values for PENK in children up to 1 year of age (median 517.3 pmol/L) than those established in healthy adults (median 45 pmol/L, 99th percentile at 80 pmol/L) [22]. Furthermore, these reference values were stable during the first year of life, in contrast to SCr [8] and CysC [15]. Nevertheless, in line with reference values for BTP [31], PENK concentrations will decrease later in childhood [39].

These higher concentrations could be explained by a lower absolute clearance due to maturation of kidney function in young children [40]. However, since the GFR of young children is not ten-fold lower than the GFR of healthy adults, increased production of enkephalins in children might also contribute to higher PENK concentrations. Concentrations of Met-enkephalin, another enkephalin derived from the same precursor as PENK, are also over tenfold higher in children than in adults [41].

Remarkably, these higher PENK concentrations were still highly associated with AKI in critically ill children. This is in concordance with a study in adults, where PENK highly correlated with measured GFR in 24 septic ICU patients [26]. Moreover, in another cohort of 101 sepsis patients, PENK was independently associated with RIFLE stages [22]. In our study, PENK concentrations were also significantly elevated with more severe AKI, even when correcting for repeated sampling and covariates. Regarding the comparison with other biomarkers, in our exploratory analysis PENK showed
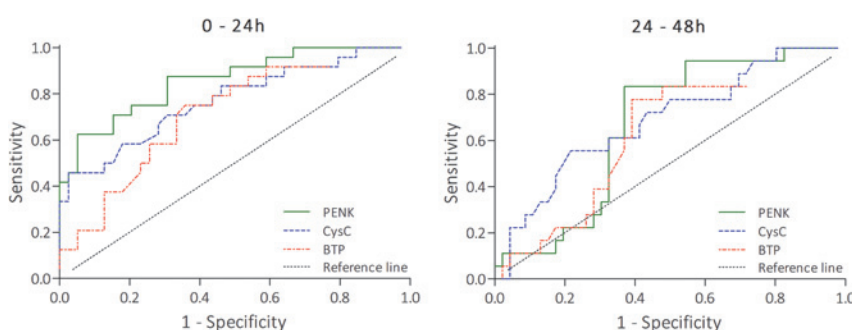

Figure 4: Performance of PENK, cystatin C and BTP for AKI diagnosis in critically ill children. Receiver-operating-characteristic (ROC) curves for PENK (green, solid line), CysC (blue, dashed line) and BTP (red, dashed-dotted line) for their association with a KDIGO stage >0 at the moment of blood sampling in two timeframes (0–24 h [left panel] and 24–48 h [right panel] after intubation).
A list of AUROC values, confidence intervals and number of samples in both time frame for each biomarker are shown in Table 2.

Table 2: Area under the receiver operating characteristic curves (AUROC) with 95 % confidence interval (CI) and number of samples for PENK, cystatin C (CysC) and β-trace protein (BTP) in two time frames.

Timeframes	Biomarker	AUROC (95 % CI)	Non-AKI samples, n	AKI samples, n
0–24 h after intubation	PENK	0.858 (0.764–0.952)	39	24
	CysC	0.770 (0.647–0.893)	39	24
	BTP	0.711 (0.580–0.842)	39	24
24–48 h after intubation	CysC	0.685 (0.542–0.829)	46	18
	PENK	0.667 (0.532–0.801)	46	18
	BTP	0.622 (0.473–0.771)	46	18

Acute kidney injury (AKI) was defined at the time of sampling based on age-adjusted creatinine reference values. Biomarkers are ranked top to bottom from highest to lowest AUROC in each time frame.

a comparable or even better association with AKI diagnosis than CysC and BTP, which are regarded to be among the best biomarkers for the diagnosis of AKI [42, 43]. Further investigation of the diagnostic potential of PENK in pediatric AKI is warranted, therefore.

Interestingly, the PENK concentrations in critically ill children without AKI were lower than those in healthy children. This can be explained by augmented renal clearance, caused by increased cardiac output and renal perfusion during critical illness, which is well documented in critically ill adults [44] and children [45]. Furthermore, extensive fluid challenges might dilute PENK concentrations in serum of critically ill patients. That this pattern was also seen for all other serum biomarkers in our study (SCr, CysC and BTP) supports this supposition.

In this study we used the outcome measure "AKI in the first 48 h after intubation" as opposed to 48 h after admission, as multiple patients were not immediately recruited and PENK concentrations in these patients were not measured until several days after admission. This resulted in a slightly higher proportion of AKI patients, mostly with a mild and temporary rise of SCr, than previously reported [33]. These mild cases could have resulted in less-pronounced differences in clinical endpoints between the AKI and non-AKI subgroups in the present study than we and others previously have shown [3, 4, 33].

Our study has some limitations. The inclusion of children up to 1 year of age limits the extrapolation to other age groups. We could not validate PENK as a biomarker for GFR since GFR formulas using SCr and/or CysC have not been validated in children under 1 year [46, 47]. Also, we identified AKI patients by SCr-based reference values for age, which is the main biomarker in clinical practice, but is less accurate than measurements of GFR using inulin or iohexol. Additionally, we lacked detailed information on urine output due to confounding by diuretic therapy, which would have allowed for a more robust AKI diagnosis

by using the KDIGO classification including urine output criteria [36]. This might have caused a relative underrepresentation of AKI patients [48]. Lastly, the majority of patients showed signs of AKI before intubation, thereby limiting the predictive capabilities of PENK in our cohort [33].

Clinical implementation of new biomarkers in pediatric AKI is challenging [49], so future research regarding PENK should focus on the following gaps in current knowledge. Firstly, age-corrected reference values for the whole pediatric age range are crucial for appropriate cut-off values in children. Secondly, the sensitivity and specificity of PENK for AKI should be compared to measured GFR as the golden standard. Additionally, the predictive capabilities for AKI should be compared between PENK, creatinine and other biomarkers by early measurements before AKI onset. These studies are essential to determine the place of PENK in the AKI biomarker landscape, whether its application in clinical practice is mostly diagnostic, prognostic, or pharmacodynamic in nature [50]. Ultimately, well validated AKI biomarkers can facilitate future biomarker-driven clinical trials for AKI treatment, that relate biomarker-based clinical decisions or pharmaceutical targets to clinical outcomes [50].

Conclusions

This research represents the first data for PENK as a biomarker for AKI in children. PENK concentrations appear stable during the first year of life, but show great inter-individual variability with considerably higher concentrations than in adults. Regardless, PENK concentrations were strongly associated with AKI diagnosis, comparable to or even better than serum biomarkers that are regarded as the best currently available. Ongoing research must validate whether PENK can improve the diagnosis and treatment of children with AKI.

Author contributions: AZ, DT and SdW designed and carried out the original trials, collected study data and samples. SH, AZ, PP, RB, MS and SdW designed the current study. NvdW carried out data cleaning and first data-analysis. Janin S, Joachim S and OH carried out the PENK biomarker analysis. JvR advised on and contributed to statistical analysis. SH carried out the final data analysis and made the final figures. SH drafted and revised the paper, with feedback and intellectual input from all co-authors. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Joachim Struck, Janin Schulte and Oliver Hartmann are employed by SphingoTec GmbH. SphingoTec GmbH holds patent rights related to the PENK assay. Prof. Pickkers reports personal fees from SphingoTec, travel and consultancy reimbursement, during the conduct of the study. Prof. De Wildt reports grants from EU: Horizon, COST, IMI2 grants, outside the submitted work.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study was approved by the ErasmusMC medical ethics review board and deferred informed consent was obtained from parents and/or caregivers of all study subjects, in concordance with the declaration of Helsinki.

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