A bedside equation to estimate 24-h proteinuria in children with nephrotic syndrome: Derivation and temporal validation

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Abstract. To date, 24-h urine protein (24hUP) excretion remains the gold standard for quantifying proteinuria and is a strong predictor of renal outcomes in glomerular diseases. Although current guidelines allow nephrotic-range proteinuria to be diagnosed using either single-void urine protein-creatinine ratio (UPCR) or 24hUP thresholds, these cut-offs primarily confirm disease presence and provide limited quantitative guidance for clinical decision-making during the acute phase of pediatric primary nephrotic syndrome (PNS). The present study thus aimed to provide a method with which to estimate 24hUP in children with PNS. A total of 251 children with PNS were included and divided into a derivation cohort (n=171) and an independent validation cohort (n=80). Log-log linear regression models were constructed using UPCR adjusted by estimated 24-h creatinine excretion derived from the Cockcroft-Gault, Hellerstein and Ghazali-Barratt equations, with age and sex incorporated as covariates. Model performance was assessed using coefficients of determination (R^2), root mean square error (RMSE), mean absolute error (MAE) and calibration analyses. The optimal model, based on Cockcroft-Gault-adjusted UPCR, demonstrated good predictive performance in external validation (RMSE, 11.39 mg/m^2/h; MAE, 9.26 mg/m^2/h; R^2, 0.91), with 93.8 and 96.3% of estimates within 20 and 30% of observed 24hUP values, respectively. The application of the model indicated that a substantial proportion of children could safely avoid immediate 24-h urine collection without missing cases of marked proteinuria. On the whole, a bedside equation using adjusted UPCR may serve as a practical surrogate for estimating 24hUP in children with PNS and facilitate clinical decision-making in routine practice.

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Introduction

Primary nephrotic syndrome (PNS) is among the most common chronic kidney diseases affecting children, and the magnitude and pattern of proteinuria remain central to diagnosis, risk evaluation, treatment selection and monitoring of response (1,2). As is known, 24-h urine protein (24hUP) excretion has long been the gold standard for quantifying protein loss and is a strong predictor of renal outcomes across glomerular diseases, underscoring the clinical need for accurate 24hUP estimates in routine care, beyond diagnosis alone (1,2). Current guidelines permit the diagnosis of nephrotic-range proteinuria using either a urine protein-creatinine ratio (UPCR) ≥200 mg/mmol or a 24hUP ≥40 mg/m^2/h (2,3). However, these diagnostic cut-offs primarily establish disease presence and do not provide the granularity required for decisions, such as initiating thromboprophylaxis, triaging for inpatient monitoring, or tailoring follow-up intensity during the acute phase (3,4).

In pediatrics, 24-h urine collections are frequently impractical due to poor cooperation, incomplete or contaminated samples, and the logistical burden on families and health systems, which compromises reliability and feasibility in routine practice (5). A single-voided UPCR is therefore widely recommended as an accessible surrogate, physiologically normalizing protein to creatinine and approximating 24hUP (2,3). Although pediatric studies have demonstrated a positive association between UPCR and 24hUP, residual heterogeneity persists due to age- and sex-related differences in creatinine generation, edema and hydration status, sampling time, assay methodology and laboratory quality control (6,7). Moreover, the association between UPCR and 24hUP is often multiplicative and heteroscedastic, predisposing to proportional bias at the lower and upper extremes on the raw scale and limiting confidence in directly inferring very high proteinuria from a single UPCR value, precisely when clinicians seek to avoid missing high-risk presentations (8,9). Population-specific kidney equations have recently been derived in Vietnamese patients, underscoring that nephrology prediction tools often

require local calibration rather than direct adoption of Western formulas (10).

Key evidence gaps include the lack of externally validated UPCR-to-24hUP equations in pediatric PNS, the limited assessment of clinical interchangeability beyond simple correlation and the sparse representation of Asian cohorts. The present study aimed to: i) Derive a pediatric UPCR-to-24hUP equation in PNS; ii) externally validate its predictive performance, including calibration and agreement; and iii) translate predictions into clinically actionable gray-zone thresholds, using prediction-interval (PI)-guided classification and decision-curve analysis (DCA) to compare net clinical benefit with threshold-based care.

Patients and methods

Study design and setting. The present study conducted a single-center observational, method-comparison study with prospectively defined derivation and validation cohorts. Consecutive pediatric patients with PNS treated at the Department of Nephrology, Can Tho Children's Hospital (Can Tho, Vietnam) between January, 2023 and October, 2025 were included. The cohort split was time-based and non-overlapping: Derivation (January, 2023 to January, 2025) and validation (February, 2025 to October, 2025). No individual contributed data to both cohorts. For the derivation cohort, encounters from January 1, 2023 to December 17, 2024 constituted a retrospective segment. No additional specimens were collected for research; all laboratory results and clinical information were routinely generated as part of standard care and were already available within the same clinical site (Can Tho Children's Hospital) in the medical records and the laboratory information system. The study was approved by the Institutional Review Board (IRB) of Can Tho University of Medicine and Pharmacy (IRB No. 4618/QĐ-ĐHYDCT/24. KY.60), approval date December 17, 2024 and received permission to conduct the study at Can Tho Children's Hospital. The retrospective component was granted a waiver of informed consent on the basis of minimal risk, impracticability of contacting all prior patients, and adequate privacy safeguards (coded identifiers, restricted access). The prospective component obtained written informed consent from a parent/legal guardian and age-appropriate child assent when applicable. Following IRB approval on December 17, 2024, study-specific data abstraction/extraction for the retrospective segment began on December 18, 2024, and data verification/cleaning continued until March 30, 2025. Data from December 18, 2024 to January 31, 2025 represented the prospective segment of the derivation cohort and were collected according to the IRB-approved protocol.

Patient selection. Children aged 1-16 years with PNS, defined per contemporary pediatric guidance (2,3), were eligible if, within 48 h of admission, a complete 24-h urine collection was available to quantify 24hUP and 24-hour urine creatinine (24hUCr), and a spot urine sample from the same episode was obtained for UPCR. Patients were excluded from the study for the following reasons: i) Secondary nephrotic syndrome; ii) decreased renal function with estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2; iii) known primary muscle disease or medications expected to materially alter creatinine generation; iv) inadequate 24-h collection, predefined as 24hUCr per body weight below the site-specific threshold (11); and (v) missing core variables after attempted retrieval.

The derivation cohort was sized using Fisher's z to target a 95% CI half-width of 0.10 around an expected log-scale correlation r_0=0.80, yielding n=60 (conservatively set to 85). For external validation, root mean square error (RMSE) precision was based on the Chi-square distribution of residual variance; the 95% relative half-width is approximately $z_{0.975}\sqrt{1/[2(n-k)]}$. Finally, 171 participants were included in the derivation cohort to develop the model and perform internal validation, whereas 80 children comprised the validation cohort for external validation to assess clinical applicability (Fig. 1).

Specimen collection and laboratory measurements. Caregivers received standardized, illustrated instructions and in-person training. After discarding the first morning void, all urine passed over the subsequent 24 h was collected, including the terminal void at 24 h. Immediately upon completing the 24-h collection, an early-morning spot urine sample was obtained from the same participant. All specimens were delivered to the laboratory within 2 h of completion. At sampling, we recorded age, sex, height/weight, serum albumin, serum creatinine and current corticosteroid or antiproteinuric therapy. Body weight was recorded as dry weight, defined as the baseline (pre-edema) weight of the child when free of clinically apparent edema or ascites. In the event that dry weight was unavailable, a height-based expected weight derived from World Health Organization (WHO) weight-for-height standards was used as a surrogate.

Total urine protein was measured by the pyrogallol red-molybdate method on a cobas c501 analyzer (Roche Diagnostics) using the Roche urine/CSF total protein calibrator (albumin-based), traceable to the manufacturer's reference materials. Urine creatinine was measured by the enzymatic (creatinase-sarcosine oxidase) method on the same analyzer and standardized to the IDMS reference measurement procedure. Both spot UPCR and the 24-h reference specimens were analyzed on the same platform to ensure methodological consistency.

Calculations. 24hUCr was estimated using three published equations: Hellerstein (12), Ghazali-Barratt (13), and Cockcroft-Gault (14). Age is represented in years and weight in kg; units are mg/24 h.

i) Hellerstein: For girls and boys <14 years: $UCr_{Hellerstein=}$ [0.17x(age)+17.1]xweight; for boys ≥14 years: $UCr_{Hellerstein=}$ [0.39x(age)+14.9]xweight.

ii) Ghazali-Barratt: $UCr_{Ghazali-Barratt}$=[0.46x(age)+15.4]x weight.

iii) Cockcroft-Gault: For boys: $UCr_{Cockcroft-Gault=}$[28-(0.2x age)]xweight; for girls: $UCr_{Cockcroft-Gault=}$[28-(0.2xage)] xweightx0.85.

Statistical analysis

Data handling. Continuous variables are summarized as the mean and standard deviation (SD) or median and interquartile range (IQR); categorical variables are presented as frequencies (percentages). Variables exhibiting right skew (including

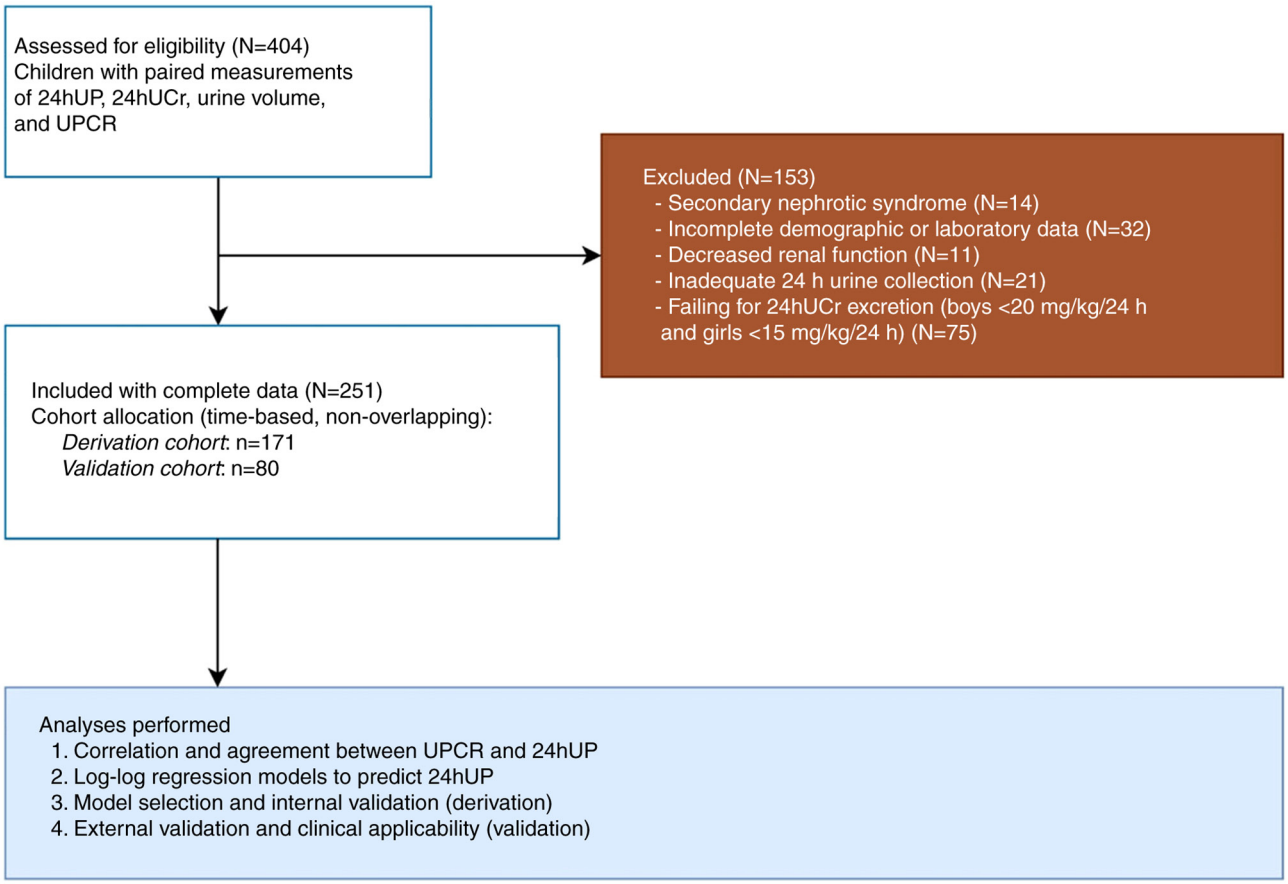

Figure 1. Study flow chart. 24hUP, 24-hour urine protein; 24hUCr, 24-hour urine creatinine; UPCR, urine protein-creatinine ratio.

UPCR and 24hUP) were analyzed on the $\log_{10}$-scale, with back-transformation to the original scale where appropriate. All statistical tests were two-sided with α=0.05; a P-value <0.05 was considered to indicate a statistically significant difference.

Correlation and agreement. Spearman's ϱ was performed for correlations between 24hUP and: i) Measured UPCR (mUPCR); ii) estimated UPCR (eUPCR) defined as mUPCR **x** 24hUCr, using measured (m24hUCr; $eUPCR_0$) or estimated (e24hUCr) from Hellerstein ($eUPCR_1$), Ghazali-Barratt ($eUPCR_2$) and Cockcroft-Gault ($eUPCR_3$) equations. A Bland-Altman analysis was performed on the $\log_{10}$-scale, reporting the geometric mean ratio (bias) and 95% limits of agreement (LoA); proportional bias was tested by regressing log-difference on log-mean. P-values for correlations were obtained from the two-sided Spearman's rank correlation test.

Model development. Log-log linear regressions were fit to predict $\log_{10}$(24hUP): $M_1=\beta_0+\beta_1 x\log10(mUPCR)+\varepsilon$; $M_2=\beta_0+\beta_1 x\log_{10}(eUPCR_0)+\varepsilon$; M_3-$M_5=\beta_0+\beta_1 x\log_{10}(eUPCR_{1\text{-}3})+\varepsilon$, using three published e24hUCr formulas: Hellerstein (12), Ghazali-Barratt (13) and Cockcroft-Gault (14). Model selection used 10-fold cross-validation (CV), prioritizing CV-RMSE/MAE (adj. R^2, AIC, BIC secondary). Standard diagnostics were applied (linearity, residuals, influence, multicollinearity); residual normality, heteroskedasticity and model specification were assessed using standard tests (e.g., Shapiro-Wilk/skewness-kurtosis; Breusch-Pagan/White/IM; Ramsey RESET; and linktest), with two-sided P-values reported for the corresponding diagnostics. HC3 robust standard errors were reported if heteroskedasticity was detected. P-values for regression coefficients were obtained from two-sided Wald t-tests using the corresponding standard errors (HC3 when applied).

Internal validation. Bootstrap resampling (B=2000) was performed to obtain optimism-corrected RMSE/MAE/R^2 and calibration (slope/intercept). Predictions were returned to the original scale using Duan's smearing estimator (smearing factor reported).

External validation. Coefficients from the best model were applied unchanged to the validation cohort. We reported RMSE, MAE, R^2 (squared Pearson correlation between observed and predicted 24hUP), and P20/P30 (percentage of predictions within 20/30% of observed 24hUP). Calibration was evaluated by intercept, slope and a calibration plot. For clinical interpretation, we generated a patient-level PI for each predicted 24hUP and applied prespecified cut-points relevant to acute pediatric PNS (100 mg/m^2/h as a lower-risk threshold and 150 mg/m^2/h as a higher-risk threshold). A case was labeled 'ruled out' if the upper PI bound was below the lower-risk threshold, 'ruled in' if the lower PI bound exceeded the higher-risk threshold, and 'gray zone' otherwise. Clinical utility was then evaluated using DCA. We compared the net benefit of the model-based classification with treat-all and treat-none strategies, as well as with fixed guideline-style cut-offs for nephrotic-range proteinuria,

Table I. Baseline characteristics of the derivation and validation cohorts.

Characteristic	Derivation cohort(n=171)	Validation cohort (n=80)
Age (mean ± SD), years	9.33±3.70	10.02±4.37
Male, n (%)	122 (71.35)	49 (61.25)
Body weight (mean ± SD), kg	32.17±11.19	33.57±15.34
Height (mean ± SD), cm	128.08±20.12	132.55±25.53
BSA (mean ± SD), m^2	1.06±0.26	1.10±0.35
BUN (median, IQR), mg/dl	12.88 (10.08, 16.24)	13.33 (10.86, 17.78)
SCr (median, IQR), mg/dl	0.57 (0.48, 0.73)	0.65 (0.52, 0.83)
eGFR (median, IQR), ml/min/1.73 m^2	93.97 (72.71, 111.62)	83.41 (70.02, 100.13)
Albumin (mean ± SD), g/l	18.89±4.59	20.32±3.45
Spot UPCR (median, IQR), mg/mmol	410.84 (287.09, 613.28)	396.74 (281.61, 471.62)
24hUP (median, IQR), mg/m^2/h	116.81 (87.81, 160.47)	107.06 (81.1, 133.99)
24hUCr (median, IQR), mg/24 h	934.03 (679.36, 1159.39)	875.49 (563.55, 1119.58)

Data are presented as the mean ± SD or median (IQR), as appropriate. BSA, body surface area; BUN, blood urea nitrogen; SCr, serum creatinine; eGFR, estimated glomerular filtration rate; UPCR, urine protein-creatinine ratio; 24hUP, twenty-four-hour urine protein; 24hUCr, 24-h urine creatinine.

across threshold probability ranges chosen a priori to reflect plausible willingness to intervene in suspected very high proteinuria.

Statistical software. Analyses were performed in R (version 4.5.1, R Foundation for Statistical Computing, Vienna, Austria).

Results

Study population characteristics. A total of 251 children were included in the present study: A total of 171 children in the derivation cohort for model development and 80 children in the validation cohort for external assessment of performance and applicability. The demographic and clinical baseline characteristics of the children are summarized in Table I. Overall, the two cohorts were generally comparable at baseline.

Correlation and agreement between 24hUP and UPCR variants in the derivation cohort. Spearman's correlation analyses demonstrated a strong correlation between mUPCR and 24hUP (ϱ=0.81, P<0.001). Adjusting by measured m24hUCr ($eUPCR_0$) markedly improved the correlation (ϱ=0.92). Using e24hUCr, the correlation increased with the Hellerstein equation ($eUPCR_1$, ϱ=0.83), was essentially unchanged with Ghazali-Barratt ($eUPCR_2$, ϱ=0.80), and improved modestly with Cockcroft-Gault ($eUPCR_3$, ϱ=0.88) (Fig. S1).

Bland-Altman plots on the $\log_{10}$-scale confirmed that 95% of observations lay within the LoA for all UPCR-derived indices (Fig. S2). Unadjusted spot UPCR substantially overestimated 24hUP [geometric mean ratio (GMR), 3.63; Fig. S2A]. Adjusting UPCR by m24hUCr ($eUPCR_0$) markedly reduced bias (GMR, 1.15; Fig. S2B), and the Cockcroft-Gault-based adjusted UPCR ($eUPCR_3$) exhibited the optimal agreement overall (GMR, 0.98 with tight ratio-scale LoA; Fig. S2E).

Development of a 24hUP prediction model. The present study developed five $\log_{10}$-linear regression models to predict 24hUP from UPCR (denoted M_1-M_5). M_2 yielded the optimal performance (CV-RMSE, 21.84; CV-MAE, 16.67; Adj. R^2 0.84; AIC, -367.64; BIC, -358.21; Table SI). However, M_2 requires measured 24hUCr urine, which is impractical in children due to continuous collection, volume errors and poor adherence. Given clinical deployability as a key criterion, M_2 was not selected. M_5 ($eUPCR_3$ via Cockcroft-Gault) provided the optimal accuracy-feasibility balance. Adding age and sex to M_5 to form M_6 further improved fit vs. M_5 alone: CV-RMSE 28.14 vs. 29.15 (Δ=-1.01; 95% CI, -1.42 to -0.78; P<0.0001) and CV-MAE 20.25 vs. 21.22 (Δ=-0.97; 95% CI: -1.19 to -0.68; P<0.0001); adjusted R^2 increased from 0.78 to 0.80, and AIC/BIC decreased.

Optimal prediction equation (M_6). The association between 24hUP and UPCR is presented as: Log_{10}[24hUP (mg/m^2/h)]=0.291+0.880 x Log_{10}(adjusted UPCR) - 0.006 x age (years) + 0.057 x sex (0=boy, 1=girl). Adjusted UPCR=mUPCR x $e24hUCr_{\text{Cockcroft-Gault}}$; when applying the model, units are harmonized so the product is expressed in mg/mm^2/h (Table II). Residuals were approximately normal, with only borderline heteroskedasticity (addressed using robust standard errors). Model diagnostics (RESET, link test, low VIFs), sensitivity analyses excluding high-influence observations or using robust regression (Table SII and Fig. S3), and component-plus-residual plots (Fig. S4) all supported a stable, approximately linear specification for M_6 without meaningful multicollinearity or functional-form misspecification.

Bootstrap optimism-corrected performance: Internal validation with 2,000 bootstrap resamples provided optimism-corrected estimates of performance and calibration. Following optimism correction, prediction error on

Table II. Multivariable linear regression analysis for Log_{10}-transformed 24hUP excretion (final model, M_6).

Predictor	β	SE (HC3)	95% CI		P-value
			Lower	Upper	
Log_{10}(adjusted UPCR)	0.880	0.036	0.810	0.951	<0.001
Age	-0.006	0.001	-0.009	-0.002	<0.001
Sex	0.057	0.017	0.024	0.090	<0.001
Intercept	0.291	0.076	0.142	0.440	<0.005

SEs are heteroskedasticity-consistent (HC3) due to borderline heteroskedasticity. Model size and fit: n=171; Adjusted R^2=0.80. Prediction equation: Log10(24hUP) (mg/m^2/h)=0.291 + 0.880 x Log10(adjusted UPCR) (mg/m^2/h) - 0.006 x age (years) + 0.057 x sex (0=boy, 1=girl); adjusted UPCR=mUPCR x e24hUCrCockroft-Gault. UPCR, urinary protein-creatinine ratio; 24hUP, 24-h urine protein; 24hUCr, 24-h urine creatinine; m, measured; e, estimated.

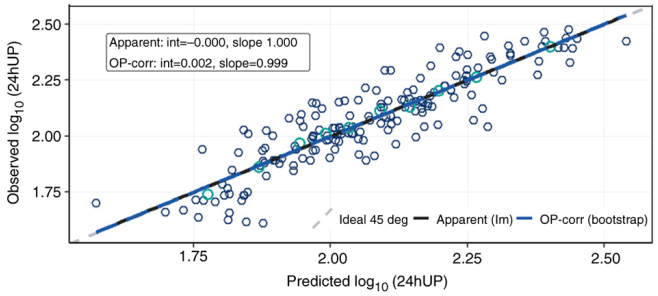

Figure 2. Calibration for model M_6: Observed Log_{10}(24hUP) vs. predicted Log_{10}(24hUP). The 45° line is the ideal reference. The solid line shows the apparent fit; the dashed line shows the optimism-corrected fit from bootstrap (B=2,000). The two lines nearly overlap (slope=0.999; intercept=0.002). Model M_6: Log_{10}[24hUP (mg/m^2/h)]=0.291 + 0.880 x Log_{10}(adjusted UPCR)-0.006xage (years)+0.057xsex (0=boy, 1=girl). Adjusted UPCR=mUPCRxe2 4hUCr$_{Cockcroft-Gault}$; n=171; adjusted R^2=0.80. 24hUP, 24-h urine protein; lm, linear model; OP-corr, optimism-corrected.

the log_{10}-scale remained low: RMSE increased by only 2.3% (95% CI, 2.0 to 2.6%), MAE by 2.2% (95% CI, 2.0 to 2.4%), and R^2 decreased minimally from 0.803 to 0.796 (-0.785 percentage points; 95% CI for R^2, 0.758 to 0.857). Calibration was near-ideal (slope 0.999, intercept 0.002; Fig. 2), indicating negligible overfitting and no need for coefficient shrinkage.

External validation of 24hUP prediction in validation cohort. For clinical implementation, a back-transformed model M_6 from the Log_{10}-scale used Duan's smearing. Using the smearing factor (SF) estimated in the derivation cohort (SF=1.022). With k=$10^{\beta 0}$=1.955, the overall constant on the original scale is C=SF **x** k=1.998. The prediction equation on the original scale is: 24hUP (mg/m^2/h)=1.998x(adjusted UPCR)$^{0.880}$x$10^{(-0.006)\ age}$x$10^{0.057\ sex}$. Applied unaltered to the independent validation cohort (n=80), the model achieved an RMSE of 11.39 mg/m^2/h (95% CI, 9.92 to 12.83), an MAE of 9.26 mg/m^2/h (7.87 to 10.78), and an R^2 of 0.912 (95% CI, 0.866 to 0.943). P20 was 93.8% (86.0 to 97.9) and P30 was 96.3% (89.4 to 99.2), indicating that the majority of predictions were within 20-30% of observed 24hUP.

The calibration of observed vs. predicted 24hUP in the validation cohort revealed a calibration intercept of -9.51 mg/m^2/h (HC3 95% CI, -17.55 to -1.47) and slope of 1.12 (HC3 95% CI, 1.05 to 1.19), indicating mild compression of predictions (slight overestimation at lower values and underestimation at higher values) but overall acceptable calibration (Fig. 3A). Bland-Altman analysis on the original scale showed a mean bias of 2.99 mg/m^2/h (observed-predicted), with LoA from -18.69 to 24.66 mg/m^2/h, and evidence of proportional bias (β=0.161; 95% CI, 0.094 to 0.229), meaning error increased at very high proteinuria (Fig. 3B).

Using the prespecified PI three-zone rule on the external validation cohort, the primary threshold at 150 mg/m^2/h allowed omission of 75% of 24-h collections with 0% false-omits (PI-95%); 23.8% fell into the gray zone and 1.3% were definite collections. At 100 mg/m^2/h, the rule omitted 23.8% and triggered 30% definite collections (PI-95%) with 0% false-omits (Table III). Consistent with these findings, DCA showed positive net benefit over treat-all and treat-none across clinically plausible threshold probabilities (Fig. S5).

Discussion

Severe PNS is critical clinically not only due to massive edema and profound hypoalbuminemia, but also due to early, potentially life-threatening complications such as thromboembolism, severe bacterial infection/sepsis and intravascular volume depletion with prerenal acute kidney injury, all of which cluster in the high-proteinuria, hypoalbuminemia phase and therefore track with the severity of proteinuria (15-18). Yet, there is currently no widely adopted pediatric tool that turns a single-void UPCR into a reliable estimate of severity tier of 24hUP to support those specific bedside decisions. UPCR correlates well with 24hUP in children; however, key gaps persist. The majority of studies stop at simple correlations and do not assess agreement, proportional bias, or calibration across the full proteinuria range (6,19,20). Numerous equations also lack external validation in independent pediatric nephrotic cohorts, particularly in Asian children, limiting use where 24-h urine collection is impractical. In addition, the UPCR-24hUP association is multiplicative (log-log), causing heteroscedastic and proportional error on the raw scale, with underestimation at the highest protein leaks, exactly where clinicians most need to identify children at highest risk (8,20,21).

Table III. Clinical impact of a PI-based three-zone rule using original M6 predictions (external validation, n=80).

Threshold T (mg/m²/h)	PI	No need 24hUP (n, %)	Measure 24hUP (n, %)	Gray zone (n, %)	False-omit (n, %)	False-do (n, %)
100	95%	19 (23.8)	24 (30.0)	37 (46.2)	0 (0.0)	0 (0.0)
150	95%	60 (75.0)	1 (1.3)	19 (23.8)	0 (0.0)	0 (0.0)
100	80%	27 (33.8)	33 (41.2)	20 (25.0)	0 (0.0)	1 (1.3)
150	80%	68 (85.0)	3 (3.8)	9 (11.2)	1 (1.3)	0 (0.0)

Rule definition: No need 24hUP if upper PI < T; Measure 24hUP if lower PI ≥T; otherwise gray zone (consideration). PI construction: PI-95%: y±1.96xRMSE; PI-80%: y±1.28xRMSE; with RMSE=11.39 mg/m²/h and y from the original M_6. Error definitions: False-omit: Classified No need 24hUP but observed ≥ T. False-do: Classified Measure 24hUP but observed < T. 24hUP, 24-hour urine protein; PI, prediction interval; RMSE, root mean squared error.

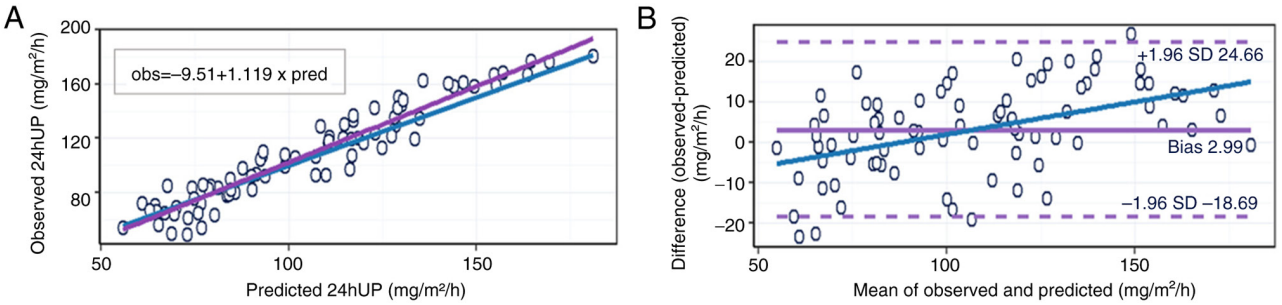

Figure 3. Calibration and agreement of the original M6 estimator on external validation. (A) Observed vs. predicted 24hUP. The blue 45° line indicates perfect calibration; the purple line is the linear calibration fit (a=-9.51, b=1.12); R^2=0.912; CCC=0.939. (B) Bland-Altman plot of the difference (observed - predicted) vs. the mean, with mean bias=2.99 mg/m²/h and 95% LoA=-18.69 to 24.66 mg/m²/h. A fitted trend line indicates proportional bias (+1.61 mg/m²/h per 10 mg/m²/h increase in the mean). 24hUP, 24-hour urine protein; CCC, concordance correlation coefficient; LoA, limits of agreement

In the present study cohort of children with PNS, a multivariable equation was developed that converts a single-void UPCR into an estimated 24-h protein excretion. Herein, three creatinine-excretion approaches (Cockcroft-Gault, Hellerstein, and Ghazali-Barratt) were compared to standardize UPCR for body-size variability. The Cockcroft-Gault-based approach provided the optimal overall agreement and the most favorable accuracy-feasibility balance in the cohort; alternative pediatric formulas did not improve performance and were therefore not retained. The final model (M_6) used a Cockcroft-Gault-based adjusted UPCR multiplied by an age and sex correction, reflecting physiologic variation in creatinine generation, muscle mass and urine concentration across childhood and adolescence, consistent with prior studies (22-25). This approach is motivated by the known limitations of 'raw' UPCR: Protein and creatinine do not scale identically with growth, edema, hydration status and lean body mass, which introduces heteroscedastic error and proportional bias, particularly at the extremes of protein loss (8,21,26).

This adjustment also aligns with the broader goal of reducing body-size-related variability when interpreting proteinuria metrics in children. The fractional excretion of total protein (FETP), the ratio of total protein clearance to creatinine clearance, has been proposed as a physiologically grounded, clearance-based index to account for body size with preserved kidney function (27). However, FETP typically requires synchronous blood measurements in addition to urine protein and urine creatinine, which were not consistently available at the time of urine sampling in the dataset in the present study; thus, calculating FETP would have introduced substantial missingness and potential selection bias. By contrast, UPCR is emphasized in the majority of pediatric nephrology guides as a practical alternative to timed urine collections for defining and monitoring nephrotic-range proteinuria, supporting our focus on UPCR for real-world, guideline-concordant implementation (1-3). Future studies with standardized paired blood-urine sampling are warranted to directly compare UPCR-based equations with clearance-based indices such as FETP to assess any incremental predictive or clinical utility.

The model used herein explained a large proportion of variability in 24hUP during development and performed robustly in a temporally independent validation cohort without refitting. In external validation, it achieved a high coefficient of determination (R^2=0.91), low absolute prediction error (9 mg/m²/h) and clinically tight accuracy (94% within 20 and 96% within 30% of observed 24hUP). Calibration remained acceptable when the original coefficients were applied unaltered, and the average bias on the original mg/m²/h scale was minimal. These findings suggest that a single-voided urine specimen can recover the approximate 24-h protein excretion of a child with clinically usable precision, not merely correlation.

The present study also examined how the model behaves at the most critical areas clinically: At the extreme upper end of proteinuria (24,28). Bland-Altman analysis in the validation

cohort revealed narrow LoA and only modest mean bias overall, but with proportional error at very high 24hUP values; specifically, the model tended to slightly underpredict the most extreme protein losses. This matters clinically as children with extremely high-grade proteinuria have the highest short-term risk for complications, such as intravascular volume depletion, severe hypoalbuminemia requiring infusion, bacterial infection and thromboembolism in the setting of marked urinary protein wasting and hemoconcentration (15,16,18). These are the very patients for whom pediatric nephrologists decide on immediate inpatient monitoring and aggressive supportive care. As proportional bias emerges at the extreme end of proteinuria, it may be unsafe to base high-stakes decisions on a single predicted value alone. Accordingly, when predicted values fall in the highest range, confirmatory testing with a timed urine collection (full 24-h urine) should be considered to support clinical decision-making. In such children, escalation decisions should incorporate the uncertainty of the model, not only its mean prediction.

To address this issue, in the present study, model output was operationalized into a three-zone decision rule based on the PI of each child, rather than relying solely on the point estimate. Of note, two clinically relevant thresholds were prespecified for 24hUP (100 mg/m^2/h as a lower-risk cut-point, and 150 mg/m^2/h as a higher-risk cut-point) consistent with levels at which clinicians begin to escalate surveillance, consider albumin/diuretic management, or are concerned about the thrombotic risk (1-3,29). For each patient, the following questions were raised: Even in a worst-plausible scenario consistent with model error (95% PI), is this child clearly below the cut-point, clearly above it, or uncertain ('gray zone')? Using this PI-based strategy at the 150 mg/m^2/h threshold, a full 24-h urine collection could have been avoided in 75% of children in the validation cohort, while directing only 1% straight to 'must collect now', and leaving 24% in a gray zone for clinician review, without falsely ruling out any truly very-high-proteinuria cases (0% false-omits). At the 100 mg/m^2/h threshold, the same framework again separated 'safe to defer collection', 'must collect', and 'gray zone' with no missed high-proteinuria cases. In practice, gray-zone results (i.e., the PI overlaps the cut-point) are most effectively handled with an iterative confirmation strategy rather than immediate escalation. The authors suggest the following: i) Repeating a spot UPCR after stabilization (e.g., next void or within 12-24 h) to reduce random variability; ii) integrating contemporaneous clinical context (edema burden, hemodynamics, urine output) and key laboratory tests (serum albumin and creatinine/eGFR); and iii) obtaining confirmatory timed urine collection (full 24-h urine) when uncertainty persists or management decisions are high-stakes. Conceptually, this functions as a triage layer on top of the numeric prediction: It concentrates limited nursing and laboratory effort on the few children whose proteinuria is most concerning, and it reassures clinicians that avoiding immediate 24-h collection in the others is unlikely to miss a dangerous presentation.

This approach is responsive to how pediatric nephrotic syndrome is actually managed. A tool that i) estimates the likely magnitude of ongoing protein loss from a single spot sample and ii) classifies the child into 'clearly below', 'clearly above', or 'uncertain relative to clinically relevant danger zones', directly mirrors bedside thinking. DCA in the external validation cohort supports this interpretation: Across plausible intervention thresholds (reflecting the tolerance of the clinician to act on suspected very high proteinuria), a model-guided strategy provided higher net clinical benefit than treating all children as high risk (i.e. obtaining 24-h collections on everyone) or treating none as high-risk. This indicates that the model is not only statistically well calibrated, but also improves how efficiently attention and resources would be allocated. This 'equation → triage rule → net benefit' chain is largely absent from prior pediatric work in nephrotic syndrome and, to the best of our knowledge, has not yet been reported in an Asian pediatric cohort.

The present study has several strengths. It uses consecutive real-world pediatric nephrotic admissions, including children with substantial hypoalbuminemia and heavy protein loss, rather than an artificially 'clean' subset. The validation cohort was acquired later in time, reducing optimism from overfitting. The present study reports discrimination-like metrics (R^2, P20/P30), absolute error (MAE, RMSE), calibration (slope/intercept), clinical agreement on the original scale and decision safety at actionable thresholds. This prediction model study is reported in accordance with the TRIPOD statement (30). Of note, the PI-based gray-zone rule achieved 0% false-omits for very high proteinuria at 150 mg/m^2/h, while suggesting that the majority of children could safely avoid a 24-h urine collection. That directly targets the operational bottleneck in pediatric nephrotic care: Deciding who truly needs an immediate timed collection as opposed to who can be managed using spot data plus close observation.

However, certain limitations of the present study should also be acknowledged. First, although the temporal split provides external validation, both cohorts were drawn from a single center. Accordingly, external validity is limited, and the equation should be externally validated in independent, multicenter and multi-ethnic pediatric cohorts prior to widespread clinical adoption. Variation in anthropometry, nutritional status, steroid exposure, supportive care practices (e.g., albumin infusion thresholds, thromboprophylaxis norms), and laboratory calibration across institutions may affect transportability (31,32). Local recalibration, particularly of the smearing factor and potentially the intercept, may be required before routine deployment elsewhere. Second, the most extreme levels of proteinuria were relatively uncommon; proportional underestimation at the very high end means clinicians should interpret predictions conservatively in profoundly edematous, hemodynamically tenuous, or thrombosis-suspect presentations. Third, the prespecified thresholds (100 and 150 mg/m^2/h) and the resulting 'rule-out/gray zone/rule-in' logic are clinically reasoned, but not yet guideline-endorsed. They should be viewed as an implementation proposal rather than a definitive standard. Fourth, the primary endpoint was proteinuria itself, not downstream hard outcomes, such as thromboembolism, albumin-requiring hypovolemia, pediatric intensive care unit transfer, or readmission. Linking the model and the gray-zone rule to those outcomes will be essential to determine whether triage truly improves safety and resource use. Finally, this model is intended for children with preserved

renal function (eGFR, ≥60 ml/min/1.73 m^2); performance in renal impairment requires dedicated validation. Although the equation uses spot UPCR as input, it was validated against measured 24-h protein excretion in children able to complete timed collections, and transportability to outpatient settings or to children unable to complete 24-h urine remains to be established. Future studies are thus required to focus on multicenter validation, recalibration in other populations and outcome-linked impact evaluation. In conclusion, if confirmed, this approach could reduce the burden of timed urine collection in hospitalized children, while preserving attention for those at greatest risk.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

QCN and HDT were responsible for the conceptualization of the study. QCN, UHAN, TTH, LTKD and PHPN performed data curation. QCN and HDT developed the study methodology. Formal statistical analyses were conducted by QCN, HDT, UHAN, TTH, LTKD and PHPN. The original draft of the manuscript was written by QCN, TTH, LTKD and PHPN. QCN, HDT, UHAN, TTH, LTKD and PHPN reviewed and edited the manuscript. HDT and QCN supervised the study. QCN and UHAN confirm the authenticity of all raw data. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB) of Can Tho University of Medicine and Pharmacy (IRB No. 4618/QĐ-ĐHYDCT/24.KY.60), approval date, December 17, 2024 and received permission to conduct the study at Can Tho Children's Hospital. All procedures adhered to the Declaration of Helsinki of 1975, as revised in 2000. The retrospective component was granted a waiver of informed consent on the basis of minimal risk, impracticability of contacting all prior patients, and adequate privacy safeguards (coded identifiers, restricted access). The prospective component obtained written informed consent from a parent/legal guardian and age-appropriate child assent when applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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