Am J Cancer Res 2025;15(2):824-832
www.ajcr.us /ISSN:2156-6976/ajcr0159224

Original Article

Evaluation of a tumor marker gastrin-releasing peptide precursor in the patients with kidney injuries

Nan Duan*, Zhihui Li*, Zhiyan Li, Lu Pang, Jialin Du, Le Chang, Haiming Huang, Haixia Li

*Department of Clinical Laboratory, Peking University First Hospital, Beijing 100034, China. *Equal contributors.*

Received July 16, 2024; Accepted February 11, 2025; Epub February 15, 2025; Published February 28, 2025

Abstract: Gastrin-releasing peptide precursor (ProGRP) is a bioactive precursor of GRP and might play an important role as an emerging tumor marker in early cancer diagnosis. It might also be abnormal in the nonmalignant disease and renal function abnormalities. The present study was undertaken to investigate the changes of ProGRP levels in patients with kidney injuries, especially with chronic kidney disease (CKD), determine the upper reference intervals and clinical diagnostic value of ProGRP in CKD, and thus help oncologists in interpreting ProGRP levels and making clinical judgments of malignances. 676 individuals were enrolled in this cross-sectional study and divided into five groups: healthy control (n=194), CKD (n=272), nephrotic syndrome (NS) (n=137), antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) (n=41), and urinary tract infection (UTI) (n=32). A total of 27 features including age, gender, and 25 laboratory markers were analyzed. Machine learning algorithms were built for the diagnostic models of CKD. Statistical analysis was performed by R software. It was shown that serum ProGRP level in CKD was significantly higher than that in healthy controls, UTI and NS ($P < 0.01$). The upper reference limit of ProGRP was 188.42 pg/ml for CKD, 245.40 pg/ml for CKD IV-V, and 97.25 pg/ml for NS. Compared with the healthy control, the level of serum ProGRP in CKD stages II, III, IV-V was significantly increased and elevated progressively with CKD grade ($P < 0.01$). Random Forest (RF) model works best among 4 building machine learning algorithms. 5 vital indicators, ProGRP, estimated glomerular filtration rate (eGFR), urea, albumin (ALB), and direct bilirubin (DBIL), were selected to establish RF model for diagnosing CKD with an area under the curve (AUC) of 0.96 (95% confidence interval [CI]: 0.94-0.97) and high sensitivity (0.89) and specificity (0.92). This study demonstrates that the level of ProGRP in patients with CKD, nephrotic syndrome or AAV, was significantly higher than that in the healthy population. The machine learning model of ProGRP with DBIL, eGFR, ALB, and urea, could provide good clinical value for CKD evaluation.

Keywords: Gastrin-releasing peptide precursor, chronic kidney disease, nephrotic syndrome, machine learning algorithms, Random Forest model

Introduction

Gastrin-releasing peptide (GRP) could modulate several gastric and intestinal functions, ranging from inducing secretion of hormones in the digestive tracts and pancreatic amylase to stimulating constriction of smooth muscle [1]. The gastrin-releasing peptide receptor (GRPR) binding to ligands such as GRP plays an important role in the pathophysiological processes of various diseases, including inflammatory diseases, neurological diseases as well as many types of tumors [1, 2], and promotes hyperuricaemia-induced tubular injury, inflammation, and renal fibrosis both in vivo and in vitro [3].

Although GRP/GRP receptor signaling has been implicated in proliferation and progression of malignancies [4], GRP is difficult to be measured in clinical practice because it is unstable in serum (half-life of approximately 1.5 minutes). As a bioactive precursor to GRP, GRP precursor (ProGRP) offers advantages of being a biomarker due to its stable structure and long half-life (ranging from 19 to 26 days), and the levels of ProGRP and GRP are highly correlated [1, 5, 6]. In clinical applications, ProGRP is used as a novel important biomarker for early cancer diagnosis, especially highly sensitive in small cell lung cancer (SCLC) with great prognostic value [7, 8]. ProGRP is also up-regulated in many types of tumors, such as pancreatic can-

https://doi.org/10.62347/CBSP3728

cer, colon cancer, prostate cancer, and central nervous system cancers [1, 5, 9]. Moreover, it has been shown that tumor markers concentrations might be abnormal in the pregnancy, inflammation, nonmalignant disease, liver function and (or) renal function abnormalities.

As kidneys are the main excretory organs of ProGRP, impaired glomerular filtration and deteriorated renal function will lead to its accumulation in blood circulation as the higher serum concentrations are observed [7, 10]. A critical challenge in the clinical application has emerged: while serum ProGRP increased above 50 pg/ml in only a small fraction of benign diseases (non-malignance), the elevation was observed in most cases of chronic kidney disease (CKD) and much higher in CKD than in other benign diseases [11]. Therefore, ProGRP evaluation in patients with renal failure was confused with its clinical value as a tumor marker [12]. Further analysis also found that the serum level of ProGRP of chronic renal failure patients was positively correlated with serum creatinine of patients, which can be used as a reliable indicator of pathogenesis and prognosis assessment of chronic renal failure patients [13]. The presence of kidney disease may pose significant problems in interpreting ProGRP level and making clinical judgment for oncologists. However, the role and utility of tumor markers such as ProGRP in cancer diagnosis in patients with renal disease remains controversial [14].

Therefore, it is vital to determine the effect of kidney injuries on the level of ProGRP in blood, to avoid misinterpretation when diagnosing and treating patients with cancers. The present study intends to investigate the level change of ProGRP in patients with kidney-related diseases including nephrotic syndrome (NS), urinary tract infection (UTI) (often developing into chronic pyelonephritis and permanently damaging the structures and functions of the kidneys [15]), antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) (kidney is commonly involved and irreversibly injured in AVV [16, 17]), and especially CKD, to preliminarily determine the upper reference intervals, so as not to confuse with early stages of malignancy. A further aim is to explore the clinical diagnostic value combining ProGRP with traditional biomarkers of renal function for CKD patients.

Materials and methods

Subjects

We enrolled a total of 676 subjects into the cross-sectional study. Of these, 548 patients of the Department of Nephrology from 1 October, 2022 to 28 February, 2023 were recruited. After excluding 66 individuals with hypertension, diabetes mellitus and other diseases (chronic liver disease, chronic bronchitis, cardiac insufficiency, heart failure, severe malnutrition, malignancies, etc.), 482 subjects were finally included in this study. These 482 subjects are divided into four groups: CKD (n=272), NS (n=137), AAV (n=41), and UTI (n=32). 194 healthy individuals without related diseases (chronic inflammatory, metabolic diseases, hypertension, cancer, coronary heart disease, diabetes mellitus, renal disorders, etc.) or abnormal laboratory examinations were selected as healthy control. The diagnostic criteria for CKD are based on the Kidney Disease Outcome Quality Initiative (KDOQI): abnormalities of kidney structure, reduced renal function evaluated by estimated glomerular filtration rate (eGFR) < 60 mL/minute/1.73 m^2 (mL/min/1.73 m^2) or a persistent proteinuria in 2 separate measurements within an interval of 3 months [18]. According to the gold standard of CKD grading followed by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI), CKD was divided into five stages (CKD I-V) [19]. Among 272 CKD subjects, there was only 24 CKD V patients and the stages IV and V of CKD patients were combined into one grade. NS is classically defined by nephrotic-range proteinuria (≥ 40 mg/m^2/hour or urine protein/creatinine ratio ≥ 200 mg/mL or 3+ protein on urine dipstick), hypoalbuminemia (< 25 g/L) and oedema [20]. This study has been reviewed and approved by Institutional Ethics Committee of Peking University First Hospital (No. 2023-research-320).

Laboratory measurement

The systemic condition of study subjects was evaluated by a comprehensive review of medical history and a series of biochemical assessments. Blood samples were collected after an overnight fast of at least 12 hours. Serum creatinine, potassium, sodium, chlorine, urea, uric acid (UA) and fasting plasma glucose (FPG) were measured concomitantly on AU5800

automatic biochemical analyzer (Beckman Coulter, Inc., CA, USA). Serum creatinine levels were determined by Jaffe's method. Serum ProGRP was detected by Roche Cobas e601 electrochemiluminescence immunoassay analyzer (Roche Diagnostics, GmbH, Mannheim, Germany). The reference interval of ProGRP is < 69.2 pg/mL in our laboratory. eGFR was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation recommended by the Kidney Disease Improving Global Outcomes [21]. CKD patients were divided into five stages: CKD I (eGFR ≥ 90 mL/min per 1.73 m^2), CKD II (eGFR 60-89 mL/min per 1.73 m^2), CKD III (eGFR 30-59 mL/min per 1.73 m^2), CKD IV (eGFR 15-29 mL/min per 1.73 m^2) and CKD V (eGFR < 15 mL/min per 1.73 m^2).

Statistical analysis

Statistical analysis was performed by R software (Version 3.6.2, https://www.r-project.org/) and the Deepwise & Beckman Coulter DxAI platform (https://dxonline.deepwise.com). Continuous variables were expressed as mean ± standard deviation or median (25^{th} percentile-75^{th} percentile), and One-way ANOVA or Kruskal-Wallis tests were used to compare the differences among the study groups. Categorical variables were presented as frequencies with percentages, and the Chi-square (χ^2) tests were applied to identify categorical variables. Reference intervals of ProGRP for CKD and nephrotic syndrome were established according to the Clinical and Laboratory Standards Institute (CLSI) guideline C28-A3 [22]. The reference upper limit was calculated by the non-parametric percentile method (95%, one-tailed). The diagnostic model was developed using R software with the rms package and displayed online through Deepwise and Beckman Coulter DxAI platform (https://dxonline.deepwise.com/). To obtain the best diagnostic performance, four machine learning algorithms, including the eXtreme Gradient Boosting (XGBoost) algorithm, Logistic regression (LR), Random Forest (RF), Gradient Boosting learning model, were built. XGBoost utilizes decision tree as base learners and employs regularization techniques to enhance model generalization. RF combines the output of multiple decision trees to reach a single result. LR is used for binary classification where it uses sigmoid function, which takes input as independent variables and produces a probability value between 0 and 1. Gradient Boosting is an algorithm that combines the predictions of multiple weak learners, typically decision trees, sequentially. The receiver operating characteristic (ROC) curve was presented to evaluate the discrimination ability of the model. The area under the ROC curve (AUC) was calculated, and an AUC of > 0.75 was considered to indicate good model performance. The *P* value of < 0.05 was considered as indicating statistical significance.

Results

Clinical characteristics and laboratory results of study subjects

The clinical and biochemical characteristics of all study subjects are summarized in **Table 1**. Of the 482 patients enrolled from the Department of Nephrology, 261 (54.15%) were male and 221 (45.85%) were female, and they were divided into four groups based on their clinical diagnoses: CKD (n=272), NS (n=137), AAV (n=41), and UTI (n=32). The healthy control (n=194) included 124 males (63.9%) and 70 females (36.1%). Patients with CKD were significantly older than healthy controls ($P < 0.01$), but not statistically different in gender between them. As showed in **Table 1**, 27 features, including ProGRP and renal function-related indicators of creatinine, eGFR and urea were significantly different between healthy controls and CKD patients ($P < 0.01$).

Comparison of ProGRP levels in study groups

As shown in **Table 1** and Supplementary Figure 1, serum ProGRP level in the CKD group was 67.14 (48.98, 100.18) pg/ml, indicating significant differences from the healthy controls (36.65 [30.29, 43.62] pg/ml) and the other groups ($P < 0.01$). ProGRP of nephrotic syndrome patients (48.95 [35.55, 65.66] pg/ml) was significantly higher than that in the healthy subjects ($P < 0.01$), but not statistically significant with the UTI patients (30.51 [26.97, 33.73] pg/ml). AAV group (125.65 [74.70, 163.20] pg/ml) had significantly higher level of ProGRP compared to the healthy control, CKD, NS and UTI ($P < 0.01$).

Table 1. Clinical characteristics and laboratory assessments of the study groups

	Healthy controls (n=194)	Chronic kidney disease (n=272)	Nephrotic syndrome (n=137)	Antineutrophil cytoplasmic antibody associated vasculitis (n=41)	Urinary tract infection (n=32)	*P*
Age (years)	40.00 (35.00, 46.75)	52.00 (39.00, 64.00)*	55.00 (40.00, 64.00)	71.00 (65.25, 75.00)	43.00 (36.00, 51.50)	< 0.001
Male (%)	63.91	54.78*	58.39	46.34	40.63	0.043
ProGRP (pg/ml)	36.65 (30.29, 43.62)	67.14 (48.98, 100.18)*	48.95 (35.55, 65.66)	125.65 (74.70, 163.20)	30.51 (26.97, 33.73)	< 0.001
eGFR (ml/min/1.73 m^2)	97.02 (88.14, 106.45)	57.12 (31.20, 77.26)*	83.96 (64.91, 99.69)	30.15 (19.90, 46.42)	90.93 (83.58, 106.97)	< 0.001
A/G	1.39 (1.29, 1.54)	1.28 (1.12, 1.50)*	1.27 (1.05, 1.40)	1.45 (1.35, 1.55)	1.42 (1.31, 1.60)	< 0.001
AG (mmol/L)	12.18 (10.98, 13.08)	12.30 (10.70, 15.03)	11.10 (9.50, 13.00)	11.25 (10.45, 13.10)	12.05 (10.20, 14.05)	< 0.001
ALB (g/L)	45.20 (44.20, 46.85)	41.35 (38.20, 44.03)*	38.10 (32.20, 42.00)	39.55 (34.45, 44.05)	44.00 (42.25, 45.75)	< 0.001
ALP (IU/L)	68.00 (56.00, 80.00)	61.00 (50.75, 71.25)*	58.00 (47.00, 71.00)	43.00 (39.75, 68.75)	62.50 (49.25, 71.50)	< 0.001
ALT (IU/L)	16.00 (10.25, 25.75)	17.00 (12.00, 26.00)	17.00 (13.00,25.00)	14.50 (11.25, 16.75)	14.00 (11.25, 16.75)	0.038
AST (IU/L)	21.00 (18.00, 25.00)	18.00 (15.00, 23.00)*	18.00 (15.00, 22.00)	17.50 (14.50, 20.00)	18.50 (16.25, 20.75)	< 0.001
Ca (mmol/L)	2.40 (2.35, 2.45)	2.31 (2.24, 2.37)*	2.28 (2.21, 2.36)	2.31 (2.25, 2.39)	2.32 (2.25, 2.35)	< 0.001
Cl (mmol/L)	103.40 (102.50, 104.58)	105.00 (104.00, 108.00)*	106.00 (104.00, 108.00)	105.50 (103.25, 107.50)	105.00 (103.25, 105.75)	< 0.001
CO_2 (mmol/L)	27.45 (26.10, 28.40)	25.85 (23.20, 27.80)*	27.10 (25.20, 28.80)	26.45 (25.98, 26.88)	27.00 (25.28, 29.23)	< 0.001
CREA (μmol/L)	81.00 (68.30, 88.52)	113.00 (86.00, 176.00)*	83.00 (72.00, 100.00)	180.00 (115.50, 208.50)	67.50 (65.25, 73.75)	< 0.001
DBIL (μmol/L)	2.40 (1.90, 3.20)	0.32 (0.20, 1.24)*	0.21 (0.20, 1.06)	0.20 (0.20, 0.57)	0.82 (0.31, 1.29)	< 0.001
GGT (IU/L)	20.00 (15.00, 33.00)	23.00 (17.00, 33.00)	25.00 (18.00, 37.00)	23.50 (16.50, 35.50)	15.50 (12.25, 19.00)	0.001
GLU (mmol/L)	4.92 (4.60, 5.31)	5.40 (4.94, 6.12)*	5.50 (5.08, 6.44)	5.04 (4.84, 6.00)	5.23 (4.97, 5.78)	< 0.001
HDL-C (mmol/L)	1.26 (1.08, 1.54)	1.22 (1.01, 1.58)	1.40 (1.16, 1.75)	1.47 (1.35, 1.83)	1.47 (1.23, 1.69)	< 0.001
IBIL (μmol/L)	11.24 (9.10, 14.13)	8.70 (6.19, 12.09)*	9.53 (6.70, 11.93)	7.49 (6.25, 9.68)	10.69 (9.41, 12.42)	< 0.001
K (mmol/L)	3.84 (3.65, 3.99)	4.10 (3.80, 4.60)*	3.90 (3.70, 4.20)	4.10 (4.03, 4.28)	3.80 (3.60, 4.00)	< 0.001
LDL-C (mmol/L)	2.79 (2.36, 3.30)	2.52 (1.94, 3.30)*	2.84 (2.29, 3.79)	2.78 (2.61, 3.94)	2.76 (2.30, 3.37)	0.001
Mg (mmol/L)	0.88 (0.85, 0.91)	0.90 (0.85, 0.97)*	0.86 (0.81, 0.92)	0.90 (0.87, 1.05)	0.90 (0.85, 0.93)	< 0.001
Na (mmol/L)	139.15 (138.05, 140.30)	140.00 (138.00, 142.00)*	140.00 (139.00, 142.00)	140.00 (139.00, 141.00)	140.00 (138.25, 142.75)	< 0.001
P (mmol/L)	1.02 (0.92, 1.12)	1.18 (1.04, 1.35)*	1.18 (1.04, 1.31)	1.22 (1.03, 1.45)	1.11 (0.93, 1.16)	< 0.001
PA (mg/L)	266.40 (240.23, 299.10)	291.35 (254.65, 339.23)*	281.30 (242.10, 332.10)	345.55 (263.23, 372.38)	238.20 (211.30, 251.10)	< 0.001
PCHE (IU/L)	8406.0 (7397.5, 9246.0)	8388.0 (6889.3, 9740.3)	8929.0 (7517.0, 10865.0)	6773.5 (5444.0, 8234.0)	7951.5 (7166.0, 8852.5)	0.001
TBA (μmol/L)	2.10 (1.33, 3.15)	2.50 (1.60, 4.70)*	2.80 (1.70, 4.35)	3.55 (1.90, 4.75)	1.80 (1.20, 2.90)	0.001
TBIL (μmol/L)	13.55 (10.93, 17.15)	9.30 (6.40, 13.40)*	10.10 (6.90, 13.10)	7.70 (6.50, 9.93)	11.50 (9.93, 13.45)	< 0.001
TCHO (mmol/L)	4.76 (4.10, 5.36)	4.62 (3.90, 5.58)	5.36 (4.26, 6.49)	5.29 (4.84, 6.56)	4.91 (4.06, 5.43)	< 0.001
TG (mmol/L)	1.02 (0.72, 1.42)	1.55 (1.09, 2.19)*	1.64 (1.27, 2.39)	1.24 (0.98, 2.17)	0.93 (0.80, 1.16)	< 0.001
TP (g/L)	78.25 (75.70, 81.00)	72.85 (68.50, 76.50)*	66.70 (59.90, 72.80)	67.75 (57.53, 72.08)	74.40 (72.63, 77.75)	< 0.001
UA (μmol/L)	337.00 (272.00, 384.25)	360.50 (299.75, 435.00)*	368.00 (318.00, 417.00)	408.50 (323.00, 469.50)	278.50 (240.25, 353.25)	< 0.001
UREA (mmol/L)	4.74 (4.15, 5.61)	8.42 (5.93, 13.36)*	6.47 (4.84, 9.00)	16.17 (10.38, 20.28)	4.27 (3.79, 4.82)	< 0.001

Data are presented as median (25^{th} percentile-75^{th} percentile) or as n (%). *$P < 0.05$, CKD group compared to healthy control group.

Table 2. The efficacy of four machine learning models

	AUC	Sensitivity	Specificity	Precision	Accuracy	NPV	PPV	F1 Score	Recall
RF	0.98	0.92	0.95	0.96	0.93	0.89	0.96	0.94	0.92
XGBoost	0.98	0.91	0.95	0.97	0.93	0.88	0.96	0.94	0.91
LR	0.97	0.90	0.94	0.96	0.92	0.87	0.95	0.93	0.90
Gradient Boosting	0.98	0.91	0.95	0.96	0.92	0.88	0.96	0.93	0.91

RF, Random Forest; XGBoost, eXtreme Gradient Boosting; LR, Logistic regression; NPV, negative predictive value; PPV, positive predictive value.

Determination of upper reference limit in CKD and NS

In the present study, histograms of ProGRP in the CKD and nephrotic syndrome group indicated non-Gaussian distributions, and ProGRP levels were not significant in age or sex. Therefore, non-parametric method was used to calculate the 95th quantile as the upper limit. The non-parametric method can be used to determine the reference interval by the percentile method, which arranges the n individual test values in order from the smallest to the largest, and sequentially assigns a rank to them. Divide the n ranks into 100 equal parts, and the number corresponding to r% rank is called the r^{th} percentile, denoted by Pr. The upper reference limit r (95th percentile) were calculated as the 95% reference intervals (one-tailed) for the assay. The upper reference limit of ProGRP was less than 188.42 pg/ml for CKD, 245.40 pg/ml for CKD IV-V patients, and less than 97.25 pg/ml for the nephrotic syndrome patients.

ProGRP levels in CKD-stages

We further compared the levels of serum ProGRP among the healthy control and the CKD stages. As showed in Supplementary Figure 2, the average ProGRP levels were 33.63 (27.09, 39.08) pg/ml, 54.35 (48.45, 64.00) pg/ml, 78.19 (64.77, 93.32) pg/ml, 139.2 (118.8, 191.8) pg/ml in CKD stages I, II, III and IV+V, respectively. ProGRP level was not significantly different between healthy subjects and CKD stage I patients (P=0.236), but it was significantly elevated in the CKD stages II, III and IV+V compared with the control ($P < 0.01$). The concentration levels also had statistical differences among different CKD stages ($P < 0.01$), showing a progressively increasing trend in CKD grading.

Clinical value of ProGRP in CKD diagnosis

A total of 27 indicators including ProGRP and eGFR were significantly different between healthy controls and CKD patients ($P < 0.01$) (**Table 1**). All the indicators with statistical differences were used to build the machine learning models. Since eGFR is calculated from serum creatinine, and creatinine was not considered in the assessment. Finally, taking AUC combined with sensitivity into account, among the four models of XGBoost, RF, LR, and Gradient Boosting (**Table 2**), the RF model could serve as the best diagnostic tool with an AUC of 0.98 for the ROC curve, a sensitivity of 0.92 and specificity of 0.95.

Moreover, the RF model was developed to further identify the important indicators for diagnosing CKD. We ranked the importance values of all the selected features (27 variables) in the machine learning model and selected the top-ranked and clinically meaningful ones of them for analysis. The feature importance of the variables of this model are ranked accordingly as direct bilirubin (DBIL), albumin, urea, eGFR, ProGRP, K, age, Cl, Ca, and total bilirubin (TBIL), etc. After repeated exclusion and analysis, we compared the model containing the five features with the highest importance (DBIL, albumin, urea, eGFR, ProGRP) with that containing the original 27 variables by the Delong test, and there was no statistical difference between them. The diagnostic performance of the top five variables (DBIL, albumin, urea, eGFR, ProGRP) for CKD diagnosis respectively in single-factor models were showed in **Table 3**. Applying these five indicators to establish the final model, the sensitivity was 0.89, specificity 0.92, accuracy 0.90, and recall 0.89. And the ROC curve of the RF model was plotted as in **Figure 1** with an AUC of 0.96 (95% CI: 0.94-0.97). Furthermore, a web-based tool (https://

Table 3. The diagnostic performance of the one-factor model for CKD diagnosis

	AUC	Cut-off value	Sensitivity	Specificity	maximal Youden's index
DBIL	0.89	1.39	0.79	0.93	0.72
eGFR	0.87	77.16	0.75	0.95	0.70
ALB	0.83	43.25	0.69	0.89	0.58
UREA	0.85	6.04	0.72	0.87	0.60
ProGRP	0.86	48.58	0.76	0.88	0.64

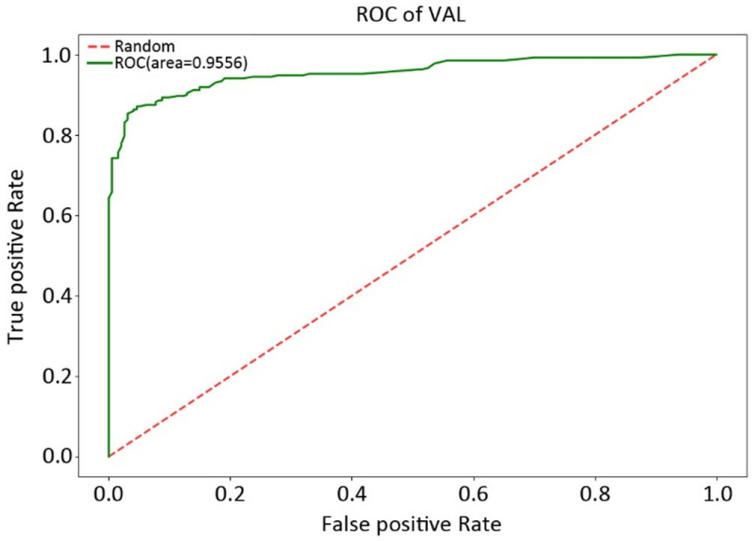


Figure 1. ROC curve of the RF model for CKD diagnosis.

dxonline.deepwise.com/prediction/index.html?baseUrl=%2Fapi%2F&id=36907&topicName=undefined&from=share&platformType=wisdom) was built for clinicians to use the proposed model. A screenshot of the webpage was shown in Supplementary Figure 3. After inputting the necessary parameters, CKD could be diagnosed or predicted with probability.

Discussion

GRP plays a critical role in cancer growth and metastasis, and its precursor, ProGRP, could serve as an emerging tumor marker in early cancer diagnosis [23, 24]. In clinical applications, ProGRP concentrations were also abnormal in some benign diseases, and related to renal function abnormalities as kidney is the major organ excreting it [7, 10]. Therefore, due to disturbing renal metabolism and excretion, application of the tumor marker ProGRP in early diagnosing and therapeutic monitoring could be misinterpreted and limited.

It was observed that serum ProGRP of chronic renal failure patients was abnormally elevated and positively correlated with serum creatinine of patients, suggesting that levels of ProGRP are correlated with the degree of kidney injury [13]. The present study showed that ProGRP levels remain normal in UTI cases, but become elevated in patients with CKD, nephrotic syndrome, and AAV. Although recurrent or persistent UTIs could develop into chronic pyelonephritis, causing permanent damage the structures and functions of the kidneys [15], our finding might indicate that ProGRP accumulation was specifically associated with damaged renal function rather than simple/temporary inflammatory conditions or other pathogeneses [10]. Notably, the significant higher level in patients with AAV compared to those with CKD and nephrotic syndrome could be linked to the clinical features of a more severe renal involvement and reduced eGFR, supporting the findings that the kidneys are most commonly and severely affected in AAV [17]. Moreover, renal disease is common in AAV with features of irreversible kidney injury (glomerulosclerosis and interstitial fibrosis), and is the most important predictor of mortality [16]. The differential elevation of ProGRP across various kidney conditions provides valuable insights into its relationship with renal pathophysiology.

Several studies reported that impaired glomerular filtration may lead to abnormal substance metabolism such as increased circulating ProGRP levels, confound the tumor marker significance of elevated values [12, 14]. Consequently, CKD and nephrotic syndrome should be considered as an interfering factor of ProGRP increase when ProGRP is applied to diagnose SCLC and other benign diseases. When a patient presents with elevated ProGRP levels, the physician's considerations should not be limited to the possibility of cancer, but could

also be the cause of kidney injuries. Based on the principle of reference interval establishment [22], the present study determined the upper reference limit of ProGRP in patients with CKD and nephrotic syndrome. If ProGRP exceeds the range, the doctor might need to consider the possibility that the patient has cancer rather than kidney diseases. Our founding support the diagnosis values of much higher ProGRP levels in SCLC (1,484 pg/ml) [25] and of ProGRP ≥ 300 pg/ml as conventional indicator for SCLC [26]. These upper references would provide clinical values for interpreting ProGRP levels in patients with kidney disease, helping to distinguish between elevations due to renal dysfunction and those potentially indicating malignancy.

To further explore the average ProGRP levels in CKD grading, we evaluated the differences of ProGRP among CKD stages. The present study indicated that ProGRP was not affected in the early stage of CKD, consistent with other researches' reports [10, 14]. When renal injury develops to the middle or late stages, ProGRP cannot pass freely through the endothelial cells and basement membrane, and thus accumulating in the blood circulation [27]. It was also observed that with the aggravation of CKD, serum ProGRP concentrations elevated significantly, reflecting glomerular filtration function change and adding further evidence to the previous studies [10]. Thus, the progressive increase in ProGRP levels across CKD stages offers particular insight into its relationship with renal function. There are several formulas available for assessing glomerular filtration rate and CKD stage, but most of them involve serum creatinine or Cystatin C [28, 29]. Currently, the diagnosis of CKD is usually based on traditional indicators including eGFR, albuminuria or proteinuria [18]. The CKD-EPI formula for calculating eGFR is mainly based on creatinine, which would be affected by various exogenous factors such as extremely high or low body size, muscle metabolism, high-protein diet, or use of drugs affecting tubular secretion of creatinine [21, 30, 31], while ProGRP is an autocrine gastrointestinal hormone which is less influenced by these exogenous factors [32] and therefore has the potential to be a more reliable marker for monitoring renal function, complementing those traditional indicators. Dai reported that serum ProGRP could complement the simplified modification of diet in renal disease (MDRD) formula in grading CKD [10], and our study showed that it might reflect glomerular filtration function as well. The above findings prompted the hypothesis that ProGRP may have clinical value in the diagnosis of CKD by incorporating ProGRP into the equation in eGFR calculation for CKD diagnosis and staging to obtain relatively accurate evaluation in the future.

We have built four machine learning algorithms using XGBoost, RF, LR and Gradient Boosting model, and chose the best diagnostic tool, the RF model, as the final algorithm to further identify the important indicators for diagnosing CKD. RF model combines the output of multiple decision trees to reach a single result, and its strength is the ability to mitigate overfitting and provide robust performance. Finally, five vital indicators (top five variables of feature weighting), including ProGRP, eGFR, urea, albumin, and direct bilirubin (DBIL), were applied to establish a CKD diagnostic model. ProGRP as well as the traditional markers like eGFR and urea, can reflect changes in renal function of CKD patients. Albumin, mainly synthesized by the liver, can assess liver function, and a low serum albumin is an increased risk for kidney failure [33]. As an endogenous product of heme catabolism, bilirubin is also a potent anti-oxidant that effectively scavenges peroxyl radicals, and it might have a protective role in progression of diabetic kidney disease with greater oxidative stress [34]. Some studies demonstrated an independent positive association between serum bilirubin and eGFR [35], and independent negative association between DBIL and CKD [36], suggesting that decreased bilirubin level would be useful as a potential risk factor for the progression of CKD [37, 38]. Therefore, the above five variables of the RF model built for diagnosing CKD in the study were rational. Moreover, our online model had better AUC and sensitivity than the single factor models, showing a good and convenient performance on the diagnosis of CKD. The integration of ProGRP into RF model alongside traditional markers might open new possibilities for improving CKD diagnosis and evaluation in daily clinical work.

However, our research has certain limitations, partly because it was single-hospital design.

The sample sizes of this study in CKD-stage and other diseases were small. Our study is also limited by retrospective and cross-sectional design, and sample collection may have been influenced by external factors. Therefore, a multi-center, longitudinal, prospective clinical trial is required to clarify and confirm our findings.

Conclusion

In conclusion, serum ProGRP levels were elevated significantly in patients with kidney diseases (such as CKD, nephrotic syndrome, and AAV patients) compared to the healthy controls. Additionally, the upper reference limit of ProGRP was 188.42 pg/ml for CKD, 245.40 pg/ml for CKD IV-V, and 97.25 pg/ml for nephrotic syndrome. The average levels of ProGRP increased significantly with the CKD stages, indicating that it might reflect glomerular filtration function change. The machine learning model of ProGRP with DBIL, eGFR, ALB and urea could provide a practical tool for integrating multiple biomarkers in CKD diagnosis and evaluation.

Disclosure of conflict of interest

None.

Address correspondence to: Haixia Li and Nan Duan, Department of Clinical Laboratory, Peking University First Hospital, No. 8 Xishiku Street, Xicheng District, Beijing 100034, China. Tel: +86-10-83575151; Fax: +86-10-83575151; E-mail: haixia.li@pkufh.com (HXL); Tel: +86-10-83575020; Fax: +86-10-83575151; E-mail: nan.duan@pkufh.com (ND)

References

[1] Sun HL, Ma QY, Bian HG, Meng XM and Jin J. Novel insight on GRP/GRPR axis in diseases. Biomed Pharmacother 2023; 161: 114497.

[2] Li C, Ma QY, Liu XQ, Li HD, Yu MJ, Xie SS, Ma WX, Chen Y, Wang JN, He RB, Bian HG, He Y, Gao L, Deng SS, Zang HM, Gong Q, Wen JG, Liu MM, Yang C, Chen HY, Li J, Lan HY, Jin J, Yao RS and Meng XM. Genetic and pharmacological inhibition of GRPR protects against acute kidney injury via attenuating renal inflammation and necroptosis. Mol Ther 2023; 31: 2734-2754.

[3] Sun HL, Bian HG, Liu XM, Zhang H, Ying J, Yang H, Zu T, Cui GQ, Liao YF, Xu MF, Meng XM and Jin J. GRP/GRPR signaling pathway aggravates hyperuricemia-induced renal inflammation and fibrosis via ABCG2-dependent mechanisms. Biochem Pharmacol 2023; 218: 115901.

[4] Laukkanen MO and Castellone MD. Gastrin-releasing peptide receptor targeting in cancer treatment: emerging signaling networks and therapeutic applications. Curr Drug Targets 2016; 17: 508-514.

[5] Peng S, Zhan Y, Zhang D, Ren L, Chen A, Chen ZF and Zhang H. Structures of human gastrin-releasing peptide receptors bound to antagonist and agonist for cancer and itch therapy. Proc Natl Acad Sci U S A 2023; 120: e2216230120.

[6] Patel O, Clyde D, Chang M, Nordlund MS, Steel R, Kemp BE, Pritchard DM, Shulkes A and Baldwin GS. Pro-GRP-derived peptides are expressed in colorectal cancer cells and tumors and are biologically active in vivo. Endocrinology 2012; 153: 1082-1092.

[7] Wojcik E and Kulpa JK. Pro-gastrin-releasing peptide (ProGRP) as a biomarker in small-cell lung cancer diagnosis, monitoring and evaluation of treatment response. Lung Cancer (Auckl) 2017; 8: 231-240.

[8] Bi H, Yin L, Fang W, Song S, Wu S and Shen J. Association of CEA, NSE, CYFRA 21-1, SCC-Ag, and ProGRP with clinicopathological characteristics and chemotherapeutic outcomes of lung cancer. Lab Med 2023; 54: 372-379.

[9] Baratto L, Song H, Duan H, Hatami N, Bagshaw HP, Buyyounouski M, Hancock S, Shah S, Srinivas S, Swift P, Moradi F, Davidzon G and Iagaru A. PSMA- and GRPR-Targeted PET: results from 50 patients with biochemically recurrent prostate cancer. J Nucl Med 2021; 62: 1545-1549.

[10] Dai Z, Zhu J, Huang H, Fang L, Lin Y, Huang S, Xie F, Sheng N and Liang X. Expression and clinical value of gastrin-releasing peptide precursor in nephropathy and chronic kidney disease. Nephrology (Carlton) 2020; 25: 398-405.

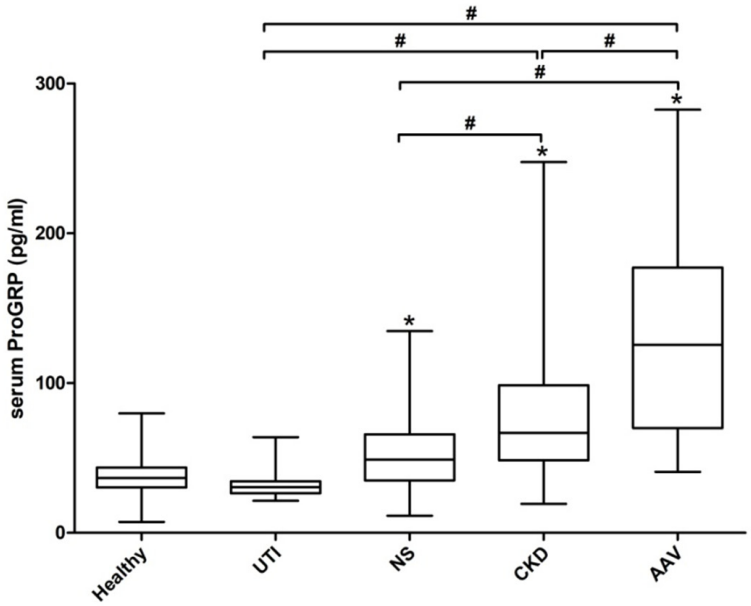
[11] Molina R, Filella X and Auge JM. ProGRP: a new biomarker for small cell lung cancer. Clin Biochem 2004; 37: 505-511.

[12] Rani BS, Suchitra MM, Srinivasa Rao PVLN and Kumar VS. Serum tumor markers in advanced stages of chronic kidney diseases. Saudi J Kidney Dis Transpl 2019; 30: 898-904.

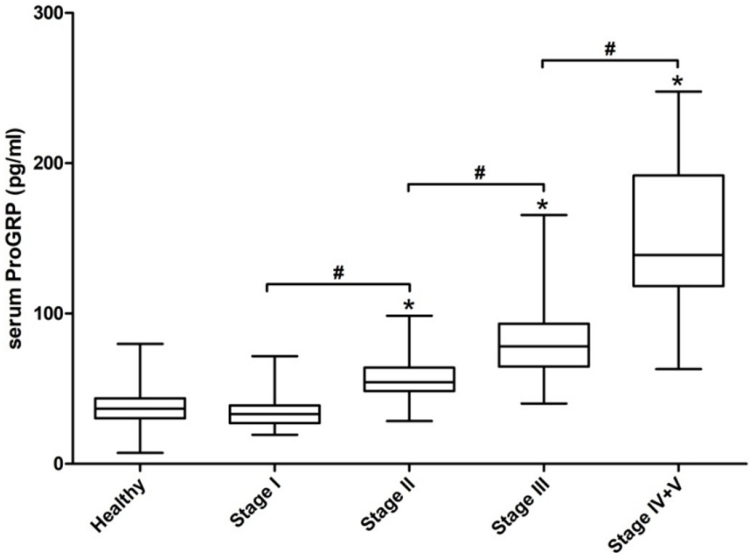
[13] Liu X, Cai J, Xun Z, Song Q, Wang R, Li G and Xu Z. Serum cystatin, chemokine, and gastrin-releasing peptide precursors and their clinical value in patients with chronic renal failure. Biomed Res Int 2022; 2022: 1775190.

[14] Miao Q, Cai B, Niu Q and Zhang J. Changes in lung cancer-related serum tumor markers in patients with chronic kidney disease and determination of upper reference limit. Front Oncol 2022; 12: 1072531.

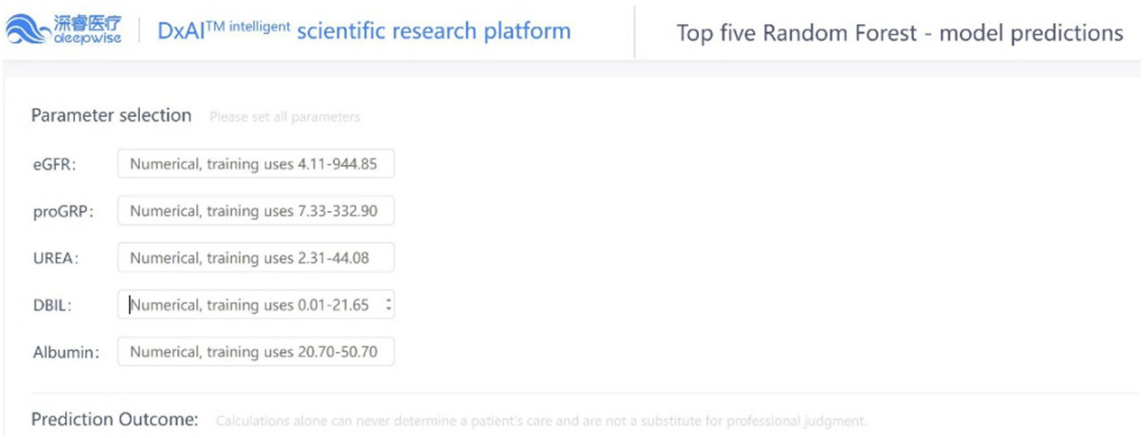
[15] Klein RD and Hultgren SJ. Urinary tract infections: microbial pathogenesis, host-pathogen interactions and new treatment strategies. Nat Rev Microbiol 2020; 18: 211-226.
[16] Geetha D and Jefferson JA. ANCA-associated vasculitis: core curriculum 2020. Am J Kidney Dis 2020; 75: 124-137.
[17] Kitching AR, Anders HJ, Basu N, Brouwer E, Gordon J, Jayne DR, Kullman J, Lyons PA, Merkel PA, Savage COS, Specks U and Kain R. ANCA-associated vasculitis. Nat Rev Dis Primers 2020; 6: 71.
[18] Levin A, Stevens PE, Bilous RW, Coresh J, De Francisco AL, and De Jong PE, Griffith KE, Hemmelgarn BR, Iseki K, Lamb EJ, Levey AS, Riella MC, Shlipak MG, Wang HY, White CT and Winearls CG. Kidney disease: improving global outcomes (KDIGO) CKD work group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl 2013; 3: 1-150.
[19] Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, Gansevoort RT, Kasiske BL and Eckardt KU. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int 2011; 80: 17-28.
[20] KDIGO GN Work Group. KDIGO clinical practice guideline for glomerulonephritis. Kidney Int 2012; 2: 139-274.
[21] Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T and Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150: 604-612.
[22] Clinical and Laboratory Standards Institute (CLSI). Defining, establishing, and verifying reference intervals in the clinical laboratory; approved Guideline. In: CLSI Document C28-A3 (3rd Edition). 2008.
[23] Li L, Zhang Q, Wang Y and Xu C. Evaluating the diagnostic and prognostic value of serum TuM2-PK, NSE, and ProGRP in small cell lung cancer. J Clin Lab Anal 2023; 37: e24865.
[24] Yu M, Yang C, Wang S, Zeng Y, Chen Z, Feng N, Ning C, Wang L, Xue L and Zhang Z. Serum ProGRP as a novel biomarker of bone metastasis in prostate cancer. Clin Chim Acta 2020; 510: 437-441.
[25] Barchiesi V, Simeon V, Sandomenico C, Cantile M, Cerasuolo D, Chiodini P, Morabito A and Cavalcanti E. Circulating progastrin-releasing peptide in the diagnosis of Small Cell Lung Cancer (SCLC) and in therapeutic monitoring. J Circ Biomark 2021; 10: 9-13.
[26] Dong A, Zhang J, Chen X, Ren X and Zhang X. Diagnostic value of ProGRP for small cell lung cancer in different stages. J Thorac Dis 2019; 11: 1182-1189.
[27] Ischia J, Patel O, Sethi K, Nordlund MS, Bolton D, Shulkes A and Baldwin GS. Identification of binding sites for C-terminal pro-gastrin-releasing peptide (GRP)-derived peptides in renal cell carcinoma: a potential target for future therapy. BJU Int 2015; 115: 829-838.
[28] Bervell J. The eGFR equation. Am J Health Promot 2024; 38: 435-436.
[29] Inker LA and Titan S. Measurement and estimation of GFR for use in clinical practice: core curriculum 2021. Am J Kidney Dis 2021; 78: 736-749.
[30] Meeusen JW, Kasozi RN, Larson TS and Lieske JC. Clinical impact of the refit CKD-EPI 2021 creatinine-based eGFR equation. Clin Chem 2022; 68: 534-539.
[31] Chen TK, Knicely DH and Grams ME. Chronic kidney disease diagnosis and management: a review. JAMA 2019; 322: 1294-1304.
[32] Torsetnes SB, Broughton MN, Paus E, Halvorsen TG and Reubsaet L. Determining ProGRP and isoforms in lung and thyroid cancer patient samples: comparing an MS method with a routine clinical immunoassay. Anal Bioanal Chem 2014; 406: 2733-2738.
[33] Banerjee T, Sebastian A and Frassetto L. Association of diet-dependent systemic acid load, renal function, and serum albumin concentration. J Ren Nutr 2023; 33: 428-434.
[34] Toya K, Babazono T, Hanai K and Uchigata Y. Association of serum bilirubin levels with development and progression of albuminuria, and decline in estimated glomerular filtration rate in patients with type 2 diabetes mellitus. J Diabetes Investig 2014; 5: 228-235.
[35] Kawamoto R, Ninomiya D, Hasegawa Y, Kasai Y, Kusunoki T, Ohtsuka N and Kumagi T. Association between serum bilirubin and estimated glomerular filtration rate among elderly persons. PLoS One 2014; 9: e115294.
[36] Zhao P, Xu H, Shi Y, Song X, Qiu G, Ding C, Zhou W, Yu C, Wang T, Zhu L, Bao H and Cheng X. Association between bilirubin and chronic kidney disease in hypertensive patients: the China hypertension registry study. J Clin Hypertens (Greenwich) 2023; 25: 1185-1192.
[37] Tanaka M, Fukui M, Okada H, Senmaru T, Asano M, Akabame S, Yamazaki M, Tomiyasu K, Oda Y, Hasegawa G, Toda H and Nakamura N. Low serum bilirubin concentration is a predictor of chronic kidney disease. Atherosclerosis 2014; 234: 421-425.
[38] Park S, Lee S, Kim Y, Lee Y, Kang MW, Kim K, Kim YC, Han SS, Lee H, Lee JP, Joo KW, Lim CS, Kim YS and Kim DK. Serum bilirubin and kidney function: a Mendelian randomization study. Clin Kidney J 2022; 15: 1755-1762.



Supplementary Figure 1. The levels of serum ProGRP in different groups. Healthy, healthy controls; UTI, urinary tract infection; NS, nephrotic syndrome; CKD, chronic kidney disease; AAV, antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis. *$P < 0.01$, compared to healthy controls. #$P < 0.01$, comparison between two groups.



Supplementary Figure 2. Comparison of ProGRP levels in CKD-stages. *$P < 0.01$, compared to healthy controls. #$P < 0.01$, comparison between two groups.



Supplementary Figure 3. A screenshot of online diagnostic model webpage.