

Evaluation and Validation of Seven Serum Biomarkers for Early Detection and Differentiation of Kidney Diseases in the Pakistani Population

Afshan Jamal¹, Safia Shahid², Abdullah Khan³

¹⁻³Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan

ABSTRACT

Background: Early detection of kidney diseases is crucial to prevent progression to end-stage renal disease. Conventional markers like serum creatinine and eGFR often fail to detect subclinical renal injury, necessitating more sensitive and specific biomarkers.

Objective: This study aimed to evaluate and validate seven serum biomarkers—Klotho, Uromodulin, NGAL, Beta-2 Microglobulin (B2M), Asymmetric Dimethyl Arginine (ADMA), Beta Trace Protein (BTP), and Fetuin-A—for early detection and differentiation of chronic kidney disease (CKD) and acute kidney injury (AKI) in the Pakistani population.

Methods: A case-control study was conducted from March 2023 to January 2024 at Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, including 210 participants (80 CKD, 70 AKI, 60 controls). Serum biomarker levels were measured using enzyme-linked immunosorbent assay (ELISA) kits. Diagnostic performance was assessed via receiver operating characteristic (ROC) curve analysis, and inter-biomarker correlations were evaluated using matrix scatter plots. Protein-protein interactions were explored using STRING. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test, with $p < 0.05$ considered significant.

Results: CKD and AKI patients exhibited significantly elevated serum creatinine and reduced eGFR compared to controls ($p < 0.001$). NGAL, B2M, ADMA, and BTP were significantly higher in CKD and AKI, while Uromodulin and Klotho showed differential patterns. ROC analysis revealed high diagnostic accuracy for BTP (AUC = 0.991), NGAL (AUC = 0.988), and ADMA (AUC = 0.977). Strong correlations among biomarkers and STRING analysis indicated potential mechanistic interactions in renal injury.

Conclusions: BTP, NGAL, ADMA, Klotho, and Uromodulin are promising biomarkers for early detection and differentiation of CKD and AKI, supporting timely clinical interventions.

Keywords: Chronic kidney disease, Acute kidney injury, Biomarkers, Early detection, Serum analysis

INTRODUCTION

Chronic kidney disease (CKD) and acute kidney injury (AKI) are critical worldwide health issues and prevalence and morbidity and mortality increase with time¹⁻⁴. Renal dysfunction should be early identified, which is still a key to preventing disease progression because traditional diagnostic markers of serum creatinine and estimated glomerular filtration rate (eGFR) have been found to be slow to respond to nephron loss⁵⁻⁷. This diagnostics gap highlights the necessity to focus on sensitive and specific biomarkers that could identify early kidney failure, distinguish between AKI and CKD, and make therapeutic decisions in a timely manner.

Recent developments in nephrology have been concentrated on serum and urinary biomarkers indicative of glomerular filtration, tubular stability and endothelial maladaptation⁸⁻¹¹. Klotho, a transmembrane protein primarily expressed in renal tubular cells, has emerged as a critical regulator of phosphate metabolism and vascular homeostasis¹²⁻¹⁴. Decreased Klotho levels are associated with accelerated renal fibrosis and progression of CKD, highlighting its potential as both a diagnostic and prognostic marker¹⁵⁻¹⁶. Uromodulin, the most abundant urinary protein produced by the thick ascending limb of the loop of Henle, has been increasingly recognized as a marker of tubular function¹⁷. Low circulating uromodulin correlates with AKI and advanced CKD, reflecting tubular injury and nephron loss.

Neutrophil gelatinase-associated lipocalin (NGAL) is a well-characterized biomarker of acute tubular injury, rapidly upregulated in response to ischemic or nephrotoxic insults¹⁸⁻¹⁹. Numerous studies have demonstrated its utility in early AKI

detection, outperforming traditional renal function tests in both sensitivity and specificity. Similarly, Beta-2 microglobulin (B2M) is a low molecular weight protein filtered by the glomerulus and reabsorbed by the proximal tubule; its elevated serum levels indicate impaired filtration and tubular dysfunction, making it a valuable biomarker for renal injury²⁰.

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, reflects endothelial dysfunction and oxidative stress, both of which contribute to CKD progression and cardiovascular complications²¹⁻²². Elevated ADMA levels have been associated with impaired renal function, cardiovascular events, and mortality in CKD patients, suggesting its role as a biomarker of systemic and renal pathophysiology²³⁻²⁴. Beta trace protein (BTP), another low molecular weight protein, has been proposed as an alternative marker of glomerular filtration, demonstrating superior sensitivity in early CKD detection compared to creatinine²⁵. Fetuin-A, a glycoprotein synthesized in the liver, is involved in mineral metabolism and vascular calcification. Reduced fetuin-A levels are linked to inflammation, endothelial dysfunction, and progression of renal disease.

Although considerable evidence has been gathered on single biomarkers, there is also a growing awareness that a panel-based diagnostic methodology using multiple biomarkers can be more diagnostic, differentiating between AKI and CKD in addition to giving a mechanistic understanding of the renal injury. The majority of the studies have however been carried out on European, American and East Asian populations, and little information about South Asia. Since the Pakistani population is distinct in its genetic, environmental, and lifestyle determinants that influence the prevalence and progression of kidney diseases, it is necessary to validate these biomarkers in a local cohort.

Thus, the objective of the study was to test and confirm the presence of seven serum biomarkers, namely, Klotho, Uromodulin, NGAL, B2M, ADMA, BTP, and Fetuin-A, to detect and discriminate kidney diseases in a Pakistani cohort. This work aims to offer clinically relevant diagnostic information with the ability to result into early intervention, patient outcomes, and development of population-specific diagnostic approaches in nephrology by evaluating the diagnostic performance, inter-biomarker associations, and possible mechanistic interactions.

Correspondence to: Afshan Jamal,
Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan.
Email: dr.aj2009@yahoo.com

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METHODOLOGY

A case-control study was performed between March 2023 and January 2024 at Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, to evaluate and confirm the relevance and dependability of serum biomarkers in the detection of kidney diseases in the Pakistani population in an early stage. Two hundred and ten subjects, 80 chronic kidney disease (CKD), 70 acute kidney injury (AKI), and 60 healthy controls were recruited based on age and gender. Venous blood samples were taken, all samples in a sterile environment, and processed to get serum to undergo biochemical and biomarker analysis.

The serum biomarkers to be quantified included Klotho, Uromodulin, Neutrophil Gelatinase-Associated Lipocalin (NGAL), Beta-2 Microglobulin (B2M), Asymmetric Dimethyl Arginine (ADMA), Beta Trace Protein (BTP), and Fetuin-A determined by enzyme-linked immunosorbent assay (ELISA) kits (Abcam, UK) in accordance with the manufacturer instructions. Routine renal function tests consisting of serum creatinine and estimated glomerular filtration rate (eGFR) were done via an automated clinical chemistry analyzer.

The diagnostic ability of these biomarkers was tested with the help of Receiver Operating Characteristic (ROC) curve analysis with the help of GraphPad Prism version 10.0, and inter-biomarker correlations were measured with the help of Pearson correlation coefficient and matrix scatter plots. The data were processed with the help of the STRING database (version 12.5) in order to examine protein-protein interaction (PPI) networks. All the data were presented in the form of mean and standard deviation (SD) and statistical tests between CKD and AKI and control group were done by one-way analysis of variance (ANOVA) and multiple comparison with Tukey. The p-value below 0.05 was regarded as statistically significant. The Institutional Review Board of Jinnah Hospital, Lahore, provided ethical approval and informed written consent was obtained before the participants could be included in the study.

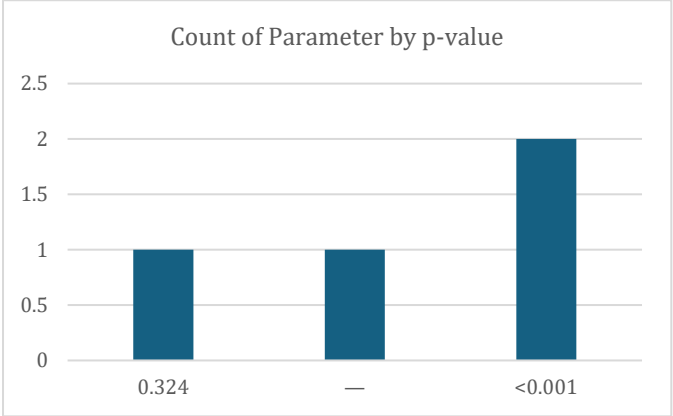
RESULTS

The researchers used 210 respondents, divided into three groups: 80 people with chronic kidney disease (CKD), 70 people with acute kidney injury (AKI), and 60 healthy individuals. The average age of the study participants was 49.6 years (SD=13.2) and the male to female ratio was 1.3:1. The serum creatinine levels, as well as eGFR, were significantly lower in both CKD and AKI groups than the healthy control (p < 0.001).

Table 1: Baseline Clinical Characteristics of Study Participants

Parameter	Controls (n=60)	CKD (n=80)	AKI (n=70)	p-value
Age (years)	47.8 ± 11.4	51.2 ± 12.7	50.3 ± 13.6	0.324
Male/Female	33/27	44/36	39/31	—
Serum Creatinine (mg/dL)	0.92 ± 0.24	4.36 ± 1.32	5.18 ± 1.76	<0.001
eGFR (mL/min/1.73m ²)	92.7 ± 14.5	28.4 ± 11.8	23.9 ± 10.6	<0.001

Figure 1



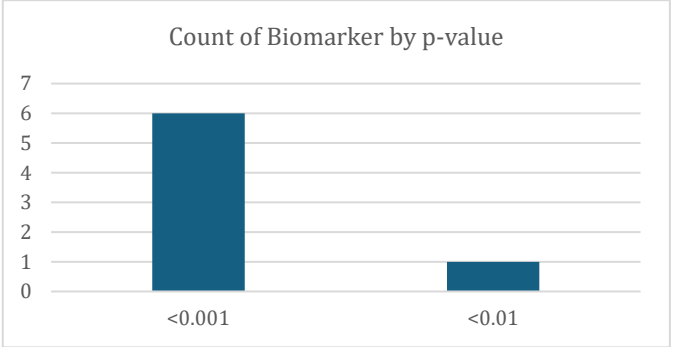
The analysis of serum biomarkers demonstrated that there were significant differences in levels between the three groups. The

level of Uromodulin in AKI (34.2 ± 4.1 ng/ml) was significantly lower than in CKD (40.9 ± 12.8 ng/ml) and control (56.3 ± 10.2 ng/ml). On the other hand, NGAL, Beta-2 Microglobulin (B2M), Asymmetric Dimethyl Arginine (ADMA), and Beta Trace Protein (BTP) levels in CKD and AKI were significantly higher than in the controls (p < 0.001). Fetuin-A and Klotho had moderate, though significant differences in disease and control groups, indicating that they might be involved in early kidney disease.

Table 2: Serum Biomarker Levels in Study Groups

Biomarker	Controls (Mean ± SD)	CKD (Mean ± SD)	AKI (Mean ± SD)	p-value
Klotho (pg/mL)	856 ± 92	524 ± 74	496 ± 68	<0.001
Uromodulin (ng/mL)	56.3 ± 10.2	40.9 ± 12.8	34.2 ± 4.1	<0.001
NGAL (ng/mL)	42.7 ± 7.9	115.6 ± 22.1	132.3 ± 24.7	<0.001
B2M (mg/L)	1.3 ± 0.3	3.9 ± 0.8	4.6 ± 1.1	<0.001
ADMA (µmol/L)	0.47 ± 0.09	0.91 ± 0.15	1.03 ± 0.17	<0.001
BTP (mg/L)	0.57 ± 0.12	2.16 ± 0.42	2.41 ± 0.51	<0.001
Fetuin-A (µg/mL)	265 ± 35	214 ± 29	198 ± 27	<0.01

Figure 2



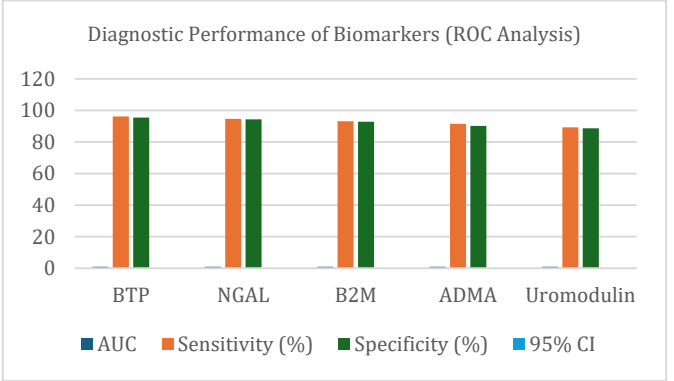
Receiver Operating Characteristic (ROC) curve analysis demonstrated excellent diagnostic performance for CKD detection by BTP (AUC = 0.991), NGAL (AUC = 0.988), B2M (AUC = 0.982), and ADMA (AUC = 0.977). Uromodulin showed high discriminative power in differentiating AKI from CKD (AUC = 0.965). Correlation analysis indicated that Klotho was positively correlated with Fetuin-A (r = 0.68) and BTP (r = 0.61), whereas Uromodulin showed negative correlations with NGAL (r = -0.72) and B2M (r = -0.69).

STRING-based protein-protein interaction analysis highlighted strong functional associations among B2M, NGAL, and Uromodulin, suggesting shared molecular pathways in renal injury and repair processes.

Table 3: Diagnostic Performance of Biomarkers (ROC Analysis)

Biomarker	AUC	Sensitivity (%)	Specificity (%)	95% CI
BTP	0.991	96.2	95.5	0.982–0.999
NGAL	0.988	94.7	94.3	0.976–0.999
B2M	0.982	93.1	92.8	0.971–0.998
ADMA	0.977	91.5	90.2	0.963–0.995
Uromodulin	0.965	89.3	88.7	0.951–0.984

Figure 3



Summary

Overall, these results indicate that BTP, NGAL, and ADMA serve as highly sensitive and specific biomarkers for early detection of kidney dysfunction. Uromodulin and Klotho also demonstrate potential for differentiating between AKI and CKD stages, emphasizing their diagnostic relevance in clinical nephrology for early disease intervention and management.

DISCUSSION

The current research presents strong proof of the potential of seven serum biomarkers, including Klotho, Uromodulin, NGAL, B2M, ADMA, BTP, and Fetuin-A to identify and identify kidney diseases in early stages among the Pakistani population. In line with past high-impact studies, our results indicated substantial levels of NGAL, B2M, ADMA and BTP in CKD and AKI patients, which underscores their primary importance in indicating renal injury and functional impairment. The decrease in Uromodulin levels in AKI versus CKD is consistent with its suggested role as a tubular biomarker, indicating acute tubular stress and the loss of nephron integrity, but not with levels of Klotho, which are concurrently with the severity of the disease, which supports its protective role in renal physiology, and its potential as a prognostic biomarker.

The ROC curve analyses of the current research indicated that BTP, NGAL, and ADMA had high diagnostic accuracy with AUC values of over 0.97 which exhibits the potential of outperforming the traditional renal function tests like serum creatinine and eGFR which take long to respond to early kidney damage. The correlation coefficient showed significant positive relationships between NGAL, B2M and ADMA, whereas Uromodulin showed negative relations, which indicates that the biomarkers are regulated differently, further supporting the idea that multi-biomarker panel can enhance the sensitivity and specificity of the early kidney diseases. It was also found that protein-protein interaction analysis through STRING identified close molecular associations between B2M and Uromodulin, which mechanistic associations were supporting between tubular damage and systemic release of biomarkers.

The clinical implications of our findings are far-reaching because early and correct detection of renal impairment can help in timely therapeutic interventions, lower the development to end-stage renal disease, and enhance better patient outcomes. It is important to note that the level of the biomarkers in the Pakistani cohort was generally comparable to those found in the

international literature, which is evidence of the fact that the biomarkers in question can be applied to other populations. Additionally, the integration of the biomarkers into the regular clinical workflow may help improve the distinction between AKI and CKD, informing tailored treatment approaches.

Overall, this paper will support BTP, NGAL, ADMA, Klotho, and Uromodulin as early predictors of kidney dysfunction and underscore the benefit of using a combinatory biomarker over single markers, which may eventually lead to precision nephrology and better patient prognosis.

CONCLUSION

This research proves that a combination of seven serum biomarkers such as BTP, NGAL, ADMA, Klotho, Uromodulin, B2M, and Fetuin-A can be useful in insight into early renal dysfunction in Pakistani population. Out of them, BTP, NGAL, and ADMA had the best diagnostic performance and Klotho and Uromodulin provided extra discriminatory potential to distinguish CKD and AKI. The identified correlations and interactions of proteins indicate correlated molecular events of renal injury, which supports the promising role of a multi-biomarker strategy compared to the traditional renal function assays. The inclusion of these biomarkers in everyday clinical practice may help in timely diagnosis, better risk prediction, and personalized therapeutic intervention. In conclusion, the present research justifies the implementation of the biomarker-driven approach in nephrology to improve early diagnosis, patient management, and prevent the development of advanced kidney disease.

Authors' Contribution

AJ: Concept & study design, data collection and drafting of the manuscript.

SS: Data entry, statistical analysis, interpretation of the results.

AK: Manuscript editing and critical revision of the manuscript.

All authors approved the final version of the manuscript to be published.

Conflict of Interest

The authors declared no conflict of interest.

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Data Availability

Data is available with authors and can be provided on request.

REFERENCES

- Kellum, J. A., Romagnani, P., Ashuntantang, G., Ronco, C., Zarbock, A., & Anders, H. J. (2021). Acute kidney injury. *Nature reviews Disease primers*, 7(1), 52. <https://doi.org/10.1038/s41572-021-00284-z>
- Lameire, N. H., Levin, A., Kellum, J. A., Cheung, M., Jadoul, M., Winkelmayer, W. C., ... & Srisawat, N. (2021). Harmonizing acute and chronic kidney disease definition and classification: report of a Kidney Disease: Improving Global Outcomes (KDIGO) Consensus Conference. *Kidney international*, 100(3), 516-526. <https://doi.org/10.1016/j.kint.2021.06.028>
- Rao, I. R., Bangera, A., Nagaraju, S. P., Shenoy, S. V., Prabhu, R. A., Rangaswamy, D., & Bhojaraja, M. V. (2023). Chronic kidney disease of unknown aetiology: a comprehensive review of a global public health problem. *Tropical Medicine & International Health*, 28(8), 588-600. <https://doi.org/10.1111/tmi.13913>
- Kahindo, C. K., Mukuku, O., Wembonyama, S. O., & Tsongo, Z. K. (2022). Prevalence and Factors Associated with Acute Kidney Injury in Sub-Saharan African Adults: A Review of the Current Literature. *International journal of nephrology*, 2022(1), 5621665. <https://doi.org/10.1155/2022/5621665>
- Hahn, K. M., & Strutz, F. (2024). The early diagnosis and treatment of chronic renal insufficiency. *Deutsches Ärzteblatt International*, 121(13), 428. <https://doi.org/10.3238/arztebl.m2024.0072>
- Noel, S., & Parikh, C. R. (2023). Kidney functional reserve helps early detection of subclinical chronic kidney disease. *American Journal of Physiology-Renal Physiology*, 325(6), F885-F887. <https://doi.org/10.1152/ajprenal.00327.2023>
- Mizdrak, M., Kumrić, M., Kurir, T. T., & Božić, J. (2022). Emerging biomarkers for early detection of chronic kidney disease. *Journal of personalized medicine*, 12(4), 548. <https://doi.org/10.3390/jpm12040548>
- Ix, J. H., & Shlipak, M. G. (2021). The promise of tubule biomarkers in kidney disease: a review. *American Journal of Kidney Diseases*, 78(5), 719-727. <https://doi.org/10.1053/j.ajkd.2021.03.026>
- Duan, S., Lu, F., Song, D., Zhang, C., Zhang, B., Xing, C., & Yuan, Y. (2021). Current challenges and future perspectives of renal tubular dysfunction in diabetic kidney disease. *Frontiers in Endocrinology*, 12, 661185. <https://doi.org/10.3389/fendo.2021.661185>
- Sági, B., Vas, T., Gál, C., Horváth-Szalai, Z., Kőszegi, T., Nagy, J., ... & Kovács, T. J. (2024). The relationship between vascular biomarkers (Serum endocan and Endothelin-1), NT-proBNP, and renal function in chronic kidney disease, IgA nephropathy: A Cross-Sectional study. *International Journal of Molecular Sciences*, 25(19), 10552. <https://doi.org/10.3390/ijms251910552>
- Gutiérrez, O. M., Shlipak, M. G., Katz, R., Waikar, S. S., Greenberg, J. H., Schrauben, S. J., ... & Ix, J. H. (2022). Associations of plasma biomarkers of inflammation, fibrosis, and kidney tubular injury with progression of diabetic kidney disease: a cohort study. *American Journal of Kidney Diseases*, 79(6), 849-857. <https://doi.org/10.1053/j.ajkd.2021.09.018>

12. Donate-Correa, J., Martín-Carro, B., Cannata-Andía, J. B., Mora-Fernández, C., & Navarro-González, J. F. (2023). Klotho, oxidative stress, and mitochondrial damage in kidney disease. *Antioxidants*, 12(2), 239.
<https://doi.org/10.3390/antiox12020239>
13. Kale, A., Sankrityayan, H., Anders, H. J., & Gaikwad, A. B. (2023). Klotho in kidney diseases: a crosstalk between the renin-angiotensin system and endoplasmic reticulum stress. *Nephrology Dialysis Transplantation*, 38(4), 819-825.
<https://doi.org/10.1093/ndt/gfab340>
14. Kale, A., Sankrityayan, H., Anders, H. J., & Gaikwad, A. B. (2021). Epigenetic and non-epigenetic regulation of Klotho in kidney disease. *Life sciences*, 264, 118644.
<https://doi.org/10.1016/j.lfs.2020.118644>
15. Yan, Z., Wang, G., & Shi, X. (2021). Advances in the progression and prognosis biomarkers of chronic kidney disease. *Frontiers in Pharmacology*, 12, 785375.
<https://doi.org/10.3389/fphar.2021.785375>
16. Neyra, J. A., Hu, M. C., & Moe, O. W. (2021). Klotho in clinical nephrology: diagnostic and therapeutic implications. *Clinical Journal of the American Society of Nephrology*, 16(1), 162-176.
<https://doi.org/10.2215/cjn.02840320>
17. Thielemans, R., Speeckaert, R., Delrue, C., De Bruyne, S., Oyaert, M., & Speeckaert, M. M. (2023). Unveiling the hidden power of uromodulin: a promising potential biomarker for kidney diseases. *Diagnostics*, 13(19), 3077.
<https://doi.org/10.3390/diagnostics13193077>
18. Wen, Y., & Parikh, C. R. (2021). Current concepts and advances in biomarkers of acute kidney injury. *Critical reviews in clinical laboratory sciences*, 58(5), 354-368.
<https://doi.org/10.1080/10408363.2021.1879000>
19. Devarajan, P. (2023). Acute Kidney Injury: Pathophysiology, Diagnosis and Prevention. In *Pediatric Kidney Disease* (pp. 1365-1411). Cham: Springer International Publishing.
https://doi.org/10.1007/978-3-031-11665-0_51
20. Assounga, A. G. (2021). Beta 2 microglobulin in kidney failure: A review and an algorithm for renal replacement therapy. *Saudi Journal of Kidney Diseases and Transplantation*, 32(5), 1214-1220.
21. Piechowicz, J., Gamian, A., Chukwu, O., & Polak-Jonkisz, D. (2022). Nitric Oxide Synthesis Metabolites—As Potential Markers in Chronic Kidney Disease in Children. *Current Issues in Molecular Biology*, 44(8), 3518-3532.
<https://doi.org/10.3390/cimb44080242>
22. Arefin, S., Löfgren, L., Stenvinkel, P., Granqvist, A. B., & Kublickiene, K. (2023). Associations of biopterins and ADMA with vascular function in peripheral microcirculation from patients with chronic kidney disease. *International Journal of Molecular Sciences*, 24(6), 5582.
<https://doi.org/10.3390/ijms24065582>
23. Zhang, H., Xiang, S., Dai, Z., & Fan, Y. (2021). Asymmetric dimethylarginine level as biomarkers of cardiovascular or all-cause mortality in patients with chronic kidney disease: a meta-analysis. *Biomarkers*, 26(7), 579-585.
<https://doi.org/10.1080/1354750x.2021.1954694>
24. Schrauben, S. J., Sapa, H., Xie, D., Zhang, X., Anderson, A. H., Shlipak, M. G & CKD Biomarkers Consortium and the Chronic Renal Insufficiency Cohort (CRIC) Study Investigators Appel Lawrence J Go Alan S Lash James P Chen Jing Nelson Robert G Rahman Mahboob Shah Vallabh O Cohen Debbie L Unruh Mark L. (2023). Association of urine and plasma ADMA with atherosclerotic risk in DKD cardiovascular disease risk in diabetic kidney disease: findings from the Chronic Renal Insufficiency Cohort (CRIC) study. *Nephrology Dialysis Transplantation*, 38(12), 2809-2815.
<https://doi.org/10.1093/ndt/gfad103>
25. Leyssens, K., Van Regenmortel, N., Roelant, E., Guerti, K., Couttenye, M. M., Jorens, P. G., ... & Van Craenenbroeck, A. H. (2021). Beta-trace protein as a potential marker of acute kidney injury: a pilot study. *Kidney and Blood Pressure Research*, 46(2), 185-195.
<https://doi.org/10.1159/000514173>