Urinary TIMP-2 and IGFBP-7 protein levels as early predictors of acute kidney injury after cardiac surgery

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Abstract

Background: Acute kidney injury (AKI) is a frequent complication associated with on-pump cardiac surgery. Early recognition may alter their prognosis. Therefore, the urinary concentrations of TIMP-2 (tissue inhibitor of metalloproteinases-2) and IGFBP7 (insulin-like growth factor-binding protein) as predictors for AKI were studied.

Methods: Repetitive blood and urine samples were collected consecutively from 50 patients. Demographic, intra-, and postoperative data were recorded prospectively. To calculate the production of the TIMP-2 and IGFBP-7 protein concentrations, urinary samples were taken preoperatively, intraoperatively at 30 and 60 min after aortic clamping and at 0, 6, 12, and 24 h after admission to the intensive care unit (ICU).

Results: AKI occurred in 14 patients (28%), all of them at Kidney Disease: Improving Global Outcomes stage 1. Predictive value for [TIMP-2] × [IGFBP7] was shown at 0 and 24 h after admission to ICU. At 0 h, the sensitivity was 84.6% and the specificity 55.6% for an ideal calculated cutoff at 0.07. After 24 h, the ideal cutoff amounted to 0.35 with a sensitivity of 53.8% and a specificity of 88.2%. The receiver operating characteristic curves demonstrated areas under the curve of 0.725 and 0.718. The suggested cutoffs of 0.3 and 2.0 could not be confirmed. The serum creatinine was reached to the peak median within 48 h after admission to ICU.

Conclusion: Postoperative risk assessment for the development of AKI can be established by [TIMP-2] × [IGFBP7]. Previously suggested cutoff values could not be confirmed. A correlation with urinary dilution parameters may enable the identification of more universal cutoffs.

KEYWORDS
acute kidney injury, cardiac surgery, early predictors, IGFBP-7, TIMP-2

Abbreviations: AKI, acute kidney injury; AUC, area under the curve; BMI, body mass index; CHD, coronary heart disease; CKD, chronic kidney disease; CPB, cardiopulmonary bypass; ICU, intensive care unit; IGFBP7, insulin-like growth factor binding protein; KDIGO, Kidney Disease: Improving Global Outcomes; LV-EF, left-ventricular ejection fraction; PVD, peripheral vascular disease; ROC, receiver operating characteristic; TIMP-2, tissue inhibitor of metalloproteinases-2.

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1 | INTRODUCTION

Acute kidney injury (AKI) is one of the most frequent complications following open cardiac surgery involving cardiopulmonary bypass (CPB). Based on current AKI definitions, the incidence is about 30% where 2.2% of these require dialysis postoperatively. Even a slight postoperative rise in serum-creatinine is an independent predictor of mortality and it increases with the increase in stage of AKI. From an economic point of view, AKI is associated with a prolonged stay in the hospital that results in increased costs. The pathogenesis of AKI is complex and multifactorial, where some include toxic, ischemic, oxidative, and inflammatory causes. Because serum-creatinine is a limited biomarker in the perioperative setting, so the use of urinary proteins like TIMP-2 (tissue inhibitor of metalloproteinase) and IGFBP7 (insulin-like growth factor-binding protein) has been promoted. Both are highly involved in the G1 cell cycle which is a known mechanism in AKI. The Sapphire study identified these two proteins and their products to be superior to the variety of biomarkers to predict AKI. The Nephrocheck™ test, which calculates the product of the TIMP-2 and IGFBP7 concentrations, was also developed.

In this pilot study, we investigated the function of the Nephrocheck™ test under clinical conditions. Additionally, we tested the assumption of urinary TIMP-2 and IGFBP7 can predict AKI in adult cardiac surgery patients at an earlier stage in comparison to the conventionally used biomarkers such as creatinine, urea, and glomerular filtration rate.

2 | MATERIALS AND METHODS

2.1 | Study design

The ethics committee of the Philipps-University Marburg approved this observational pilot study (Az 178/13). The Standards for Reporting of Diagnostic accuracy (STARD) were applied. Fifty male patients requiring elective coronary artery bypass grafting and/or valve surgery with the use of CPB were screened for study admission. Exclusion criteria included those patients with a Cleveland Score of more than 7, younger than 35 years, acute infection, emergency surgery, and end-stage renal disease. Written informed consents were received from all patients for enrollment.

Urinary samples were taken just after anesthetic induction, intraoperatively 30 and 60 min after clamping of the aortic arch and 0, 6, 12, and 24 h after admission to the intensive care unit (ICU). The product of urinary TIMP-2 and IGFBP7 concentrations was measured with the Nephrocheck™ Test (Astute Medical). [TIMP-2] × [IGFBP7] was correlated with urinary dilution parameters as the urinary osmolality. Results did not add to the clinical decision process.

Preoperative descriptive patient characteristics as well as intraoperative risk factors for AKI were recorded. Postoperative complications and course were registered. AKI risk stratification was obtained using the Cleveland and Leicester scores.

2.2 | Study endpoints

Any stage of AKI defined by the Kidney Disease: Improving Global Outcomes (KDIGO) classification was the primary endpoint—a serum creatinine rise by 0.3 mg/dl within 48 h, 1.5-fold within 7 days or a diuresis below 5 ml/kg/h for a minimum of 6 h. Occurrence of AKI was only administered as a study endpoint if happened within 72 h after ICU admission, as this is the suggested time frame for both cardiac surgery induced AKI and exposure relevant biomarker measurements.

2.3 | TIMP-2 and IGFBP7 measurements

The NephroCheck™ Test (Astute Medical) is a fluorescence-immunoassay that quantifies TIMP-2 and IGFBP7 concentrations by converting fluorescent signals with an Astute 140 meter® (Astute Medical). It is a point-of-care test that calculates the product of the concentrations of both urinary proteins as [TIMP-2] × [IGFBP7] resulting in an AKI risk score.

2.4 | Statistical analysis

The statistical analysis was performed and graphics were created using the Microsoft® Excel® add-in XLSTAT (2016.7) for Mac (macOS Sierra 10.12.2) and Windows 10 (2016, 15.28).

Normality was examined with Shapiro–Wilk tests. To describe the study population, absolute and relative frequencies were used for categorical variables, mean values, and standard deviations for metric ones. Comparisons of the subgroups with and without AKI were performed with two-sided Mann–Whitney U-tests due to a lack of normality. NephroCheck™ test results were also set in ratio with urinary osmolality, urea, and uric acid concentrations to perform Mann–Whitney U-tests. Fisher’s exact tests were used for categorical variables. Significance was assumed for p-values < .05.

Previously suggested cutoff values for [TIMP-2] × [IGFBP7] of 0.3 and 2.0 as well as the ideal calculated cutoffs were described by sensitivity, specificity, negative and positive predictive values. The receiver operating characteristic curve (ROC) to analyze the predictive values were calculated and specified by the area under the curve (AUC).

3 | RESULTS

3.1 | Descriptive statistic and risk profile for AKI

For this study, 50 male patients were approached for participation and none were excluded from the analysis. Among these, 36% of patients received isolated coronary artery bypass grafting, 20% isolated valve surgery, and 44% combined coronary and valvular surgical interventions. Table 1 summarizes the descriptive statistic for the
entire patient collective and for the subgroups with and without AKI. Besides, it shows $p$-values for subgroup differences. Patients with postoperative AKI had a worse median baseline creatinine of 1.11 mg/dl compared to those without AKI with a baseline of 0.89 mg/dl. Most patients showed arterial hypertension, coronary heart disease, or heart failure as a comorbidity. The prevalence of patients with chronic kidney disease or severely reduced left ventricular function was 6%. Both the Cleveland- and Leicester-score showed a higher risk for AKI for the subgroup with postoperative AKI. The median Leicester-score for the patients with AKI was 29% compared to 18% for those without. For the entire patient collective, the Leicester-score anticipated an AKI incidence of 21%. The actual rate of AKI was 28%. Postoperatively the length of stay in ICU was a median of 6 days for those with AKI compared to 4 for those without postoperative AKI. No significant subgroup differences could be shown for age, CPB, or cross-clamp time. No patient in this study suffered an in-hospital death or required dialysis.

Table 2 shows the distribution of the Cleveland-score for the entire study population and for the subgroups in absolute and relative frequencies. In the group without AKI, 50% of the patients had a Cleveland-score of zero opposed to only 21.4% in the group with AKI. A patient without AKI and a Cleveland-score of 7 due to chronic kidney injury, congestive heart failure, insulin-dependent diabetes, and a combined surgical intervention stand out.

### Table 1 Baseline characteristics for the entire patient collective and for the subgroups with and without acute kidney injury

|                | Total (n = 50) | AKI (n = 14) | No AKI (n = 36) | p   |
|----------------|---------------|-------------|----------------|-----|
| Hypertension   | 42 (84)       | 12 (86)     | 30 (83)        | 1   |
| CHD            | 44 (88)       | 13 (93)     | 31 (86)        | .663|
| Heart failure  | 47 (94)       | 14 (100)    | 33 (92)        | .55 |
| LV-EF < 30%    | 3 (6)         | 2 (14)      | 1 (3)          | .186|
| PVD            | 8 (16)        | 2 (14)      | 6 (17)         | 1   |
| Diabetes       | 17 (34)       | 5 (36)      | 12 (33)        | 1   |
| COPD           | 3 (6)         | 2 (14)      | 1 (3)          | .186|
| CKD            | 8 (16)        | 3 (21)      | 5 (14)         | .67 |
| Age            | 68.5 (58–74)  | 71.5 (66–75)| 64.5 (57.75–74)| .082|
| Baseline Creatinine, mg/dl | 0.97 (0.83–1.14) | 1.11 (0.98–1.275) | 0.89 (0.80–1.05) | .016|
| BMI, kg/m²     | 28.5 ± 3.6    | 28.5 ± 3.3  | 28.5 ± 3.7     | .934|
| Cleveland-Score| 1 (0–1)       | 1.5 (1–2.75)| 0.5 (0–1.25)   | .049|
| Leicester-Score| 0.21 (0.13–0.30)| 0.29 (0.19–0.36)| 0.18 (0.10–0.24)| .025|
| CPB-time, min  | 97.5 (77–134) | 100.5 (85–122)| 95.5 (77–134) | .666|
| X-clamp, min   | 53 (44–66)    | 55 (43–66)  | 53 (44–67)     | .921|
| Time in ICU, days | 4 (3–6)    | 6 (5.25–12.5)| 4 (3–5)        | .005|
| Dialysis       | 0 (0)         | 0 (0)       | 0 (0)          | 1   |
| Mortality      | 0 (0)         | 0 (0)       | 0 (0)          | 1   |

Note: The $p$-value is shown for subgroup differences.

Abbreviations: BMI, body mass index; CHD, coronary heart disease; CKD, chronic kidney disease; LV-EF, left-ventricular ejection fraction; PVD, peripheral vascular disease.

### Table 2 Distribution of the Cleveland-scores for the total study population and the subgroups

| Cleveland-score | Total Absolute (relative) | AKI Absolute (relative) | No AKI Absolute (relative) |
|-----------------|---------------------------|-------------------------|-----------------------------|
| 0               | 21 (42)                   | 3 (21.4)                | 18 (50)                     |
| 1               | 13 (26)                   | 4 (29)                  | 9 (25)                      |
| 2               | 8 (16)                    | 3 (21.4)                | 5 (13.9)                    |
| 3               | 5 (10)                    | 3 (21.4)                | 2 (5.6)                     |
| 4               | 2 (4)                     | 1 (7.1)                 | 1 (2.8)                     |
| 5               | 0                         | 0                       | 0                           |
| 6               | 0                         | 0                       | 0                           |
| 7               | 1 (2)                     | 0                       | 1 (2.8)                     |
3.2 | Analysis of [TIMP-2] × [IGFBP7] and its ratio

Both pre- and intraoperative measurements of [TIMP-2] × [IGFBP7] and its dilution quotients showed no significant subgroup differences.

Figure 1 shows boxplots of the postoperative [TIMP-2] × [IGFBP7] values and the p-values for the respective time points. Significant differences could be shown at the time of admission to ICU and 24 h thereafter with p-values of .017 and .022. The corresponding ROC curves in Figures 2 and 3 show an AUC of 0.725 and 0.718. The ideal calculated cutoff values for [TIMP-2] × [IGFBP7] were calculated at 0.07 and 0.35 and compared to the previously suggested cutoffs of

| TABLE 3 | Analysis of measured collective's ideal cutoff and previously published cutoffs for [TIMP-2] × [IGFBP7] to predict the likelihood of acute kidney injury |
|---------|----------------------------------|
| AUC     | Cutoff | Sensitivity | Specificity |
| 0 h     | 0.725  | 0.07        | 0.846       | 0.556       |
|         | 0.3    | 0.308       | 0.917       |
|         | 2.0    | 0.077       | 1.000       |
| 24 h    | 0.718  | 0.35        | 0.538       | 0.882       |
|         | 0.3    | 0.538       | 0.853       |
|         | 2.0    | 0           | 0.971       |

Note: Previous cutoffs cited from Bihorac et al.13
At admission to ICU, the sensitivity of the predefined cutoff 0.3 was at 30.8% compared to 84.6% of the ideal calculated 0.07 cut-off. Similar results were shown in the boxplots for the dilution-adjusted \([\text{TIMP-2}] \times [\text{IGFBP7}]\) in Table 4 and Figure 4. For the significant subgroup differences of \([\text{TIMP-2}] \times [\text{IGFBP7}]\) at admission to ICU and 24 h afterward ROC curves are illustrated in Figures 5 and 6 with AUCs of 0.739 and 0.767. The ideal calculated cutoff values for \([\text{TIMP-2}] \times [\text{IGFBP7}]\) showed sensitivities just above 80% and specificities around 70%.

### 3.3 | Analysis of urinary dilution

The median urine osmolality for the entire population was 374 osmol/kg at admission to ICU compared to 572 osmol/kg 24 h later. This difference was significant with a \(p\)-value below .0001 and is shown in Figure 7. Similar differences could be shown for the subgroups with and without AKI.

### 4 | DISCUSSION

AKI is a common postoperative complication of on-pump cardiac surgery.\(^3\) Even minimal increase in serum-creatinine is associated with worse outcome and an increase in costs.\(^4\) To depict the relevance of small changes in kidney function the KDIGO proposed a low threshold for the definition of AKI. Besides, the American Society of Nephrology stated in 2005 the importance of developing biomarkers for AKI. Kashani et al.\(^5\) suggested \([\text{TIMP-2}] \times [\text{IGFBP7}]\) as a risk-score for AKI of KDIGO-stage two and three in a broad intensive care setting. Previous studies showed the feasibility of the test in a setting of patients after cardiac surgery, especially in populations at intermediate to high risk for AKI.\(^11,12\) Hahn and Zdolsek\(^12\) postulated that there is a need to correlate \([\text{TIMP-2}] \times [\text{IGFBP7}]\) results with urinary dilution parameters. This may be especially true for patients after on-pump cardiac surgery when considering the immense volume imbalances. Previously suggested cut-off values by Bihorac et al.\(^13\) could not be confirmed in the populations after cardiac surgery, possibly due to volume status and urinary dilution.\(^11–13\)

Therefore, we included 50 patients of lower risk for AKI in our study anticipating lower AKI stages than \([\text{TIMP-2}] \times [\text{IGFBP7}]\) has previously been evaluated for. As even low AKI stages correlate with...
with a worse prognosis, testing for AKI should include these patients. Besides we aimed to extend the range of test limitation by correlating its results with parameters for urinary dilution. Women were excluded from this study to create a more homogenous patient collective at the time points 0 and 24 h after admission to ICU (AUC = 0.767). AUC, area under the curve; ICU, intensive care unit; ROC, receiver operating characteristic.

At postoperative admission on ICU TIMP-2 \times [IGFBP7] showed a ROC curve with an AUC of 0.725 and 24 h afterward the AUC amounted to 0.718. Previously suggested cutoff values could not be confirmed at administration to ICU. The correlation of TIMP-2 \times [IGFBP7] with the urine osmolality showed AUCs of 0.739 and 0.767 at admission to ICU and 24 h later, respectively. A significant superiority of TIMP-2 \times [IGFBP7] over TIMP-2 \times [IGFBP7] could not be shown. The discrepancy of the ideal calculated cutoff values between administration to the ICU and 24 h thereafter was lower for TIMP-2 \times [IGFBP7] than for TIMP-2 \times [IGFBP7].

This study showed the feasibility of TIMP-2 \times [IGFBP7] for the prediction of AKI stage 1 at the cost of a reduced predictive power when compared to higher AKI of higher stages.\textsuperscript{7} Intraoperative measurements of TIMP-2 \times [IGFBP7] showed no power to predict AKI. Our study demonstrated the tests validity 4 h earlier than Meersch et al.\textsuperscript{9} potentially enabling an earlier intervention to prevent further kidney injury.

Kashani et al.\textsuperscript{6} suggested cutoff values of 0.3 and 2.0 for the product of TIMP-2 and [IGFBP7] depending on whether prioritizing a good sensitivity or specificity. The ideal cut-offs proposed for patients after cardiac surgery range from 0.15 to 0.89.\textsuperscript{9,11} The range in this study alone goes from 0.07 at admission to ICU to 0.35 measured 24 h later. It seems like cutoff values just after cardiac surgery tend to be lower than those measured later. We showed that the urinary osmolality just after cardiac surgery is lower than 24 h later, hence the urine is more diluted, which may explain the lower concentrations of TIMP-2 and [IGFBP7] just after surgery. Meersch et al.\textsuperscript{9} correlated TIMP-2 \times [IGFBP7] with the urinary creatinine which did not improve the tests predictive power. Hahn and Zdolsek\textsuperscript{12} described TIMP-2 \times [IGFBP7] in a healthy population without AKI. A great variability was shown. Furthermore, they stated that when measuring concentrations it is only logical that they are susceptible to dilution. Possibly the above-mentioned inclusion of parameters for urinary dilution will provide more universal cutoff values that are more independent of the study population in the future. Thus, the impact of possible biases as the heart-lung-machines priming volume and institutionally unique fluid resuscitation protocols on the TIMP-2 \times [IGFBP7] results, may be reduced.

Kashani et al.\textsuperscript{6} suggested a pathophysiological model of AKI which involved TIMP-2 and IGFBP7 in the initiation of a G1-cell-cycle-arrest. For IGFBP7 the sources give no clear statement. For oncology, Benatar et al.\textsuperscript{14} showed an IGFBP7 involvement in stopping the cell cycle when transferring from the G2- to S-phase.

Unfortunately, our study population was small, thus putting our results into perspective. Although TIMP-2 \times [IGFBP7] has been developed and verified for AKI stages 2 and 3, our lower risk population showed that it may also be applied to predict the development of AKI stage 1 with only a minor deterioration of the predictive value. When evaluating this there are a few institutional peculiarities. Priming of the heart-lung machine with mannitol and postoperative treatment of oliguria with torasemide may greatly influence both the urinary dilution and the diuresis as criteria for AKI. Furthermore,
the serum creatinine concentration is a very versatile parameter due to volume redistribution and makes the indiscriminate application of KDIGO-criteria in the peri-cardiac surgery setting complicated.

Recently Meersch et al.\textsuperscript{15} showed that the implementation of KDIGO-criteria for the prevention of AKI in patients with [TIMP-2] × [IGFBP7] values bigger than 0.3 showed a significant reduction of AKI-incidence. Another promising approach may be the early initiation of renal replacement therapy to reduce mortality and the time spent on ICU.\textsuperscript{13,16} Advances in early risk prediction with [TIMP-2] × [IGFBP7] may change the time of therapeutic intervention.

After cardiac surgery, [TIMP-2] × [IGFBP7] can predict AKI stage 1 with a slightly reduced predictive power compared to stage 2/3. The correlation of [TIMP-2] × [IGFBP7] with urinary dilution parameters may help to identify more universal cutoff values which also apply to patients after cardiac surgery.

So, the question remains if a new test promises better data for earlier recognition of AKI. Hereof, in 2014, the US Food and Drug Administration (FDA) approved the point-of-care urinary biomarker assay, NephroCheck\textsuperscript{™} (Astute Medical), for predicting the risk of AKI by quantitative measurement of [TIMP-2] × [IGFBP7] in human urine on a bench/table-top analyzer. Lameire et al.\textsuperscript{17} reported in his review the reference intervals for these biomarkers measured by the NephroCheck\textsuperscript{™} test in apparently healthy adults and those with stable chronic morbid conditions without AKI. But there was no statistical difference between reference intervals for the apparently healthy and stable chronic morbid cohorts.\textsuperscript{17} They suggested that the parameter values obtained in critically ill patients should only be used in conjunction with patient condition and clinical signs/symptoms. The authors pronounce the development of this test to assess the risk of AKI and was not intended as a sole indicator for the diagnosis of AKI. Here in, stable chronic disease patients suffering from active cancer were included. The test population in other studies included 25% and 27% cancer patients, respectively. Biomarker values are significantly affected by comorbidities even in the absence of AKI. These findings challenge the robustness and utility of cell cycle arrest biomarkers for the prediction of AKI in general ICU patients and also suggest that their performance may decrease markedly in general ICU patients with heterogeneous diagnoses, differing in comorbidities and multiple sources of inflammation.\textsuperscript{17} The clinical impact of the test is still unproven. Although the complicated AKI critical illness is highly heterogeneous in severity, etiology and timing the biomarker combination of [TIMP-2] × [IGFBP7] appears as a positive step in search of robust and accurate means of early diagnosis of AKI. In the same year Vijayan et al. on behalf of the American Society of Nephrology Acute Kidney Injury Advisory Group pointed out the optimal role of this biomarker combination in the diagnosis, management, and prognosis of AKI in different clinical settings, it still requires further clarification.\textsuperscript{18} To date, Nephrocheck\textsuperscript{™} is the only commercially available test for [TIMP-2] × [IGFBP7] as one of the most promising candidate biomarkers.\textsuperscript{19} But the current state of clinical implementation is irritating. Especially, in case of identification of early AKI after on-pump cardiopulmonary surgery. Wetz et al.\textsuperscript{20} found [TIMP-2] × [IGFBP7] concentration useful as a diagnostic test to identify patients at increased risk of AKI after heart surgery on the first postoperative day, only. At earlier time points, no significant difference in [TIMP-2] × [IGFBP7] concentration was found between patients classified as KDIGO 0 or KDIGO 1 or 2. In a comment on that article, Edwards\textsuperscript{21} said that previous studies reported that this diagnostic test could predict elevated risk of AKI as early as 4 h after cardiac surgery. In contrast, Wetz et al.\textsuperscript{20} did not limit their cohort to patients at high risk of AKI. Furthermore, they could not confirm previously published cut-off points of the multiplied biomarker concentrations for diagnosis of AKI. They attribute these divergent results to differences in patient cohort composition, surgical setting and methodologies. The question remains how precise is the test to predict AKI after cardiac surgery. There is a trend toward moderate sensitivity and specificity on the first postoperative day and the test was considered most applicable in patients at high risk of AKI, and less precise in those at lower risk.\textsuperscript{21} Hence, in case of emergency room patients, the predictable capacity of AKI is limited. Recently, Nalesso et al.\textsuperscript{22} stressed again the urinary [TIMP-2] × [IGFBP7] becomes an important tool for the early detection of patients at high risk (major surgery, cardiac surgery, sepsis, those with hemodynamic instability) for AKI and requests its integration with the local ICU experience provided as a multidisciplinary management of AKI. In the earlier mentioned article from Wetz et al.\textsuperscript{20} limited action of the test in heart surgery is pronounced again although Nalesso et al.\textsuperscript{22} suggest an evaluation of diagnostic test accuracy by building a multivariate logistic regression model for postoperative AKI prediction. Although the urinary [TIMP-2] × [IGFBP7] is recommended in US guidelines for perioperative care of cardiac surgery patients "laboratory medicine and nephrology expert groups have not officially endorsed its use."\textsuperscript{23} The National Institute for Health and Care Excellence (NICE) in the UK and the American Association for Clinical Chemistry’s AACC Academy in the US, both recently reviewed the evidence and do not recommend urinary [TIMP-2] × [IGFBP7] for clinical use.\textsuperscript{23} Its performance has varied widely in several studies depending on the cutoff used, the population tested and the testing time. In patients with low prevalence of AKI the number of falsely test positive appears the pivotal point. Even our study cannot confirm the given cutoff values. An improvement of the assay could result from a better risk- stratification in the patients’ group but in this concern experiences are missing. Normalizing the data to urine osmolality has potential to improve. The ideal calculated cut-off values for [TIMP-2] × [IGFBP7] in our study showed sensitives just above 80% and specificities around 70%. It sounds encouraging but leaves the question for better reference intervals still open. Indeed, El-Khoury\textsuperscript{24} concludes the present status in the best way stating "urinary [TIMP-2] × [IGFBP7] is not ready for clinical implementation due to its poor specificity." The parameter appears helpful when "implemented as part of care bundles." But many questions have to be addressed. For instance, "there are no reported biological variability studies to help us understand how urinary [TIMP-2] × [IGFBP7] changes throughout the day in healthy individuals act as an essential step to determine the change in reference values."
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