

Functional–Structural Heterogeneity in AKI Recovery Across Renal Perfusion Phenotypes in Critical Illness

Hongxiang Li

The First Hospital of Jilin University

Yao Fu

The First Hospital of Jilin University

Yuting Li

The First Hospital of Jilin University

Youquan Wang

The First Hospital of Jilin University

Dong Zhang

zhangdong@jlu.edu.cn

The First Hospital of Jilin University

Research Article

Keywords: acute kidney injury, renal perfusion, cystatin C, neutrophil gelatinase-associated lipocalin, renal recovery, critical illness, phenotyping

Posted Date: October 13th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11080075/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

1 **Functional–Structural Heterogeneity in AKI Recovery Across Renal Perfusion**

2 **Phenotypes in Critical Illness**

3 Running title: Functional–Structural AKI Recovery Phenotypes

4 Hongxiang Li ¹, Yao Fu ¹, Yuting Li ¹, Youquan Wang¹, Dong Zhang^{1*}

5 ¹Department of Critical Care Medicine, The First Hospital of Jilin University,

6 Changchun, China

7

8 *** Correspondence author:**

9 Dong Zhang, PhD, Department of Critical Care Medicine, The First Hospital of Jilin

10 University, No. 1 Xinmin Street, Changchun 130021, China. Email:

11 zhangdong@jlu.edu.cn

12

13 Clinical trial registry: ClinicalTrials.gov

14 Trial registration number: NCT05866250

15 Date of registration: 10 May 2023

16

17

18

19

20

21

22

Abstract

Background: Acute kidney injury (AKI) is heterogeneous, and renal perfusion, functional impairment, and tubular injury may not evolve synchronously. We investigated whether functional and structural biomarkers further characterize AKI course and recovery after renal perfusion phenotyping.

Methods: This biomarker substudy was nested within a prospective multicentre cohort of critically ill adults undergoing serial semiquantitative renal perfusion (SQP) assessment at ICU admission and 24 and 48 h. Previously derived high-stable and low-perfusion trajectories were used without repeating trajectory modelling. Among 224 patients enrolled at the principal study centre, 172 had serum cystatin C and neutrophil gelatinase-associated lipocalin (NGAL) measured within 24 h; complete-case analyses included 163 patients. Biomarker profiles were compared across perfusion trajectories and no, transient, and persistent AKI. Exploratory combined perfusion–biomarker states were evaluated against day-7 AKI status among 153 patients after excluding 10 patients who died before day 7.

Results: Among 163 patients, 94 had high-stable and 69 had low-perfusion trajectories. The low-perfusion group had higher cystatin C than the high-stable group (median 1.71 vs 1.34 mg/L; $P=0.007$) and higher NGAL (183.0 vs 127.2 ng/mL; $P=0.043$). Cystatin C increased across no AKI, transient AKI, and persistent AKI (1.2, 1.7, and 2.4 mg/L; overall $P<0.001$) and distinguished transient from persistent AKI (Holm-adjusted $P=0.014$). NGAL showed a parallel gradient (109.6, 186.4, and 313.9 ng/mL; overall $P<0.001$) but did not distinguish transient from persistent AKI

(Holm-adjusted $P=0.093$). Day-7 AKI status differed across the combined perfusion–cystatin C states (overall $P=0.043$). No day-7 AKI occurred among patients with cystatin C ≤ 1.07 mg/L, irrespective of perfusion trajectory; among those with elevated cystatin C, day-7 AKI occurred in 8.8% of the high-stable group and 19.6% of the low-perfusion group.

Conclusions: Renal perfusion phenotypes provided a physiological context for functional and structural biomarker heterogeneity in AKI. Cystatin C and NGAL increased across no, transient, and persistent AKI, with cystatin C additionally distinguishing transient from persistent AKI; exploratory combined perfusion–cystatin C states showed heterogeneous patterns of AKI at day 7. These findings support further evaluation of multidimensional AKI phenotyping in larger multicentre cohorts.

Keywords: acute kidney injury; renal perfusion; cystatin C; neutrophil gelatinase-associated lipocalin; renal recovery; critical illness; phenotyping

1. Introduction

Acute kidney injury (AKI) is one of the most common complications in critically ill patients and is associated with prolonged hospitalization, progression to chronic kidney disease, and increased mortality [1]. Despite advances in supportive care, recovery after AKI remains highly heterogeneous [2]. Patients with similar serum creatinine (SCr)-defined AKI severity often experience markedly different clinical courses, ranging from rapid renal recovery to persistent kidney dysfunction [2, 3].

These observations suggest that conventional functional markers alone incompletely capture the underlying biological heterogeneity of AKI and therefore provide limited information regarding renal recovery potential.

To better characterize this heterogeneity, increasing attention has shifted beyond static severity classification toward dynamic assessment of renal perfusion [2]. We previously demonstrated that serial semiquantitative renal perfusion (SQP) assessment identified distinct renal perfusion trajectories associated with different risks of persistent AKI [4]. These findings suggest that dynamic renal perfusion assessment captures clinically meaningful heterogeneity beyond conventional clinical evaluation. However, the biological basis underlying these renal perfusion trajectories remains unclear. Dynamic renal perfusion reflects renal hemodynamics, whereas functional and structural biomarkers provide complementary information regarding glomerular filtration and tubular injury, respectively [5]. Together, these modalities represent complementary dimensions of AKI. Whether functional and structural biomarkers can biologically characterize distinct renal perfusion trajectories remains unknown.

Therefore, we aimed to characterize distinct renal perfusion trajectories using functional and structural kidney biomarkers, examine biomarker profiles across 72-h AKI courses, and explore the relationship between combined perfusion–biomarker states and AKI status at day 7.

2. Methods

2.1 Study design and participants

This biomarker substudy was nested within a prospective multicentre cohort of 456

critically ill patients recruited from six ICUs in Jilin Province, China, between August and December 2023 (ClinicalTrials.gov, NCT05866250; registered May 10, 2023). As reported previously [4], serial SQP measurements at ICU admission and 24 and 48 h identified two renal perfusion trajectories: high-stable perfusion and low perfusion. The present study used these previously assigned trajectories without repeating the trajectory modelling. Among 224 patients enrolled at the First Hospital of Jilin University, 172 underwent biomarker assessment within 24 h of ICU admission. After excluding nine patients without available cystatin C measurements, 163 constituted the complete biomarker analysis cohort. The eligibility criteria and study procedures have been reported previously [4]. The study was approved by the ethics committees of the participating institutions, and written informed consent was obtained from patients or their legal representatives.

2.2 Renal perfusion assessment and trajectory assignment

Renal perfusion was assessed using colour Doppler ultrasound at ICU admission and 24 and 48 h by trained ICU physicians following a standardized study protocol. SQP was graded separately for each kidney on a 0–3 ordinal scale according to the extent of visible intrarenal perfusion: 0, no detectable flow signal; 1, sparse signals limited to the renal hilum; 2, prominent hilar signals with limited interlobar arterial signals at the corticomedullary junction; and 3, diffuse signals extending to the arcuate artery level. Bilateral scores were averaged, allowing half-point increments; when only one kidney could be reliably assessed, the available measurement was used. Marked bilateral discrepancies were reassessed, and persistently unreliable examinations were

excluded according to the parent-study protocol. Operators were not formally blinded to clinical information because examinations were performed at the bedside. Measurements were scheduled relative to ICU admission rather than AKI onset. The present analysis used the previously assigned high-stable and low-perfusion trajectories without repeating trajectory modelling [4].

2.3 Biomarker measurement

Venous blood samples were collected within 24 h of ICU admission, irrespective of the presence or absence of AKI at the time of sampling. Biomarker sampling was therefore not necessarily performed before AKI onset and was intended for phenotypic characterization rather than early diagnostic testing. After centrifugation, serum aliquots were stored at -70°C until analysis, and repeated freeze–thaw cycles were avoided. All measurements were performed centrally. Serum cystatin C was measured using an immunoturbidimetric assay on a Beckman Coulter automated chemistry analyser (instrument no. 5841), with a cystatin C reagent kit supplied by Beckman Coulter Laboratory Systems (Suzhou) Co., Ltd., China. Serum neutrophil gelatinase-associated lipocalin (NGAL) was measured using an immunofluorescence assay on a Jet-iStar 1000 immunoassay analyser, with an NGAL assay kit supplied by Zhonghan Shengtai Biotechnology Co., Ltd., China. Cystatin C and NGAL concentrations were expressed in mg/L and ng/mL, respectively.

2.4 Definitions and outcomes

AKI was defined and staged according to KDIGO serum creatinine criteria. Baseline serum creatinine was determined using the hierarchical approach described previously

[4]. Key exclusion criteria included chronic kidney disease stage III-V, kidney transplantation or renal vascular disease, pregnancy, cardiac arrhythmias precluding Doppler assessment, hospital stay <72 h, refusal of consent, and missing baseline data as previously reported [4]. Based on AKI status during the first 72 h after ICU admission, patients were classified as having no AKI, transient AKI, or persistent AKI; persistent AKI was defined as AKI lasting ≥ 72 h.

The primary clinical outcome was AKI course during the first 72 h after ICU admission, classified as no AKI, transient AKI (TAKI), or persistent AKI (PAKI). AKI was defined and staged according to KDIGO serum creatinine criteria. The primary outcome was AKI course during the first 72 h after ICU admission, classified as no AKI, TAKI, or PAKI. TAKI was defined as AKI that resolved within 72 h, whereas PAKI was defined as AKI persisting for at least 72 h. The secondary outcome was AKI status at day 7. Patients who died before day 7 were not included in analyses requiring assessment of this outcome.

2.5 Statistical analysis

No formal sample-size calculation was performed for this substudy; the sample size was determined by the availability of complete biomarker data. Continuous variables are presented as median (interquartile range), and categorical variables as number (percentage). The high-stable and low-perfusion trajectory groups were compared using the Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. Effect sizes for continuous biomarker comparisons between trajectories were quantified using Cliff's delta. For binary biomarker status

comparisons, odds ratios with 95% confidence intervals were calculated with the high-stable trajectory as the reference group.

Overall comparisons across the no-AKI, transient-AKI, and persistent-AKI groups were performed using the Kruskal–Wallis test for continuous variables and Fisher’s exact test for categorical variables. Pairwise comparisons of biomarker concentrations were conducted using Dunn’s test with Holm correction for multiple comparisons. Differences in day-7 AKI status across the four integrated perfusion–cystatin C states were assessed using Fisher’s exact test. Analyses of day-7 AKI status were restricted to patients alive at day 7.

Exploratory perfusion–biomarker groups were constructed using manufacturer-provided reference limits. Cystatin C concentrations >1.07 mg/L and NGAL concentrations ≥ 180 ng/mL were classified as elevated. These thresholds were not derived through outcome-based optimization in the present cohort. No missing values were imputed, and analyses were based on available data. All tests were two-sided, with $P < 0.05$ considered statistically significant. Analyses were performed using R version 4.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

2.6 Use of generative artificial intelligence

During manuscript preparation, the authors used ChatGPT (OpenAI) and DeepSeek to assist with language editing and refinement of academic expression. The authors independently reviewed and revised all AI-assisted text and verified the scientific content and references. All study design decisions, statistical analyses, interpretation of the results, and final conclusions were made and approved by the authors. The

authors take full responsibility for the accuracy, integrity, and content of the manuscript.

3. Results

3.1 Study population

Among 224 patients enrolled at the principal study centre, 52 were excluded from biomarker assessment because of incomplete required clinical or laboratory information ($n = 26$), unavailable or insufficient stored serum ($n = 15$), or samples that failed pre-analytical quality-control requirements, including haemolysis ($n = 11$). Biomarker assessment was therefore performed in 172 patients. After excluding nine patients without an available cystatin C measurement, 163 patients constituted the complete biomarker analysis cohort (Figure 1). Their median age was 63.0 years (IQR 51.0–72.0), 89 (54.6%) were men, and 64 (39.3%) received vasopressors. During the first 72 h, 80 patients (49.1%) had no AKI, 51 (31.3%) had transient AKI, and 32 (19.6%) had persistent AKI. Ninety-four patients (57.7%) belonged to the high-stable perfusion trajectory and 69 (42.3%) to the low-perfusion trajectory (Table 1).

3.2 Biomarker profiles across renal perfusion trajectories

Patients in the low-perfusion trajectory had higher cystatin C concentrations than those in the high-stable trajectory [1.71 (1.36–2.36) vs 1.34 (1.07–2.12) mg/L; $P=0.007$; Cliff's $\delta=-0.246$, 95% CI -0.406 to -0.071]. NGAL concentrations were also higher in the low-perfusion group [183.0 (90.5–388.6) vs 127.2 (84.1–216.8) ng/mL; $P=0.043$; Cliff's $\delta=-0.186$, 95% CI -0.352 to -0.008] (Figure 2 and Table 2). Cystatin C concentrations >1.07 mg/L occurred in 59 of 69 patients (85.5%) in the

low-perfusion group and 70 of 94 patients (74.5%) in the high-stable group; however, the difference was not statistically significant (OR 2.02, 95% CI 0.90–4.57; $P=0.118$). In contrast, NGAL concentrations ≥ 180 ng/mL occurred in 35 of 69 patients (50.7%) and 32 of 94 patients (34.0%), respectively (OR 1.99, 95% CI 1.06–3.77; $P=0.037$).

3.3 Biomarker gradients across 72-hour AKI courses

Cystatin C increased progressively across the no-AKI, transient-AKI, and persistent-AKI groups, with median concentrations of 1.2 (1.0–1.6), 1.7 (1.3–2.5), and 2.4 (1.9–3.0) mg/L, respectively (overall $P<0.001$). Pairwise differences remained significant for no AKI versus transient AKI, no AKI versus persistent AKI (both Holm-adjusted $P<0.001$), and transient versus persistent AKI (Holm-adjusted $P=0.014$).

NGAL showed a similar overall gradient, increasing from 109.6 (80.6–149.6) ng/mL in patients without AKI to 186.4 (92.4–391.5) ng/mL in transient AKI and 313.9 (183.4–523.1) ng/mL in persistent AKI (overall $P<0.001$). NGAL differed between no AKI and both transient and persistent AKI (both Holm-adjusted $P<0.001$), but not between transient and persistent AKI (Holm-adjusted $P=0.093$) (Figure 3 and Table 3). The proportion of patients with a low-perfusion trajectory increased from 33.8% in the no-AKI group to 49.0% in transient AKI and 53.1% in persistent AKI, although the overall difference was not statistically significant ($P=0.094$).

3.4 Biomarker status and subsequent AKI course

After excluding 10 patients who died before day 7, 153 patients were evaluable for day-7 AKI status. Transitions from early biomarker status to 72-h AKI persistence and

AKI status at day 7 are shown in Figure 4. Patients with elevated cystatin C or NGAL more frequently transitioned to persistent AKI and remained classified as having AKI on day 7 than patients without biomarker elevation. The separation was more apparent for cystatin C than for NGAL, consistent with the pairwise biomarker comparisons across the 72-h AKI courses.

3.5 Integrated perfusion–biomarker states and day-7 AKI status

Among the 153 patients evaluable at day 7 after excluding 10 patients who died before day 7, AKI status differed across the four integrated renal perfusion–cystatin C states (overall Fisher’s exact test, $P=0.043$; Figure 5). The highest proportion of day-7 AKI was observed among patients with low perfusion and cystatin C >1.07 mg/L (10/51, 19.6%), followed by those with high-stable perfusion and elevated cystatin C (6/68, 8.8%). No day-7 AKI occurred among patients with cystatin C ≤ 1.07 mg/L, irrespective of renal perfusion trajectory.

4. Discussion

In this biomarker substudy, renal perfusion phenotypes provided a physiological framework within which functional and structural kidney profiles further characterized the heterogeneity of AKI persistence and recovery. Patients with a low-perfusion trajectory had higher serum cystatin C and NGAL concentrations than those with a high-stable trajectory. Both biomarkers increased from no AKI through transient to persistent AKI, but only cystatin C distinguished transient from persistent AKI, suggesting that sustained impairment of filtration was more closely related to AKI persistence than the magnitude of the tubular-injury signal alone. Exploratory

consideration of renal perfusion trajectory together with cystatin C status showed heterogeneous patterns of AKI at day 7, with the highest observed frequency among patients with low perfusion and elevated cystatin C. Collectively, these findings support a multidimensional framework in which perfusion, functional impairment, and structural injury provide related but non-equivalent information about AKI course. Renal perfusion phenotypes should be interpreted as physiological contexts rather than direct surrogates for parenchymal injury or recovery. In a prospective multicentre study, single Doppler-based resistive index and semiquantitative perfusion measurements showed limited ability to discriminate persistent AKI, indicating that bedside perfusion signals alone do not fully capture the subsequent renal course [6]. An ancillary analysis of the same cohort subsequently demonstrated similarly poor performance for predicting de novo AKI among patients without AKI at admission [7]. Particularly in sepsis and shock, kidney dysfunction may also arise from microcirculatory alterations, dysregulated inflammation, and cellular metabolic reprogramming, allowing perfusion, cellular injury, and filtration to become partly dissociated [8]. Current consensus and recent biomarker frameworks therefore recommend integrating clinical information with functional and damage biomarkers rather than relying on any single dimension [5, 9]. Our findings extend this framework by showing that functional and structural biomarker profiles remain heterogeneous even after patients have been classified according to their renal perfusion trajectories.

Cystatin C and NGAL showed related but distinct patterns across AKI courses.

Cystatin C exhibited a consistent gradient from no AKI to transient and persistent AKI and distinguished transient from persistent AKI, whereas NGAL identified a tubular-injury gradient but did not clearly separate the two AKI courses. This difference should not be interpreted as evidence that cystatin C is superior to NGAL; rather, functional impairment and structural tubular injury capture different components and potentially different phases of AKI [5, 10]. As a filtration marker with faster kinetics than serum creatinine, cystatin C may detect functional impairment earlier [11]; in a recent study of 9,424 critically ill patients, cystatin C-based staging identified clinically relevant AKI not captured by creatinine-based criteria [12]. Conversely, NGAL is rapidly released in response to tubular stress but may vary with the timing and nature of kidney injury. Previous studies have linked plasma NGAL to renal recovery [13], although its performance is context-dependent, particularly in sepsis, where activated neutrophils and extra-renal tissues can increase circulating NGAL independently of tubular injury [8, 14]. Cystatin C also remains susceptible to inflammation, body composition, and other non-GFR determinants [15, 16]. Thus, the observed discordance supports complementary functional–structural phenotyping rather than substitution of one biomarker for another.

Exploratory integration of perfusion trajectory with cystatin C revealed concordant and discordant physiological states. No day-7 AKI occurred among patients with cystatin C ≤ 1.07 mg/L, irrespective of perfusion trajectory, whereas the highest observed frequency occurred among patients with low perfusion and elevated cystatin C. This pattern suggests that cystatin C status was the principal source of separation in

287 this exploratory analysis, while perfusion trajectory provided physiological context
288 rather than demonstrated incremental prognostic information. The discordant groups
289 remained biologically informative: high-stable perfusion with elevated cystatin C
290 suggested functional impairment despite a favourable perfusion trajectory, whereas
291 low perfusion with normal cystatin C indicated that reduced Doppler perfusion was
292 not invariably accompanied by filtration impairment. Such mismatch is biologically
293 plausible because haemodynamic recovery, filtration, and cellular repair need not
294 occur simultaneously [3, 8, 17]. Consistent with functional–damage biomarker
295 frameworks [5, 18], discordance between perfusion and biomarker status may provide
296 complementary biological information. By incorporating renal perfusion phenotype,
297 our findings conceptually extend existing functional–damage frameworks [5, 10] with
298 an additional physiological dimension.

299 The clinical implication is not that these combinations define treatment thresholds or a
300 prediction rule. Rather, they support a multidimensional framework—perfusion
301 phenotype, functional state, structural injury, and recovery course—for interpreting
302 renal heterogeneity. This framework may explain why normalization of mean arterial
303 pressure, improvement in Doppler perfusion, or a high SQP does not necessarily
304 indicate resolution of functional impairment or tubular injury. Conversely, an
305 unfavourable perfusion phenotype may not imply irreversible damage when
306 functional markers remain preserved. Combining these domains could improve
307 biological characterization and support future biomarker-guided enrichment studies
308 [19, 20]. Clinical application will nevertheless require external validation, serial

assessment, and evidence that phenotype-directed management improves patient-centred outcomes.

This study has several strengths, including its prospective design, standardized serial assessment of renal perfusion, and integration of physiological, functional, and structural kidney domains with prospectively ascertained AKI courses. Several limitations should also be acknowledged. Although the parent cohort was multicentre, biomarker assessment was restricted to participants enrolled at the principal study centre, which may limit generalizability. The sample size was modest, particularly for analyses of persistent and day-7 AKI. Exclusions related to incomplete clinical or laboratory information, unavailable or insufficient stored serum, pre-analytical quality-control requirements, and missing cystatin C measurements may also have introduced selection bias. Cystatin C and NGAL were measured only once within 24 h of ICU admission; therefore, their temporal relationships with changing perfusion, injury, and recovery could not be assessed. Manufacturer-provided reference limits were used to categorize biomarker status, and the resulting integrated states were exploratory rather than validated prognostic categories. In addition, 10 patients who died before day 7 could not be included in analyses of day-7 AKI status; these findings are therefore conditional on survival to day 7 and may be susceptible to survivor selection. Moreover, day-7 AKI status provides only an early measure of renal course and may not reflect subsequent recovery, progression to acute kidney disease, or long-term kidney outcomes. Finally, the observational design precludes causal inference. These findings should therefore be considered a phenotypic

characterization of AKI heterogeneity rather than the development of a prediction model and require validation in larger multicentre cohorts with serial biomarker measurements and longer follow-up.

5. Conclusion

Renal perfusion phenotypes provide a physiological context within which functional and structural biomarkers characterize AKI heterogeneity. Cystatin C and NGAL both increased across no, transient, and persistent AKI, whereas only cystatin C distinguished transient from persistent AKI. Exploratory combined perfusion–cystatin C states showed heterogeneous patterns of AKI at day 7. Together, these findings support a multidimensional framework linking renal perfusion, functional impairment, tubular injury, and AKI course, warranting validation in larger multicentre studies.

Abbreviations

AKI, acute kidney injury

ICU, intensive care unit

IQR, interquartile range

KDIGO, Kidney Disease: Improving Global Outcomes

NGAL, neutrophil gelatinase-associated lipocalin

PAKI, persistent acute kidney injury

SCr, serum creatinine

SQP, semiquantitative perfusion score

TAKI, transient acute kidney injury

APACHE II, Acute Physiology and Chronic Health Evaluation II

MAP, mean arterial pressure

RRT, renal replacement therapy

Supplementary Information

Not applicable.

Declarations

Funding

Biomarker assay kits and laboratory testing support were provided by Jilin Taisite Medical Technology Co., Ltd. The company had no role in the study design, participant recruitment, data collection, data analysis, interpretation of the findings, preparation of the manuscript, or the decision to submit the manuscript for publication.

Authors' contributions

HXL and YF conceived and designed the biomarker substudy. YF and QYW coordinated data collection and quality control. YTL and QYW contributed to data curation, statistical analysis, and interpretation. HXL and YF drafted the manuscript. DZ supervised the study and contributed to the interpretation of the findings and critical revision of the manuscript. All authors reviewed and approved the final manuscript and accept responsibility for the integrity of the work.

Acknowledgements

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This biomarker substudy was conducted within a prospective multicentre cohort registered at ClinicalTrials.gov (NCT05866250). The study protocol was approved by the Ethics Committee of the First Hospital of Jilin University (23K071-001) and the institutional review boards of the other participating hospitals. Written informed consent was obtained from all participants or their legally authorized representatives before enrolment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no financial or non-financial competing interests. Jilin Taisite Medical Technology Co., Ltd. provided support for biomarker testing but had no role in the conduct, analysis, interpretation, or reporting of the study.

References

- 396 1. Hoste EA, Kellum JA, Selby NM, Zarbock A, Palevsky PM, Bagshaw SM, Goldstein SL,
397 Cerdá J, Chawla LS: **Global epidemiology and outcomes of acute kidney injury.** *Nature*
398 *Reviews Nephrology* 2018, **14**(10):607-625.
- 399 2. Kellum JA, Romagnani P, Ashuntantang G, Ronco C, Zarbock A, Anders H-J: **Acute kidney**
400 **injury.** *Nature reviews Disease primers* 2021, **7**(1):52.
- 401 3. Chawla LS, Bellomo R, Bihorac A, Goldstein SL, Siew ED, Bagshaw SM, Bittleman D, Cruz
402 D, Endre Z, Fitzgerald RL: **Acute kidney disease and renal recovery: consensus report of**
403 **the Acute Disease Quality Initiative (ADQI) 16 Workgroup.** *Nature Reviews Nephrology*
404 2017, **13**(4):241-257.
- 405 4. Yao Fu LK, Cheng Lv, Yongjie Wang, Shuyun Zhang, Jingguo Yan, Xinmei Zhang, Weiheng
406 Liu, Yang Hu, Dong Zhang, Hongxiang Li: **Early renal perfusion phenotypes and renal**
407 **recovery in critically ill patients with or at risk for acute kidney injury: a prospective**
408 **multicenter cohort study.** *Shock* . 2026 Sep 7. doi: 10.1097/SHK.0000000000002936..
- 409 5. Ostermann M, Zarbock A, Goldstein S, Kashani K, Macedo E, Murugan R, Bell M, Forni L,
410 Guzzi L, Joannidis M: **Recommendations on acute kidney injury biomarkers from the**
411 **acute disease quality initiative consensus conference: a consensus statement.** *JAMA*
412 *network open* 2020, **3**(10):e2019209.
- 413 6. Darmon M, Bourmaud A, Reynaud M, Rouleau S, Meziani F, Boivin A, Benyamina M,
414 Vincent F, Lautrette A, Leroy C *et al*: **Performance of Doppler-based resistive index and**
415 **semi-quantitative renal perfusion in predicting persistent AKI: results of a prospective**
416 **multicenter study.** *Intensive Care Med* 2018, **44**(11):1904-1913.

- 417 7. Schnell D, Bourmaud A, Reynaud M, Rouleau S, Merdji H, Boivin A, Benyamina M, Vincent
418 F, Lautrette A, Leroy C *et al*: **Performance of renal Doppler to predict the occurrence of**
419 **acute kidney injury in patients without acute kidney injury at admission.** *Journal of*
420 *Critical Care* 2022, **69**:153983.
- 421 8. Zarbock A, Nadim MK, Pickkers P, Gomez H, Bell S, Joannidis M, Kashani K, Koyner JL,
422 Pannu N, Meersch M *et al*: **Sepsis-associated acute kidney injury: consensus report of the**
423 **28th Acute Disease Quality Initiative workgroup.** *Nat Rev Nephrol* 2023, **19**(6):401-417.
- 424 9. Meersch-Dini M, Kellum JA: **Biomarkers for acute kidney injury: a pragmatic approach.**
425 *Intensive Care Medicine* 2026, **52**(4):803-806.
- 426 10. Ostermann M, Legrand M, Meersch M, Srisawat N, Zarbock A, Kellum JA: **Biomarkers in**
427 **acute kidney injury.** *Annals of Intensive Care* 2024, **14**(1):145.
- 428 11. Hooper L, Heung M, Kenes M, Stringer KA, Mueller BA, Pai MP: **The Kinetics of Cystatin**
429 **C and Serum Creatinine in AKI.** *Clin J Am Soc Nephrol* 2025, **20**(4):477-484.
- 430 12. Helmersson-Karlqvist J, Byberg L, Ärnlov J, Bell M, Mårtensson J, Dardashti A, Svensson
431 MK, Larsson A, Marks-Hultström M, Lipcsey M: **Development and Validation of a Cystatin**
432 **C-based Staging of AKI in Critically Ill Patients.** *Kidney International Reports* 2026,
433 **11**(6):106514.
- 434 13. Srisawat N, Murugan R, Lee M, Kong L, Carter M, Angus DC, Kellum JA: **Plasma**
435 **neutrophil gelatinase-associated lipocalin predicts recovery from acute kidney injury**
436 **following community-acquired pneumonia.** *Kidney Int* 2011, **80**(5):545-552.

- 437 14. Mårtensson J, Bell M, Oldner A, Xu S, Venge P, Martling CR: **Neutrophil**
438 **gelatinase-associated lipocalin in adult septic patients with and without acute kidney**
439 **injury**. *Intensive Care Med* 2010, **36**(8):1333-1340.
- 440 15. Knight EL, Verhave JC, Spiegelman D, Hillege HL, de Zeeuw D, Curhan GC, de Jong PE:
441 **Factors influencing serum cystatin C levels other than renal function and the impact on**
442 **renal function measurement**. *Kidney Int* 2004, **65**(4):1416-1421.
- 443 16. Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, Froissart M, Kusek JW, Zhang
444 YL, Coresh J *et al*: **Factors other than glomerular filtration rate affect serum cystatin C**
445 **levels**. *Kidney Int* 2009, **75**(6):652-660.
- 446 17. Forni LG, Darmon M, Ostermann M, Oudemans-van Straaten HM, Pettilä V, Prowle JR,
447 Schetz M, Joannidis M: **Renal recovery after acute kidney injury**. *Intensive Care Med* 2017,
448 **43**(6):855-866.
- 449 18. Basu RK, Wong HR, Krawczeski CD, Wheeler DS, Manning PB, Chawla LS, Devarajan P,
450 Goldstein SL: **Combining functional and tubular damage biomarkers improves**
451 **diagnostic precision for acute kidney injury after cardiac surgery**. *J Am Coll Cardiol* 2014,
452 **64**(25):2753-2762.
- 453 19. Bhatraju PK, Zelnick LR, Herting J, Katz R, Mikacenic C, Kosamo S, Morrell ED,
454 Robinson-Cohen C, Calfee CS, Christie JD *et al*: **Identification of Acute Kidney Injury**
455 **Subphenotypes with Differing Molecular Signatures and Responses to Vasopressin**
456 **Therapy**. *Am J Respir Crit Care Med* 2019, **199**(7):863-872.

457 20. Stanski NL, Rodrigues CE, Strader M, Murray PT, Endre ZH, Bagshaw SM: **Precision**
458 **management of acute kidney injury in the intensive care unit: current state of the art.**
459 *Intensive Care Med* 2023, **49**(9):1049-1061.

460

461 **Table 1. Clinical characteristics and outcomes according to 72-hour AKI course**

Variable	Overall N = 163	No AKI N = 80	TAKI N = 51	PAKI N = 32	P value
Age, years	63.0 (51.0– 72.0)	60.0 (52.0– 70.0)	60.0 (49.0– 72.0)	64.0 (51.0– 72.0)	0.854
Male sex, n (%)	89 (54.6%)	51 (63.8%)	23 (45.1%)	15 (46.9%)	0.069
Body weight, kg	70.0 (60.0– 75.0)	70.0 (60.0– 75.0)	65.0 (60.0– 70.0)	70.0 (60.0– 75.0)	0.422
APACHE II score	12.0 (9.0– 15.0)	11.0 (7.5– 13.0)	13.0 (9.0– 16.0)	15.0 (13.5– 21.0)	<0.001
Mean arterial pressure, mmHg	86.7 (79.3– 96.3)	90.0 (82.0– 99.8)	85.7 (78.3– 94.3)	81.8 (70.0– 90.0)	0.009
Vasopressor use, n (%)	64 (39.3%)	18 (22.5%)	25 (49.0%)	21 (65.6%)	<0.001
Baseline creatinine, μmol/L	59.3 (49.5– 75.3)	52.5 (44.6– 63.0)	65.3 (55.0– 77.7)	71.0 (70.5– 88.0)	<0.001
Creatinine at enrollment, μmol/L	77.2 (52.1– 115.8)	57.3 (46.6– 70.2)	108.9 (86.0– 161.9)	125.6 (87.3– 225.8)	<0.001
AKI stage at enrollment					<0.001
Stage 0	91 (55.8%)	80 (100.0%)	3 (5.9%)	8 (25.0%)	
Stage 1	26 (16.0%)	0 (0.0%)	22 (43.1%)	4 (12.5%)	
Stage 2	16 (9.8%)	0 (0.0%)	13 (25.5%)	3 (9.4%)	
Stage 3	30 (18.4%)	0 (0.0%)	13 (25.5%)	17 (53.1%)	
Primary AKI etiology					<0.001

Shock	88 (54.0%)	17 (21.3%)	44 (86.3%)	27 (84.4%)	
Hypoperfusion	9 (5.5%)	6 (7.5%)	1 (2.0%)	2 (6.3%)	
Intrarenal injury	5 (3.1%)	1 (1.3%)	2 (3.9%)	2 (6.3%)	
Sepsis without shock	4 (2.5%)	4 (5.0%)	0 (0.0%)	0 (0.0%)	
No AKI risk	57 (35.0%)	52 (65.0%)	4 (7.8%)	1 (3.1%)	
Renal perfusion trajectory					0.094
High-stable	94 (57.7%)	53 (66.3%)	26 (51.0%)	15 (46.9%)	
Low perfusion	69 (42.3%)	27 (33.8%)	25 (49.0%)	17 (53.1%)	
Cystatin C, mg/L	1.5 (1.1–2.3)	1.2 (1.0–1.6)	1.7 (1.3–2.5)	2.4 (1.9–3.0)	<0.001
NGAL, ng/mL	134.2 (86.7–294.5)	109.6 (80.6–149.6)	186.4 (92.4–391.5)	313.9 (183.4–523.1)	<0.001
CRRT use by day 3, n (%)	15 (9.2%)	0 (0.0%)	0 (0.0%)	15 (46.9%)	<0.001
28-day mortality, n (%)	20 (12.3%)	4 (5.0%)	2 (3.9%)	14 (43.8%)	<0.001

Values are presented as median (interquartile range) or n (%). P values were calculated using the Kruskal–Wallis test for continuous variables and Fisher’s exact test for categorical variables. Abbreviations: AKI, acute kidney injury; APACHE II, Acute Physiology and Chronic Health Evaluation II; CRRT, continuous renal replacement therapy; NGAL, neutrophil gelatinase-associated lipocalin; PAKI, persistent acute kidney injury; TAKI, transient acute kidney injury.

462 **Table 2. Functional and structural biomarker profiles according to renal perfusion**
463 **trajectory**

Variable	High-stable N = 94	Low perfusion N = 69	P value	Effect estimate (95% CI)
Cystatin C, mg/L	1.34 (1.07–2.12)	1.71 (1.36–2.36)	0.007	Cliff’s δ = –0.246 (–0.406 to –0.071)
NGAL, ng/mL	127.2 (84.1–216.8)	183.0 (90.5–388.6)	0.043	Cliff’s δ = –0.186 (–0.352 to –0.008)
Cystatin C >1.07	70/94 (74.5%)	59/69 (85.5%)	0.118	OR=2.02 (0.90–4.57)

mg/L, n/N (%)

NGAL ≥ 180 ng/mL, 32/94 (34.0%) 35/69 (50.7%) 0.037 OR=1.99 (1.06–3.77)
n/N (%)

Continuous variables are presented as median (interquartile range) and were compared using the Wilcoxon rank-sum test; effect sizes are reported as Cliff's delta with 95% confidence intervals. Cliff's delta was calculated as high-stable versus low perfusion, with negative values indicating higher biomarker concentrations in the low-perfusion group. Categorical variables are presented as n/N (%) and were compared using Fisher's exact test. Odds ratios compare low perfusion with high-stable perfusion as the reference group. Elevated biomarker status was defined using manufacturer-provided reference limits: cystatin C >1.07 mg/L and NGAL ≥ 180 ng/mL. Abbreviations: CI, confidence interval; NGAL, neutrophil gelatinase-associated lipocalin; OR, odds ratio.

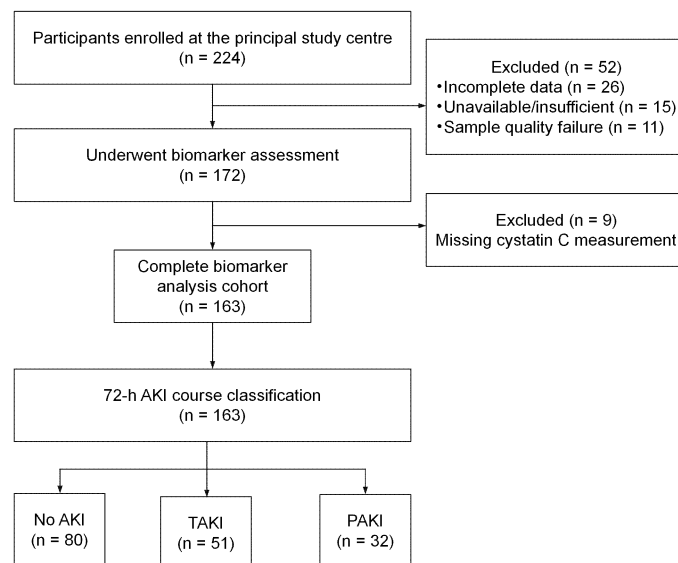
Table 3. Pairwise comparisons of biomarker concentrations across 72-hour AKI course groups

Biomarker	Overall P value	Comparison	Holm-adjusted P value	Cliff's δ (95% CI)
Cystatin C	<0.001	No AKI vs TAKI	<0.001	−0.419 (−0.572 to −0.238)
		No AKI vs PAKI	<0.001	−0.702 (−0.803 to −0.560)
		TAKI vs PAKI	0.014	−0.355 (−0.557 to −0.114)
NGAL	<0.001	No AKI vs TAKI	<0.001	−0.371 (−0.532 to −0.184)
		No AKI vs PAKI	<0.001	−0.578 (−0.715 to −0.399)
		TAKI vs PAKI	0.093	−0.232 (−0.456 to 0.021)

Overall group differences were assessed using the Kruskal–Wallis test. Pairwise comparisons were performed using Dunn's test with Holm correction for multiple testing. Cliff's delta is expressed for the first group relative to the second group; negative values indicate lower biomarker concentrations in the first group. Abbreviations: AKI, acute kidney injury; CI, confidence interval; NGAL, neutrophil gelatinase-associated lipocalin; PAKI, persistent acute kidney injury; TAKI, transient acute kidney injury.

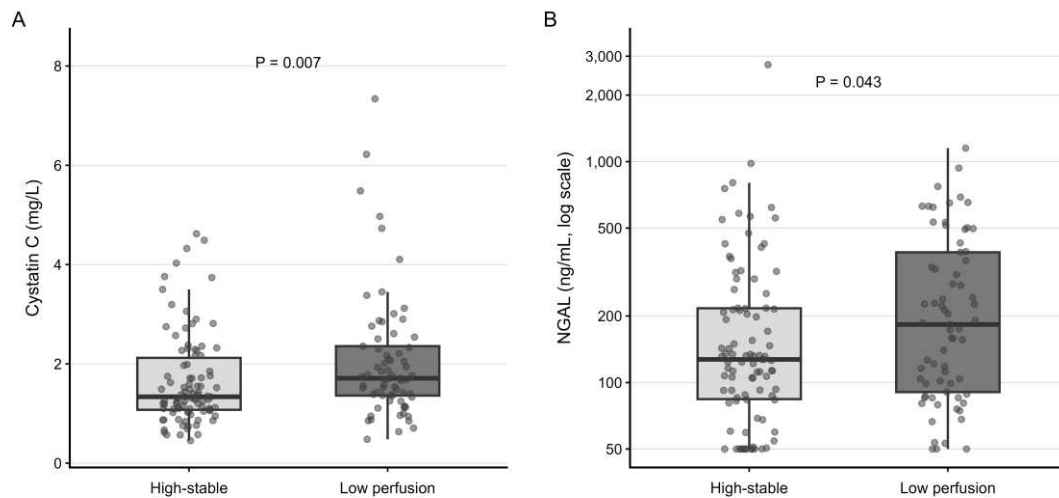
Figure legends

Figure 1. Study flow diagram.



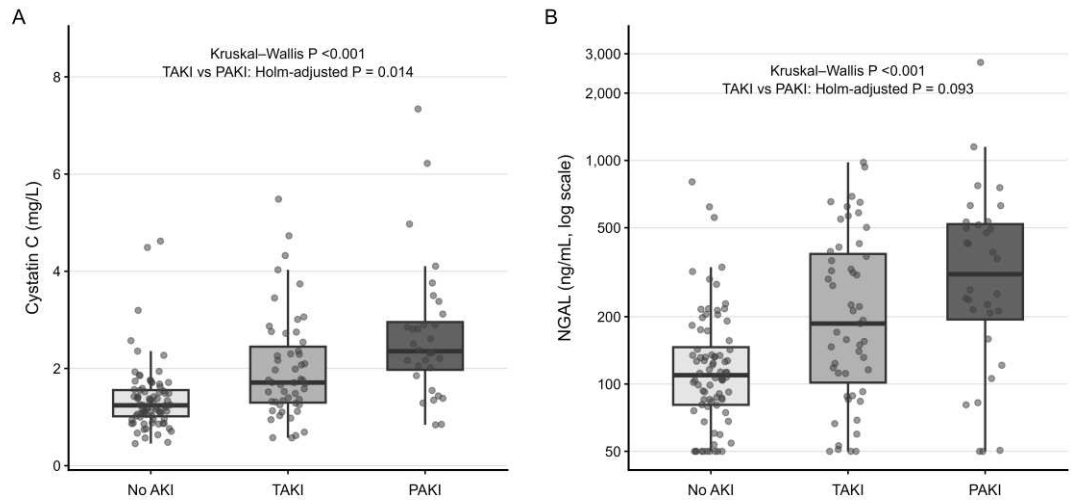
The biomarker substudy included participants enrolled at the principal study centre of the 456-patient multicentre parent cohort. Of 224 participants enrolled at this centre, 52 did not undergo biomarker assessment and 172 were assessed. After excluding nine patients without available cystatin C measurements, 163 constituted the complete biomarker analysis cohort and were classified according to their 72-h AKI course as no AKI (n = 80), TAKI (n = 51), or PAKI (n = 32).

Figure 2. Functional and structural biomarker profiles according to renal perfusion trajectory



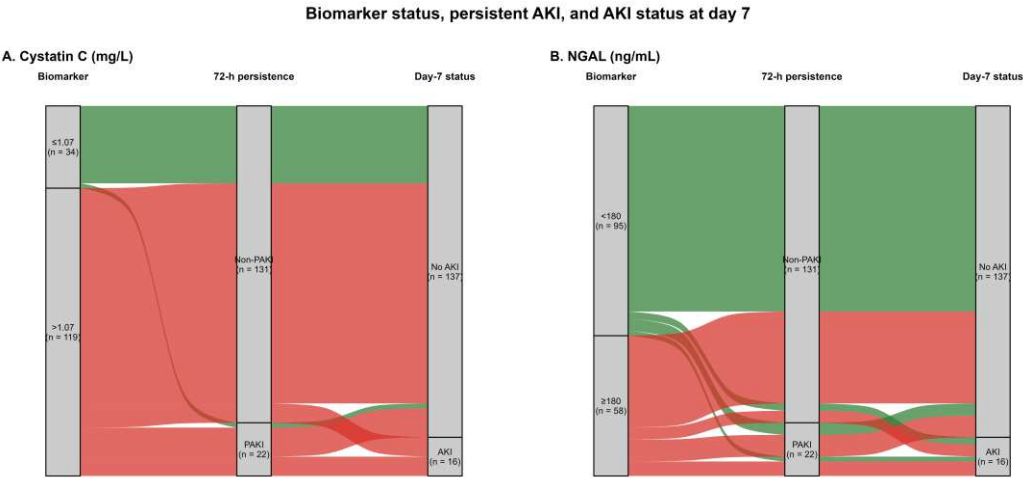
(A) Distribution of serum cystatin C concentrations according to renal perfusion trajectory. Patients with a low perfusion trajectory exhibited significantly higher cystatin C levels than those with a high-stable trajectory (Wilcoxon rank-sum test, $P = 0.007$; Cliff's $\delta = -0.246$). (B) Distribution of serum NGAL concentrations according to renal perfusion trajectory. Patients with a low perfusion trajectory also showed higher NGAL levels than those with a high-stable trajectory (Wilcoxon rank-sum test, $P = 0.043$; Cliff's $\delta = -0.186$). The y-axis is displayed on a logarithmic scale because of the right-skewed distribution of NGAL. Continuous variables are presented as box-and-whisker plots with individual observations overlaid. Boxes indicate the interquartile range (IQR), the horizontal line indicates the median, whiskers represent $1.5 \times \text{IQR}$, and points represent individual patients. Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; IQR, interquartile range.

Figure 3. Biomarker concentrations across 72-hour AKI course groups.



Boxplots show the distribution of serum cystatin C (Panel A) and NGAL (Panel B) according to the 72-hour AKI course (No AKI, transient AKI [TAKI], and persistent AKI [PAKI]). Boxes represent the interquartile range, horizontal lines indicate the median, whiskers extend to $1.5 \times$ the interquartile range, and individual points represent individual patients. Overall group differences were assessed using the Kruskal–Wallis test, followed by Dunn's test with Holm correction for pairwise comparisons. NGAL is displayed on a logarithmic scale. Abbreviations: AKI, acute kidney injury; TAKI, transient acute kidney injury; PAKI, persistent acute kidney injury; NGAL, neutrophil gelatinase-associated lipocalin; IQR, interquartile range.

Figure 4. Transitions from biomarker status to persistent AKI and AKI status at day 7.



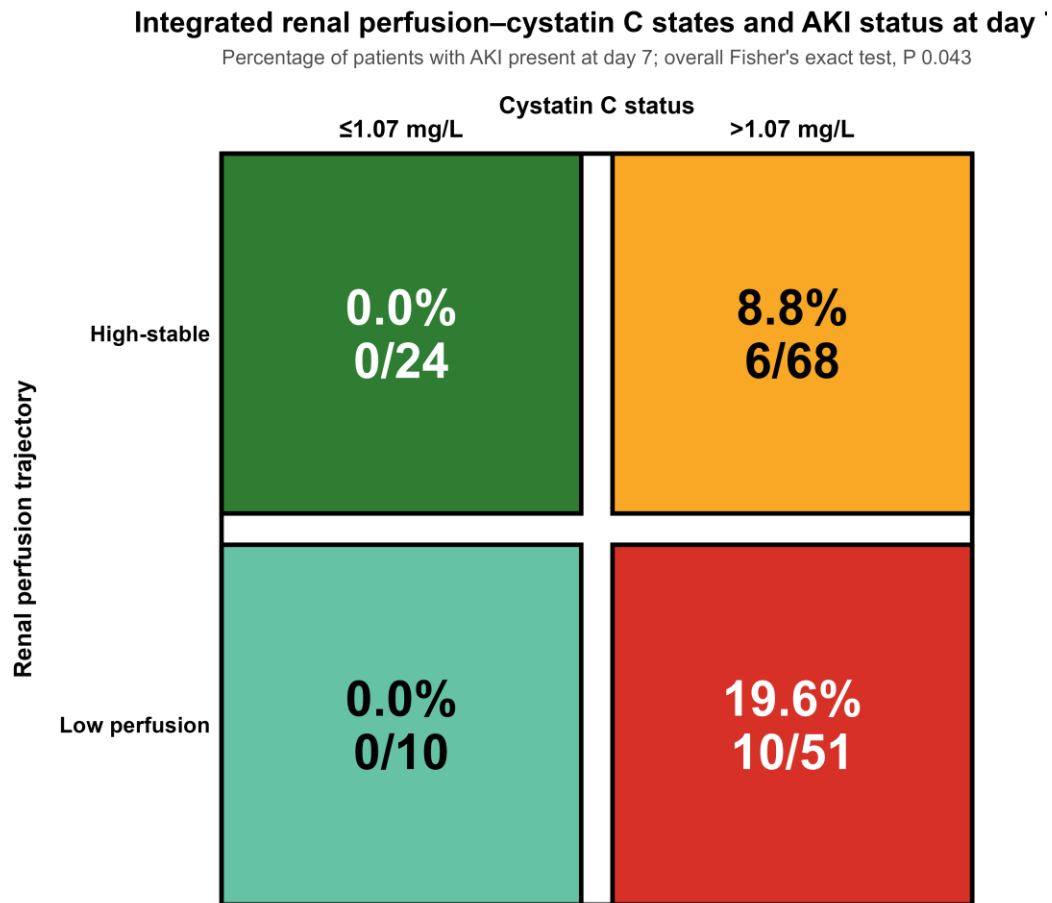
Sankey diagrams illustrate the flow of patients from biomarker status within 24 h of ICU admission to 72-hour AKI persistence and subsequent AKI status at day 7.

Analyses included 153 patients evaluable at day 7; 10 patients who died before day 7 were not included.

Panel A shows transitions according to cystatin C status, and Panel B according to NGAL status. Elevated biomarker status was defined as serum cystatin C >1.07 mg/L and NGAL ≥180 ng/mL. The width of each flow is proportional to the number of patients represented. Persistent AKI (PAKI) was defined as AKI persisting for ≥72 hours, whereas Non-PAKI included patients without AKI or with transient AKI.

Abbreviations: AKI, acute kidney injury; PAKI, persistent acute kidney injury; NGAL, neutrophil gelatinase-associated lipocalin.

Figure 5. Integrated renal perfusion–cystatin C states and AKI status at day 7.



Values within each quadrant indicate the percentage and number of patients with AKI at day 7 among the 153 patients evaluable at day 7. Patients with low perfusion and cystatin C >1.07 mg/L had the highest proportion of day-7 AKI (19.6%), whereas no patient with cystatin C ≤1.07 mg/L had AKI at day 7. Differences across the four integrated states were statistically significant (overall Fisher's exact test, $P=0.043$). Elevated cystatin C was defined as >1.07 mg/L.