Proenkephalin A 119-159 (penKid)
A unique biomarker for real-time assessment of kidney function

Deborah Bergmann, MSc
Head of Marketing and MSLM
deborah.bergmann@sphingotec.com

December 2021/Version 01
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Acute kidney injury (AKI) affects many critically ill patients, making timely information on kidney function highly important to early initiate nephroprotective strategies (1). Existing creatinine-based estimations of the glomerular filtration rate (GFR) routinely used in critical care settings are unspecific, error-prone, and have a substantial time delay (2).

An emerging body of evidence demonstrates that the biomarker proenkephalin A 119-159 (penKid) overcomes these limitations by indirectly measuring the kidney stimulating hormone enkephalin, which better reflects the kidney function(3). As a new biomarker for diagnosing AKI, penKid can be measured earlier at admission to the emergency department (ED) or intensive care unit (ICU), anticipating worsening renal function within 48 hours (4, 5). PenKid is detectable with the CE-IVD marked quantitative immunoassays - sphingotest® penKid® and the point-of-care (POC) IB10 sphingotest® penKid®.

More than 30 peer-reviewed publications and additional submitted clinical studies investigated the significance of penKid in more than 40,000 patients allowing a clear understanding of biological values and distribution to use penKid in critical care. In addition, measuring penKid levels reveals kidney function in real-time and offers a blood-based alternative for the in vivo measurement of the true GFR (6,7).

In summary, penKid rapidly provides physicians at the ED and ICU with urgently needed information on kidney function, which is complementary to the current diagnostic toolbox and enables early assessment of renal function. Thus, penKid may support clinicians in a wide range of clinical cases: guide nephrotoxic drug administration, inform weaning decisions from renal replacement therapy by assessing renal function during the therapy, or management of patients attending catheterization-laboratory intervention.
Impaired kidney function affects a broad number of critically ill patients and largely contributes to fatal outcomes. One in three ICU patients develops AKI (8, 9), a condition that represents a global burden with 13 million cases per year (8). In addition, AKI accounts for many complications in patients with sepsis or septic shock (1); about 30% of AKI cases in ICU are due to nephrotoxic drugs (10). A high mortality rate and extensive hospital costs are also reported, besides additional costs estimated at $42,000 per case complicated by AKI in the US (11). Therefore, timely information on renal function is of high importance to early initiate nephroprotective strategies. Although several biomarkers of kidney damage have been developed, they have not made it to clinical routine. Currently, only two biomarkers for kidney function are traditionally used to define AKI: serum creatinine (sCr) and urine output. However, they give partial and delayed information on changes during kidney injury, and have low sensitivity and specificity (2).

Scientific experts agree on the significant need for new biomarkers that timely mirror kidney function, thereby improving the prediction and monitoring of AKI, major adverse kidney events (MAKE), and the necessity of renal replacement therapy (RRT) (2). In addition, many other concrete clinical cases may also benefit from these developments, for instance, guidance to start or stop RRT, the guidance of nephrotoxic drug administration, hospitalization decisions after catheterization-laboratory procedures, the prediction of delayed graft function, and assessment of contrast-induced nephropathy.

GFR is considered to be the best indicator of kidney function. However, the gold standard technique for determining the true GFR is based on complex, time-consuming, expensive and invasive interventions using inulin, iothalamate or iohexol that are not feasible in daily practice. Today’s routine standard relies on determining an estimated GFR (12). However, this method is affected by non-renal factors (age, gender, muscle mass, and others) and based on sCr. The inability of sCr to detect mild kidney insufficiency is because its levels only begin to rise above the normal value when approximately 50% of renal function is already lost; this is known as the creatinine-blind area (13).

PenKid is the new biomarker for kidney function detectable by sphingotest® penKid® immunoassays and overcomes routine standards’ limitations (3, 7, 14). PenKid is a stable prohormone fragment of the proenkephalin A precursor molecule. It is released into the blood by the kidney and heart and produced in equal molar concentrations to unstable enkephalin – the kidney-stimulating hormone. Studies have shown that penKid strongly correlates with the true GFR (6, 7, 15).

Hollinger and colleagues showed that penKid predicts the future change in serum creatinine up to two days in advance independently from common comorbidities (e.g. CKD, Hypertension, and Diabetes Mellitus) (4), therefore providing physicians with urgently needed information on top of standard of care.
PenKid has been evaluated as a biomarker of kidney function in several clinical conditions (4-6). The assessment of penKid identifies patients at high risk of AKI in EDs and ICUs and delivers information on the GFR in healthy and hospitalized patients (4, 5, 7). PenKid values measured with the sphingotest® penKid® reflect the true GFR, being specific to kidney function and not influenced by inflammation (5).

**PenKid robustness and value in patients with AKI**

**PenKid is a reliable surrogate for measuring true GFR (6)**

**PenKid has high specificity for renal function (5)**

**PenKid predicts the incidence of AKI within 48h (4)**

**PenKid to monitor survival in septic patients (5)**

**Clinical Significance**

PenKid robustness and value in patients with AKI

PenKid is a reliable surrogate for measuring true GFR (6)

PenKid has high specificity for renal function (5)

PenKid predicts the incidence of AKI within 48h (4)

PenKid to monitor survival in septic patients (5)

**Figure 1.** Correlation of penKid concentration as a function of measured GFR (iothalamate clearance). Modified from (6).

**Figure 2.** PenKid’s correlation with renal SOFA score in septic patients. PenKid increased only upon renal dysfunction (subscores 1 – 4), remaining normal even if massive inflammation is present, as in sepsis or septic shock (subscore 0) (5). Line indicates the upper normal range.

**Figure 3.** PenKid concentration in septic or septic shock patients admitted to the ICU (boxplot). According to AKI levels, penKid shows a stepwise increase with AKI severity (P<0.001) (4).

**Figure 4.** Kaplan-Meier survival curves up to 90 days for patients divided into four groups according to their penKid concentrations on days 1 and 7, below or above a pre-defined clinical cut-off of 100 pmol/L. There is a clear indication of mortality risk with increasing penKid concentrations (red and orange lines) (5).
**PenKid measurement**

Sphingotest® penKid® is a CE-IVD marked quantitative immunoassay available as chemiluminescence-based immunoassay format or as a test on the Nexus IB10 point-of-care technology (POCT) (IB10 sphingotest® penKid®).

**Measurement principle**

**Healthy state**

- Kidney Stimulation
- Proenkephalin
- Enkephalins

Enkephalin stimulates kidney function

Kidney function is stimulated by the hormone enkephalin which remains hard to detect. Sphingotest® penKid® overcomes this limitation by measuring a stable fragment that results out of the kidney’s enkephalin production.

**Disease state**

- Kidney Stimulation
- Proenkephalin
- Enkephalin 119-159 (penKid)

Loss of kidney function triggers a compensatory stimulation and enkephalin levels rise

When kidney function is low, enkephalin levels rise to stimulate the kidneys. By indirectly measuring the enkephalin production, high penKid levels indicate an impaired kidney function.

**Measuring platforms**

- **Nexus IB10®** for penKid at the bedside
  - Nexus IB10®: is a rapid and user-friendly POCT; fully automated assay for bedside testing.
  - **Results:** in 20 minutes
    - Detection range: 50-500 pmol/L
    - Sample volume: 500 µL EDTA whole blood or plasma

- **Microtiter plate (MTP)** for penKid at the hospital laboratory
  - MTP: is an appropriate platform for high throughput analysis; manual assay for the laboratory.
  - **Results:** in 1 hour
    - Detection range: 30-1,200 pmol/L
    - Sample volume: 50 µL EDTA plasma
More than 30 peer-reviewed publications and additional submitted clinical studies have investigated the significance of penKid biomarker in more than 40,000 patients allowing a clear understanding of penKid distribution and its clinical value of in critical care.

PenKid has already compiled clinical evidence for many indications in critical care and beyond, proving value to the existing standard of care markers.

### Table 1: Main clinical published indications where penKid showed value as a kidney function marker.

<table>
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<tr>
<th>Clinical indication</th>
<th>Outcome parameters</th>
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<td><strong>AKI in sepsis</strong></td>
<td>ED: progression of renal SOFA, 28-day mortality (17); 7-day mortality in ED (18). ICU: MAKE, transient AKI, WRF, renal recovery (4); the need for RRT, improvement in renal function, 90-day mortality (5); the need for RRT, 30-day mortality (19).</td>
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<td><strong>Heart failure</strong></td>
<td>Acute: WRF, in-hospital mortality (20); 1-year mortality, heart failure rehospitalization, WRF (21); WRF, 180-day mortality (22). Chronic: MACE (23); AMI, MACE, hospitalization, 2-years mortality (24); HFpEF patients, rehospitalization at 2 years (25).</td>
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<tr>
<td><strong>AKI after major surgery</strong></td>
<td>Cardiac surgery: prediction of AKI at 48h (26); TAAA: in-hospital mortality at 12h post-surgery (AUC = 0.827); increased peri-operative penKid levels in patients with AKI at 48h (27).</td>
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<td><strong>Organ transplantation</strong></td>
<td>Predicting risk of graft failure in renal transplant receptors (AUC 0.87) (28).</td>
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<td><strong>Renal replacement therapy in sepsis</strong></td>
<td>Need for RRT on day one or later during hospitalization (P&lt;0.0001; AUC 0.755) (5,14).</td>
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<td><strong>Pediatric AKI (&lt; 1-year-old)</strong></td>
<td>Establishment of penKid reference value in healthy infants; discrimination between AKI and non-AKI children (KDIGO) (p&lt;0.001) (16).</td>
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<td><strong>Burned patients</strong></td>
<td>PenKid highly associated with 90-day mortality (p=0.0001) and with the development of AKI (p&lt;0.0001) (29).</td>
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- **WRF**: worsening of renal function.
- **MACE**: major adverse cardiac events.
- **MAKE**: major acute kidney events.
- **HFpEF**: heart failure with preserved ejection fraction.
- **AMI**: acute myocardial infarction.
- **TAAA**: thoraco-abdominal aortic aneurysm.
Summary

- PenKid is an innovative biomarker aiding in the early diagnosis of AKI in critically ill patients.
- Publications showed that penKid correlates with the gold standard of the true GFR and enables early assessment of worsening and improving kidney function.
- PenKid has been accessed in over 40,000 patients.
- Scientific evidences suggested that penKid blood level is not influenced by inflammation.

Disease state
Impaired kidney function, acute kidney injury

Healthy state:
Normal kidney function

Scheme displaying the rise of penKid blood levels (1) predicting AKI up to 48 hours earlier than today's standard of care; the decrease of penKid levels (2) indicates the normalization of renal function.

Literature showing kidney function assessment with penKid

Clinical settings already evaluated
- Acute myocardial infarction (24).
- AKI (7).
- Burns (29).
- Cardiac arrest (30).
- Cardiac surgery (26, 27, 31).
- Cardiogenic shock (32).
- Chronic kidney disease (33, 34).
- Contrast-induced nephrotoxicity (35).
- ICU (16, 40-42).
- Renal transplantation (28).
- Sepsis (4, 5, 14, 15, 17-19, 43-45).

Other potential applications where penKid might benefit
- Follow kidney functions during RRT.
- Guidance of nephrotoxic drug administration.
- ICU discharge.
- Management of patients attending catheterization intervention.
- Prediction of AKI after heart surgery.
- Risk stratification before contrast media application.
- Triaging.
References


### Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<td>AKI</td>
<td>Acute kidney injury</td>
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<tr>
<td>AMI</td>
<td>Acute myocardial infarction</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<td>CKD</td>
<td>Chronic kidney disease</td>
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<td>ED</td>
<td>Emergency department</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<td>GFR</td>
<td>Glomerular filtration rate</td>
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<td>HFpEF</td>
<td>Heart failure with preserved ejection fraction</td>
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<td>ICU</td>
<td>Intensive care unit</td>
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<td>KDIGO</td>
<td>Kidney disease: improving global outcomes</td>
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<tr>
<td>MACE</td>
<td>Major adverse cardiac event</td>
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<tr>
<td>MAKE</td>
<td>Major adverse kidney events</td>
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<td>MTP</td>
<td>Microtiter plate</td>
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<tr>
<td>PenKid</td>
<td>Proenkephalin A 119-159</td>
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<td>RRT</td>
<td>Renal replacement therapy</td>
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<td>POC</td>
<td>Point-of-care</td>
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<td>POCT</td>
<td>Point-of-care technology</td>
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<tr>
<td>SOFA</td>
<td>Sepsis-relate organ failure assessment</td>
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<td>sCr</td>
<td>Serum creatinine</td>
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<tr>
<td>TAAA</td>
<td>Thoraco-abdominal aortic aneurysm</td>
</tr>
<tr>
<td>WRF</td>
<td>Worsening of renal function</td>
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Disclaimer

This communication is for educational use only. It is not intended to guide or imply medical decision making.

CE-IVD-marked diagnostic immunoassays for penKid, namely sphingotest® penKid® and IB10 sphingotest® penKid®, are intended for the in vitro quantitative determination of Proenkephalin A 119-159 in human EDTA whole blood and/or plasma.

sphingotest® penKid® and IB10 sphingotest® penKid® are designed for professional use only and may be used in hospital central laboratories (sphingotest® penKid® and IB10 sphingotest® penKid®) or in alternate care settings such as emergency departments, critical care units, and other sites where near-patient testing is practiced (IB10 sphingotest® penKid®).

The results of sphingotest® penKid® and IB10 sphingotest® penKid® should be evaluated and interpreted in conjunction with the patient’s medical history, symptoms and other clinical information.

sphingotest® penKid® and IB10 sphingotest® penKid® are CE-IVD-marked and hence certified and approved for use in the European Economic Area (EEA) only. Human diagnostic use of such products may be subject to local regulations.

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SphingoTec GmbH
Neuendorfstr. 15A, 16761 Hennigsdorf
Telefon: +49 3302 20565 0
E-Mail: info@sphingotec.com
www.sphingotec.com