Point-of-Care Urinary Biomarker Testing for Risk Prediction in Critically Injured Combat Casualties

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BACKGROUND: Risk prediction is important during combat operations because resources are limited and triage decisions must be rapid and accurate. We evaluated 2 point-of-care urinary biomarker tests for risk prediction in combat casualties.

STUDY DESIGN: This was an observational cohort study of critically injured military personnel admitted to Craig Joint Theater Hospital in Afghanistan from October 2012 to December 2013. We collected urine within 3 hours of admission and measured urinary biomarkers with NephroCheck and a neutrophil gelatinase-associated lipocalin dipstick (NGALds) to evaluate their ability to predict a combined end point of need for renal replacement therapy or death. Odds ratios (ORs) were calculated and receiver operator characteristic curves were generated for both tests.

RESULTS: A total of 89 patients were included for analysis. The median Injury Severity Score was 18 and the combined end point occurred in 12 (13.5%) patients. NephroCheck was not associated with the combined end point (OR 1.56; 95% CI 0.81 to 3.03; $p = 0.19$) and the area under the curve of the receiver operator characteristic curve was 0.65. The NGALds was highly associated with the combined end point (OR 4.93; 95% CI 2.18 to 11.14; $p < 0.001$) and the area under the curve of the receiver operator characteristic curve was 0.84. The NGALds remained significantly associated with the combined end point in a logistic regression model that included Injury Severity Score as a covariate (OR 4.10; 95% CI 1.74 to 9.67; $p = 0.001$).

CONCLUSIONS: Measurement of urinary biomarkers with an NGALds, but not NephroCheck, predicts poor outcomes in combat casualties. An NGALds is a simple urine dipstick that could be deployed to combat zones to prioritize aeromedical evacuation, help with triage decisions, and predict resource use. (J Am Coll Surg 2019;229:508−515. Published by Elsevier Inc. on behalf of the American College of Surgeons.)

CME questions for this article available at http://jacscme.facs.org

Disclosure information: Authors have nothing to disclose. Timothy J Eberlein, Editor-in-Chief, has nothing to disclose.

Support: This study was supported by the US Air Force, Headquarters, Office of the Surgeon General.

Disclaimer: The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Air Force, the Department of the Army, the Department of Defense, or the United States Government. The NGALds kits were provided free of charge by the manufacturer. BioPorto Diagnostics did not have any role or input into the study design or analysis. The authors have no other disclosures or conflicts of interest to report.

Presented at the Military Health System Research Symposium, Kissimmee, FL, August 2018; American College of Surgeons Region 13 Resident Trauma Paper Competition, Boston, MA, October 2018.

Received April 25, 2019; Accepted July 8, 2019.

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https://doi.org/10.1016/j.jamcollsurg.2019.07.003
ISSN 1072-7515/19

Abbreviations and Acronyms

AKI = acute kidney injury
AUC = area under the curve
CJTH = Craig Joint Theater Hospital
IGFBP7 = insulin-like growth factor binding protein-7
ISS = Injury Severity Score
NGALds = neutrophil gelatinase-associated lipocalin dipstick
OR = odds ratio
RRT = renal replacement therapy
TIMP-2 = tissue inhibitor of metalloproteinase-2
UB = urinary biomarker

Systematic and objective prediction of a patient's clinical course based on admission variables has been a goal of critical care research for many years. Although initial efforts were focused solely on prediction of mortality, risk prediction models have become more complex over time and now include forecasts for individual organ systems and hospital lengths of stay.[1] Accurate risk prediction models facilitate improved prognostication for patients and families, healthcare resource allocation, and group comparisons for quality improvement. For example, the standardized mortality ratio compares the expected and observed mortality rates and is a mandatory measure of ICU quality in the Veterans Health Administration.[2]

Risk prediction is especially important in a combat zone, where resources are limited and triage decisions must be rapid and accurate. The current era of combat casualty care, characterized by readily available aeromedical evacuation for the vast majority of casualties, can be replaced with increasing instances of prolonged field care.[3,4] Patient selection for transport is paramount, as resource-intensive treatments, such as renal replacement therapy (RRT), are limited in the deployed environment.

Current risk prediction in trauma patients is primarily accomplished with anatomic scoring systems and the use of basic physiologic data, such as vital signs. Popular examples include the Injury Severity Score (ISS), Revised Trauma Score, and Trauma Injury Severity Score.[5-7] The integration of laboratory data into trauma scoring systems can improve their prognostic capabilities.[8] Urinary biomarkers (UBs) can also have a role to play in risk prediction for trauma patients and are attractive due to their non-invasive nature.[9] Although previous work has shown that UBs offer useful prognostic information in combat casualties, those methods were designed for a research laboratory environment.[10]

Given the limited utility of benchtop assays in the austere setting of a combat zone, we sought to evaluate the prediction potential of field ready, point-of-care tests of UBs. The NephroCheck (Astute Medical) test evaluates the levels of tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor binding protein-7 (IGFBP7) in urine and NGALds is a dipstick test for urine neutrophil gelatinase-associated lipocalin (BioPorto Diagnostics). Tissue inhibitor of metalloproteinase-2 and IGFBP7 are G_1 cell-cycle arrest proteins. Under normal conditions, they are minimally expressed by renal tubular cells. However, they are produced by the tubular cells in response to stress or renal damage due to a wide variety of insults.[11] Neutrophil gelatinase-associated lipocalin is a siderophore that binds iron and is believed to be a component of innate immunity. Similar to TIMP-2 and IGFBP7, it is minimally expressed under normal conditions, but is upregulated in the setting of ischemic or nephrotoxic acute kidney injury (AKI).[12] We hypothesized that NephroCheck and NGALds would predict a combined end point defined as the need for RRT or death.

METHODS

The study was approved by the US Army Medical Research and Materiel Command IRB. Informed consent was deemed necessary if the subject could provide consent. However, given the inability to access legally authorized representatives and the low-risk nature of the study, this requirement was waived for patients unable to consent. This was an observational cohort study of injured US military personnel admitted to Craig Joint Theater Hospital (CJTH) in Bagram Airfield, Afghanistan, from October 2012 to December 2013. The methods used in this study have been described previously.[10] Patients with a traumatic injury requiring ICU level of care were included. Patients were excluded for anuria, the absence of a Foley catheter, admission to the ICU more than 48 hours after injury, or the failure to obtain a urine sample within 3 hours of admission.

Data were collected both prospectively and retrospectively. Data on demographics, ISS, blood transfusions, and laboratory values were collected prospectively by the investigators at CJTH. Massive transfusion was defined as receiving 10 or more units of packed RBCs in a 24-hour period. Data on events that occurred after evacuation from CJTH, including need for RRT and in-hospital death, were collected retrospectively. Renal replacement therapy was defined as any use of intermittent or continuous hemodialysis, hemofiltration, or peritoneal dialysis. Specific criteria to start RRT were not part of the protocol and physicians who were not involved in the study determined the need for RRT. Within 1 hour of collection, urine samples were centrifuged at 2,000*g* for 10 minutes and then frozen at −80°C. Samples were

batched and shipped back to the US. A temperature logger was included in the shipment to ensure that the samples remained at a temperature below −40°C during transport. The samples were subsequently stored at −80°C and thawed immediately before analysis in laboratories in the US.

The TIMP-2 and IGFBP7 were measured using NephroCheck according to the manufacturer's recommendation. The NephroCheck test score is calculated as the product of the measured concentrations of the 2 biomarkers, measured in ng/mL, divided by 1,000. The score can range from 0.04 to 10.[13] For sensitivity and specificity calculations, we used the manufacturer's cutoff of a score >0.3 for the NephroCheck test. Urine NGAL was measured using the NGALds kit according to the manufacturer's protocol. Briefly, all reagents were brought to room temperature before use and 3 drops of buffer were added to the provided tube containing a pellet. Ten microliters of urine were added to the tube using equipment included in the kit, which was then incubated for 5 minutes. The dipstick was then placed in the urine mixture for 10 minutes. Each dipstick was scored visually by comparing the intensity of the test line with the intensity of the manufacturer's control line. This score was recorded according to the manufacturer's scale as either low risk (<50 ng/mL), inconclusive risk (50 to 150 ng/mL), moderate risk (150 to 300 ng/mL), or high risk (>300 ng/mL) by 3 different investigators. The same 3 investigators performed all scoring and were blinded to each other's scores. The mode of their scores was used for subsequent analysis. Representative dipsticks from each risk level are show in eFigure 1.

The combined end point was defined as either the need for RRT or death during initial hospitalization. We used the Kidney Disease Improving Global Outcomes criteria to define the presence of AKI.[14] Results are presented as mean ± SD or median (interquartile range) for parametric and non-parametric results, respectively. Continuous variables were compared using *t*-tests when normally distributed, or Wilcoxon rank-sum tests otherwise. Categorical variables were compared using Fisher's exact test. The κ-statistics were calculated to evaluate agreement in visual dipstick scoring between investigators for NGALds. Odds ratios (OR) were calculated considering the score of each diagnostic test as a continuous variable. Receiver operator characteristic curves were generated for both tests and areas under the curves (AUCs) were calculated to assess diagnostic capability. For diagnostic tests that showed predictive value, a logistic regression model including ISS was performed to assess whether the test provided additional information beyond existing scoring systems. A p value <0.05 was considered significant. Statistical analysis was performed using STATA, version 14.2 (StataCorp).

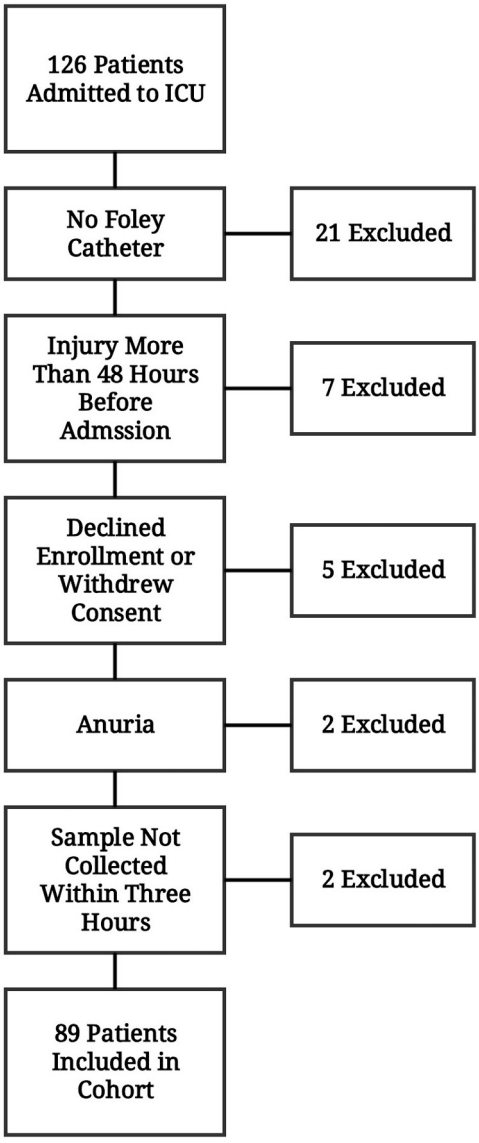

Figure 1. Cohort derivation of critically injured patients at Craig Joint Theater Hospital, Bagram Airfield, Afghanistan.

RESULTS

During the study period, 126 patients were admitted to the ICU at CJTH and 37 patients were excluded. Patients were excluded for not having a Foley catheter (21 patients), injury more than 48 hours before admission (7 patients), declined enrollment (4 patients), anuria (2 patients), urine sample not collected within 3 hours (2 patients), or withdrawal of consent (1 patient). A total of 89 patients were included in the final analysis (Fig. 1).

The baseline characteristics and rates of AKI in this cohort have been described previously and are presented in Table 1.[10,15] Most subjects were males (96.6%) aged 26.9 ± 5.3 years. The median ISS was 18 (interquartile range 11 to 38), denoting severe injury. Median serum lactate concentration at admission was 1.8 mmol/L (interquartile range 1.1 to 2.9 mmol/L), and a massive transfusion was required in 37.1% of patients. Patients with the combined end point had higher ISS, higher serum lactate concentration at admission, and a greater proportion receiving massive transfusion. Six (6.7%) patients required RRT and 9 (10.1%) patients died. Median hospital day of death was 4 (interquartile range 3 to 13) and ranged from days 2 to 38. Three of the 6 patients who received RRT died. The combined end point occurred in 12 (13.5%) patients. Urine samples were drawn 19.7 ± 10.8 hours after injury. Eighteen percent (n = 16) of patients had AKI when urine was sampled. Acute kidney injury developed later in 17 additional patients, resulting in an overall AKI rate of 37.1%.

The NephroCheck device had an error message for 2 study subjects, therefore, this analysis included only 87 patients. There were 57 patients with a NephroCheck test score <0.3, and the combined end point developed in 5 (9%) in this group. In the group of patients with a NephroCheck test score above the cutoff, the combined end point did not develop in 20 (74%) patients, and did develop in 7 (26%) patients. The sensitivity, specificity, positive predictive value, and negative predictive value were 58%, 74%, 26%, and 92%, respectively. When considered as a continuous variable, the NephroCheck test score was not associated with the combined end point (OR 1.56; 95% CI 0.81 to 3.03; p = 0.19). The receiver operator characteristic curve generated for the NephroCheck test is shown in Figure 2 and the AUC is 0.65 (95% CI 0.44 to 0.86).

There was substantial interrater agreement for visual scoring of the NGALds (κ = 0.71, SE 0.04; p < 0.001). Table 2 shows the frequency of the combined end point stratified by NGALds level. The sensitivity, specificity, positive predictive value, and negative predictive value at the different NGALds cutoffs are shown in Table 3. At the point of optimal performance (moderate risk or higher), the NGALds had sensitivity of 75%, specificity of 82%, positive predictive value of 39%, and negative predictive value of 95%. When considered as a continuous variable, the NGALds test was highly associated with the combined end point (OR 4.93; 95% CI 2.18 to 11.14; p < 0.001). The receiver operator characteristic curve generated for the NGALds test is shown in Figure 2 and the AUC is 0.84 (95% CI 0.71 to 0.98). The NGALds score remained significantly associated with the combined end point in a logistic regression model that included ISS as a covariate (OR 4.10; 95% CI 1.74 to 9.67; p = 0.001). When the AUCs of the NephroCheck and NGALds were compared, however, the difference was not significant (p = 0.073).

DISCUSSION

Urine measurement within 3 hours of admission to the ICU with the NGALds test, unlike the NephroCheck system, was predictive of need for RRT or death in combat casualties. In addition, this relationship remained significant, even after adjusting for injury severity. This suggests that NGALds could potentially be integrated into existing trauma risk prediction models to improve their accuracy. Additionally, this point-of-care urine dipstick does not require specialized equipment or electricity. Therefore, it can be deployed to help prioritize aeromedical evacuation and predict the need for RRT in the austere environment of a combat zone.

Table 1. Baseline Characteristics of 89 Critically Injured Patients at Craig Joint Theater Hospital, Bagram Airfield, Afghanistan

Characteristic	Full cohort	Without combined end point* (n = 77)	With combined end point* (n = 12)	p Value
Age, y, mean ± SD	26.9 ± 5.3	26.8 ± 5.4	27.8 ± 4.9	0.57
Male, %	96.6	96.1	100	1
Injury Severity Score, median (IQR)	18 (11–38)	18 (9–34)	46 (28.5–67)	0.001
Lactate, mmol/L, median (IQR)	1.8 (1.1–2.9)	1.5 (1.1–2.5)	4.3 (2.1–6.7)	0.001
Massive transfusion, %	37.1	31.2	75.0	0.008
Acute kidney injury, %	37.1	27.3	100.0	—
Renal replacement therapy, %	6.7	0.0	50.0	—
Mortality, %	10.1	0.0	75.0	—
Combined end point,* %	13.5	0.0	100.0	—

*Combined end point is need for renal replacement therapy or death.
IQR, interquartile range.

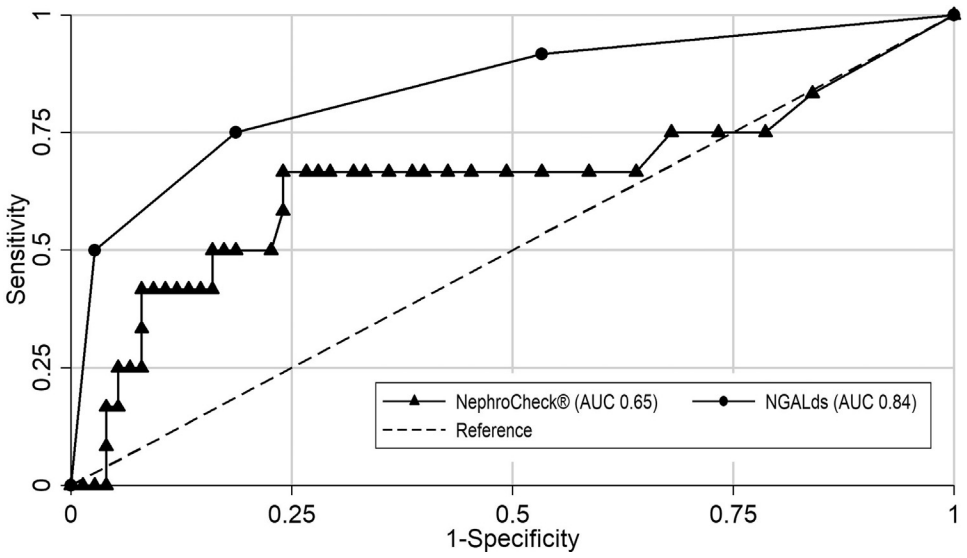

Figure 2. NephroCheck and neutrophil gelatinase-associated lipocalin dipstick (NGALds) receiver operator characteristic curves. The area under the curve (AUC) for the NephroCheck test is 0.65 and the AUC for the NGALds test is 0.84.

The NephroCheck system is dependent on the concentrations of TIMP-2 and IGFBP7. These UBs are associated with G_1 cell-cycle arrest events early after renal tubular cell injury.[16,17] The SAPPHIRE and TOPAZ trials validated this combination panel as a risk stratification tool for the prediction of moderate to severe AKI in patients admitted to the ICU with AUCs of 0.80 and 0.82, respectively.[11,18] The NephroCheck test score performed similarly well for predicting AKI in sub-groups with different comorbidities.[19] In a secondary analysis of the SAPPHIRE trial, TIMP-2 and IGFBP7 levels were associated with a combined end point of mortality and need for RRT at 9 months after hospitalization.[20]

It is unclear why the NephroCheck test did not perform as well in our study. One possible explanation for our results is that AKI prediction was not the end point used in this study, although a link between AKI and these outcomes has been described previously in combat casualties.[21,22] In addition, in a cohort of patients experiencing out-of-hospital cardiac arrest, TIMP-2 and IGFBP7 urine concentrations were predictive of AKI, but not mortality.[23] Another possible explanation for our findings is the unique cohort studied. The initial study validating TIMP-2 and IGFBP7 included a heterogenous civilian patient population that was 62% male with a median age of 64 years.[11] These biomarkers might not perform as well in an overwhelmingly male, young, and traumatically injured military population.

Urinary NGAL is perhaps the most widely studied UB and this study reinforces its predictive value. Neutrophil gelatinase-associated lipocalin is a small protein involved in iron transport for cell proliferation and differentiation. It is expressed at very low levels in many tissues, but is massively upregulated in injured epithelial cells.[24] Our previous work with this patient cohort showed that urinary NGAL with a cutoff of 1.51 ng/mg creatinine had a positive predictive value of 53% and a negative predictive value of 95% for the same combined end point.[10] The only other study of NGAL as a UB in trauma patients showed an AUC of 0.98 for the prediction of AKI within

Table 2. Neutrophil Gelatinase-Associated Lipocalin Dipstick Results Stratified by the Combined End Point

	Without combined end point*		With combined end point*	
NGALds visual score	**n**	**%**	**n**	**%**
Low risk (<50 ng/mL)	36	40.4	1	1.1
Inconclusive risk (50 to 150 ng/mL)	27	30.3	2	2.2
Moderate risk (150 to 300 ng/mL)	12	13.5	3	3.4
High risk (>300 ng/mL)	2	2.2	6	6.7

*Combined end point is need for renal replacement therapy or death.
NGALds, neutrophil gelatinase-associated lipocalin dipstick.

Table 3. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value at Different Neutrophil Gelatinase-Associated Lipocalin Dipstick Visual Score Cutoffs

Risk	Sensitivity	Specificity	PPV	NPV
Inconclusive risk or higher, %	92	47	21	97
Moderate risk or higher, %	75	82	39	95
High risk, %	50	97	75	93

NPV, negative predictive value; PPV, positive predictive value.

5 days after injury.[9] In a study of 90 patients with burns >20% of their total body surface area, urinary NGAL levels were independently associated with development of AKI and death.[25] In 510 general surgery patients, urinary NGAL drawn within 3 hours of operation differentiated transient and prolonged AKI with an AUC of 0.85.[26] A recent meta-analysis evaluating the capability of urinary NGAL to predict the initiation of RRT showed a pooled AUC of 0.72.[27]

The heterogeneity of diagnostic precision for urinary NGAL reflects the different populations and outcomes used in the studies. Neutrophil gelatinase-associated lipocalin is a marker of cell injury and therefore has a better capability to predict AKI compared with the initiation of RRT. The initiation of RRT is a clinical decision dependent on additional factors beyond the discrete level of renal injury, including patient comorbidities, fluid status, and electrolyte concentrations. Despite this, NGALds performed well in this study as a predictor of our combined end point, which included the initiation of RRT. In addition, the NGALds had an excellent negative predictive value at all scores, which allows increased confidence in triage decisions to continue care in the combat theater and avoid difficult or dangerous aeromedical evacuation flights. Although the role of urinary biomarker testing in civilian trauma systems remains unclear, the NGALds test could assist with decisions about which patients to transfer to higher levels of care or with triage decisions during mass casualty natural disasters, such as earthquakes.

Acute kidney injury is a diverse entity with a variety of causes, different molecular pathways, and potentially varied treatments.[28] Combat casualties are at a high risk for AKI. Identified risk factors in this population include ISS, amputation, burn injury, hypotension, and rhabdomyolysis.[22,29] Although there is an overall paucity of UB research in trauma patients, urinary NGAL seems to have a relatively better diagnostic ability in this patient population. This might reflect the specific molecular mechanisms of AKI in traumatically injured patients and could be exploited to improve risk prediction models for mortality, need for RRT, and other outcomes.[30] Future research should evaluate to what extent there are differences in urinary NGAL expression and predictive capability in trauma patients compared with other patient populations.

The effectiveness of UBs for risk prediction is constrained by the need for a urine specimen. In this study, 21 patients were excluded due to the absence of a urinary catheter. Other biomarker sources, such as saliva swabs or finger-stick blood samples, might be easier to obtain in prehospital or field conditions.[31] In addition, anuric patients cannot be tested, although anuria itself portends poor outcomes. There is also insufficient data evaluating UBs long-term predictive capabilities, although there is some evidence that urine NGAL might have the ability to predict outcomes years after the initial insult.[32,33] In the future, the best risk prediction models will likely rely on a variety of inputs, including UBs, biomarkers from other sources, physiologic data, and clinical information.

Our study has several limitations. First, we initially planned to enroll 226 subjects but were unable to achieve this due to the reduction of combat forces in Afghanistan during the study period. The smaller cohort of 89 patients limits the statistical power of the study and necessitated the use of a combined end point. Second, the NephroCheck testing was performed in August 2015 and the NGALds testing was performed in January 2018, so there is the potential for degradation of UBs in the samples during storage. However, studies have shown stability of frozen UBs with storage times ranging from 18 months to 5 years.[34,35] Additionally, the assays were run in a laboratory setting and not in the field, which might affect performance. Third, some of the data had to be collected retrospectively due to the chain of aeromedical evacuation. Last, although the NephroCheck device is approved for use, the NGALds test has not been approved by the US Food and Drug Administration and is still experimental. These limitations should be considered in the context of the resource-poor combat environment in which the study was conducted.

CONCLUSIONS

Measurement of urinary biomarkers with the NGALds, but not NephroCheck, predicted poor outcomes in

combat casualties. The NGALds is a simple, point-of-care urine dipstick that could be deployed forward to austere locations. The prognostic information provided by this non-invasive test could be used by medics or clinicians to prioritize aeromedical evacuation, help with triage decisions, and predict resource use. Additional research should focus on integrating UB results with clinical factors and other biomarkers to continue to improve risk prediction models.

Author Contributions

Study conception and design: Gómez, Tercero, Babcock, Chung, Stewart

Acquisition of data: Burmeister, Stewart

Analysis and interpretation of data: Beyer, Burmeister, Gómez, Tercero, Babcock, Walker, Hoareau, Sosnov, Chung, Stewart

Drafting of manuscript: Beyer

Critical revision: Beyer, Burmeister, Gómez, Tercero, Babcock, Walker, Hoareau, Sosnov, Chung, Stewart

Acknowledgment: The authors would like to thank the members of the US Central Command Joint Combat Casualty Research Team who assisted with data collection.

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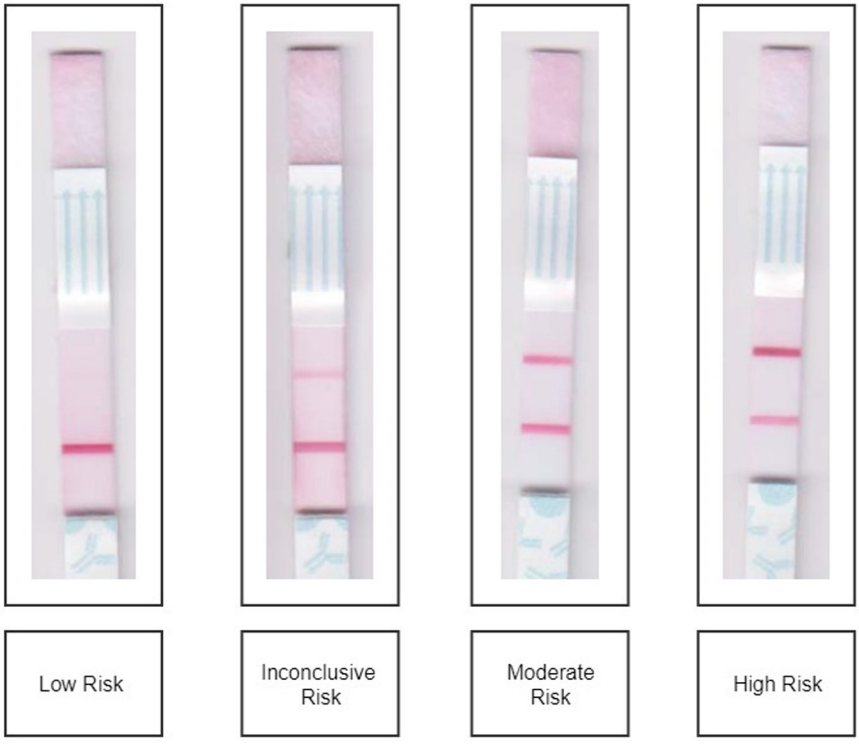

eFigure 1. Representative images of neutrophil gelatinase-associated lipocalin dipstick (NGALds) at each risk level.