# Circulating RIPK3 Levels are Associated with Mortality and Organ Failure During Critical Illness

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## Supplemental text

**Supplemental Tables: 2**

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**Supplemental Text**

**WCM:**

Study subjects were adult (≥18 years old) patients with critically illness patients hospitalized in the Biobank of Critical Illness (BOCI) cohort in the MICU of New York-Presbyterian Hospital-Weill Cornell Medical Center (WCM), New York, USA (enrollment started on 10/2014), IRB 1405015116A005. Within 48 hours after ICU admission, whole blood (10 mL) was drawn from each patient into EDTA-coated blood collection tubes (BD Pharmingen, San Jose, CA). The collected blood samples were kept at 4°C and centrifuged within 4 hours at 480g at 4°C for 10 minutes. Plasma was subsequently divided into aliquots and kept at -80°C. Patients were excluded if they were unwilling to provide biological samples for research, mentally handicapped, unable to provide informed consent, admitted merely to receive comfort care, unwilling to be transfused, or with a hemoglobin level of <7 g/dL.

**BWH**

Study subjects were adult (≥18 years old) patients admitted to the MICU at the Research Registry and Human Sample Repository for the Study of the Biology of Critical Illness (RoCI) in the MICU of Brigham and Women's Hospital (BWH), Boston, USA (enrollment started 2008, IRB 2008-P-000495). Blood samples are drawn within 24 hours of enrollment to the biobank registry in EDTA-coated Vaccutainer tubes (BD Pharmigen, San Jose, CA) and kept at 4°C. All samples were processed within 4 hours after blood collection. The samples were centrifuged at 480g at 4°C for 10 minutes. The plasma was subsequently divided into aliquots and kept frozen at -80°C for future analysis. Patients were excluded if they were unwilling to provide biological samples for research, mentally handicapped, unable to provide informed consent, admitted

merely to receive comfort care, unwilling to be transfused, or with a hemoglobin level of <8 g/dL.

**SMC**

Study subjects were adult (≥18 years old) patients admitted to the MICU at Samsung Medical Center (SMC), Seoul, Korea (enrollment started 12/2012, IRB 2012-12-033). Blood samples are drawn within 24 hours of enrollment to the biobank registry in EDTA-coated Vaccutainer tubes (BD Pharmigen, San Jose, CA) and kept at 4$^{o}$C. All samples were processed within 4 hours after blood collection. The samples were centrifuged at 480g at 4$^{o}$C for 10 minutes. The plasma was subsequently divided into aliquots and kept frozen at -80$^{o}$C for future analysis. Patients were excluded if they were unwilling to provide biological samples for research, mentally handicapped, unable to provide informed consent, admitted merely to receive comfort care, unwilling to be transfused, or with a hemoglobin level of <8 g/dL.

**ASAN**

Critically ill patients (>18 years) were enrolled in the cohorts within 24 hours of their admission in medical ICU. The following exclusion criteria were applied: cognitive impairment, inability to provide informed consent (or lack of an appropriate legal representative to do so), uncontrolled hematologic malignancy, terminal stage of solid tumor. Comprehensive clinical information, such as demographics and past medical history, was collected for enrolled patients, along with laboratory values, severity of illness scoring, presence of sepsis, ARDS and other organ failures. Within 48 hours after ICU admission, plasma was obtained.

Whole blood was drawn from each patient into EDTA-coated blood collection tubes. The samples were stored at 4$^{o}$C and centrifuged within 4 hours at 3200 rpm for 10 min. Plasma was separated and kept frozen at -80$^{o}$C until further analysis.

**PSHMC**

The Penn State Hershey Medical Center (PSHMC) Critical Illness Registry (CIR) is an active, IRB-approved, observational follow-up study being conducted in the medical intensive care unit (MICU) PSHMC in which critically ill patients are enrolled on a rolling basis within 48 hours of their admission to the medical ICU. For all study subjects, we abstracted clinical information daily from the electronic medical record throughout the duration of each subject's admission to the ICU. We collected follow up regarding mortality and functional status at both 28-days and 1-year following admission to the ICU. Blood samples were drawn within 48 hours of ICU admission in $K_2$EDTA-coated vacutainer tubes ( ). The samples were centrifuged at 2000g for 10 minutes and subsequently divided into aliquots and kept frozen at -80$^{o}$C for future analysis.

Extensive phenotypic data (such as demographics, medical history, laboratory parameters, and admission diagnosis or infection status) was prospectively collected and recorded.

**Table E1:** Distribution of individual SOFA components among all cohorts

| | | WCM | BWH | SMC | ASAN | PSHMC | Overall |
|---|---|---|---|---|---|---|---|
| **Characteristic** | | | | | | | |
| Respiratory | Score | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) |
| | 0 | 44 (28) | 53 (33) | 62 (25) | 12 (4) | 3 (3) | 174 (19) |
| | 1 | 23 (15) | 26 (16) | 41 (16) | 27 (10) | 5 (5) | 123 (13) |
| | 2 | 47 (30) | 49 (31) | 83 (33) | 68 (25) | 49 (49) | 296 (32) |
| | 3 | 31 (20) | 19 (12) | 42 (17) | 107 (40) | 28 (28) | 226 (24) |
| | 4 | 10 (6) | 12 (8) | 23 (9) | 54 (20) | 14 (14) | 113 (12) |
| | N/A | 0 (0) | 0 (0) | 0 (0) | 1 (0) | 21 (18) | 22 (2) |
| Cardiovascular | | | | | | | |
| | 0 | 29 (19) | 56 (35) | 60 (24) | 37 (14) | 47 (39) | 229 (24) |
| | 1 | 54 (35) | 51 (32) | 68 (27) | 38 (14) | 26 (22) | 238 (25) |
| | 2 | 20 (13) | 1 (1) | 4 (2) | 5 (2) | 1 (2) | 31 (3) |
| | 3 | 8 (5) | 51 (32) | 35 (14) | 49 (18) | 27 (22) | 169 (18) |
| | 4 | 44 (28) | 0 (0) | 84 (33) | 139 (52) | 19 (13) | 286 (30) |
| | N/A | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (0) |
| Neurologic | | | | | | | |
| | 0 | 81 (54) | 89 (56) | 187 (75) | 75 (28) | 41 (34) | 473 (50) |
| | 1 | 14 (9) | 18 (11) | 21 (8) | 158 (59) | 20 (17) | 231 (24) |
| | 2 | 23 (15) | 10 (6) | 9 (4) | 24 (9) | 9 (8) | 75 (8) |
| | 3 | 22 (15) | 20 (13) | 18 (7) | 11 (4) | 48 (40) | 119 (13) |
| | 4 | 9 (6) | 22 (14) | 16 (6) | 0 (0) | 2 (2) | 49 (5) |
| | N/A | 6 (4) | 0 (0) | 0 (0) | 1 (0) | 0 (0) | 7 (1) |
| Hematologic | | | | | | | |
| | 0 | 69 (45) | 87 (55) | 141 (56) | 118 (44) | 75 (62) | 490 (51) |
| | 1 | 26 (17) | 33 (21) | 37 (15) | 43 (16) | 13 (11) | 152 (16) |
| | 2 | 25 (16) | 25 (16) | 39 (16) | 54 (20) | 16 (13) | 159 (17) |
| | 3 | 20 (13) | 6 (4) | 23 (9) | 33 (12) | 11 (9) | 93 (10) |
| | 4 | 15 (10) | 8 (5) | 11 (4) | 20 (7) | 5 (4) | 59 (6) |
| | N/A | 0 (0) | 0 (0) | 0 (0) | 1 (0) | 0 (0) | 1 (0) |
| Renal | | | | | | | |
| | 0 | 53 (34) | 88 (55) | 170 (68) | 134 (50) | 45 (38) | 490 (51) |
| | 1 | 48 (31) | 28 (18) | 41 (16) | 38 (14) | 22 (18) | 177 (19) |
| | 2 | 21 (14) | 15 (9) | 16 (6) | 35 (13) | 19 (16) | 106 (11) |
| | 3 | 17 (11) | 9 (6) | 12 (5) | 16 (6) | 13 (11) | 67 (7) |
| | 4 | 16 (10) | 19 (12) | 12 (5) | 45 (17) | 21 (18) | 113 (12) |
| | N/A | 0 (0) | 0 (0) | 0 (0) | 1 (0) | 0 (0) | 1 (0) |
| Hepatic | | | | | | | |
| | 0 | 69 (50) | 114 (78) | 168 (67) | 165 (62) | 54 (64) | 570 (64) |
| | 1 | 36 (26) | 16 (11) | 29 (12) | 38 (14) | 13 (15) | 132 (15) |
| | 2 | 27 (20) | 13 (9) | 33 (13) | 35 (13) | 11 (13) | 119 (13) |
| | 3 | 4 (3) | 3 (2) | 13 (5) | 16 (6) | 3 (4) | 39 (4) |
| | 4 | 1 (1) | 1 (1) | 8 (3) | 14 (5) | 3 (4) | 27 (3) |
| | N/A | 18 (12) | 12 (8) | 0 (0) | 1 (0) | 36 (30) | 67 (7) |

**Table E2:** Univariate and Multivariate Logistic Regression of RIPK3 and Mortality for each individual cohort. APACHE II = Acute Physiology and Chronic Health Evaluation II, OR = Odds Ratio, CI = Confidence Interval. WCM = Weill Cornell Medicine, BWH = Brigham and Women's Hospital, SMC = Samsung Medical Center, ASAN = Asan Medical Center, PSHMC = Penn State Hershey Medical Center.

| | | Univariable | | Multivariable | |
|---|---|---|---|---|---|
| | | OR (95% CI) | p-value | OR (95% CI) | p-value |
| WCM | | | | | |
| | APACHE II | - | - | 1.12 (1.05, 1.19) | 0.0006 |
| | Log RIPK3 | 1.81 (1.00, 3.27) | 0.049 | 1.29 (0.69, 2.39) | 0.42 |
| BMH | | | | | |
| | APACHE II | - | - | 1.03 (0.97, 1.09) | 0.38 |
| | Log RIPK3 | 3.73 (1.13, 12.35) | 0.031 | 2.97 (0.83, 10.70) | 0.095 |
| SMC | | | | | |
| | APACHE II | - | - | 1.06 (1.02, 1.11) | 0.0080 |
| | Log RIPK3 | 4.59 (2.36,8.92) | <0.0001 | 3.09 (1.52, 6.26) | 0.0018 |
| ASAN | | | | | |
| | APACHE II | - | - | 1.07 (1.02, 1.12) | 0.0026 |
| | Log RIPK3 | 1.54 (1.17, 2.02) | 0.002 | 1.37 (1.04, 1.82) | 0.027 |
| PSHMC | | | | | |
| | APACHE II | - | - | 1.11 (1.05, 1.18) | 0.0006 |
| | Log RIPK3 | 2.63 (1.36, 5.09) | 0.004 | 1.68 (0.86, 3.28) | 0.13 |

**Figure E1:** Association between RIPK3 level and lactate for n=838 patients from WCM, BWH, SMC, ASAN. RIPK3 analyzed on the log-10 scale, and points colored by survival status. Line of best fit shown, with corresponding R-squared and F-statistic p-value.

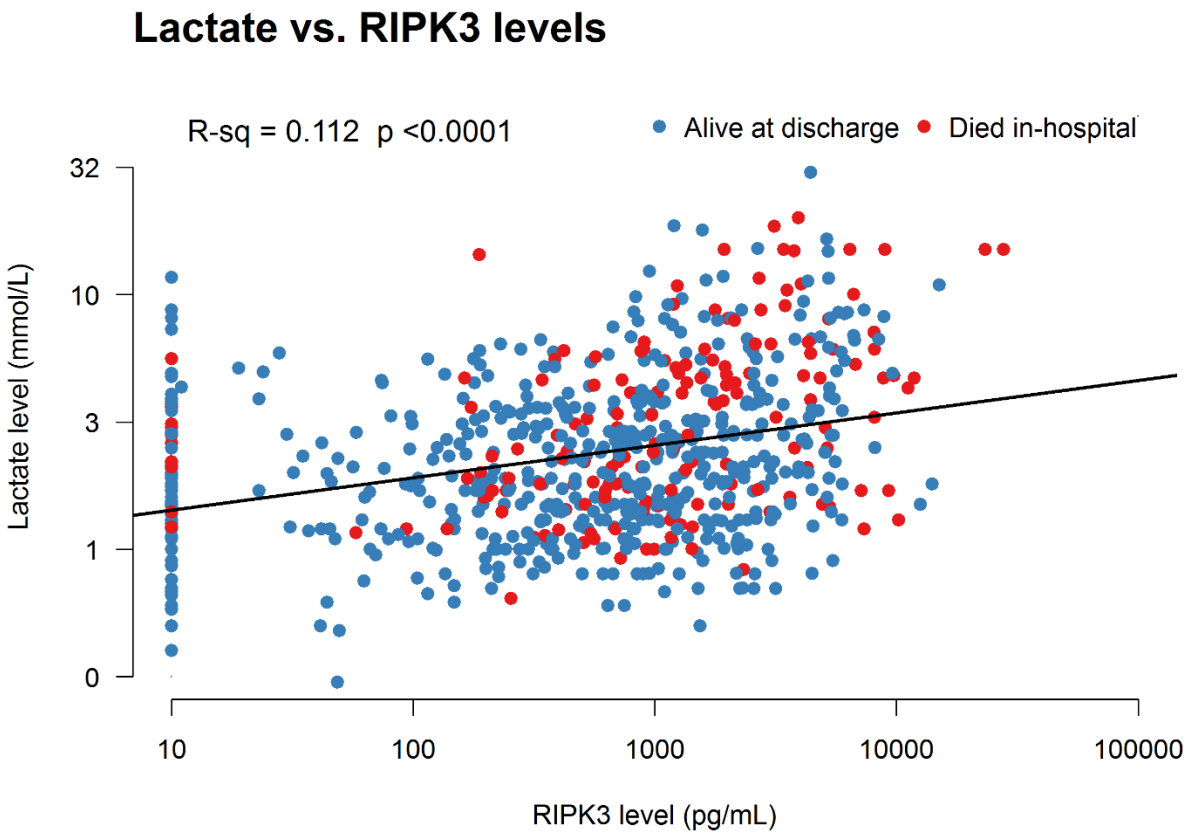

**Figure E2**: Association between RIPK3 level and LDH levels for n=121 patients in the Cornell cohort. RIPK3 and LDH both analyzed on the log-10 scale, and points colored by survival status. Line of best fit shown, with corresponding R-squared and F-statistic p-value.