Plasma catalytic iron, AKI, and death among critically-ill patients

Leaf DE *et al.*

SUPPLEMENTAL MATERIAL

Supplemental Methods

Plasma Catalytic Iron Measurement:

Plasma catalytic iron (µmol/l) was measured using the modified bleomycin assay as described by us previously.[1] The measurement of catalytic iron in plasma samples is based on the reaction of the antitumor antibiotic bleomycin with DNA in the presence of iron and suitable reducing agent to bind and degrade it. The DNA degradation products react with thiobarbituric acid to form a chromogen to be measured at 532 nm spectrophotometrically. The assay detects iron capable of catalyzing free radical reactions in plasma. A Beckman (DU800) spectrophotometer was used to measure the intensity of the colored assay product.

Plasma Free Hemoglobin Measurement:

Plasma free hemoglobin (fHb, mg/dl) was measured using an ELISA kit (catalogue # MBS 564144, "My Biosource" company). fHb present in samples is sandwiched between two anti hemoglobin antibodies, one bound to the wall of the microtitre well while the other is conjugated to horseradish peroxidase enzyme. The quantity of bound enzyme varies directly with the concentration of the fHb in the sample. The intensity of the colored product developed by the enzyme from specific chromogenic substrate added to the assay is measured quantitatively at 450 nm using a Rayto RT 2100 C microplate reader manufactured by Rayto Diagnostics, China.

Plasma Total Iron Measurement:

Total plasma iron (µg/dl) was measured using the Ferene colorimetric assay using a commercial kit (Teco Diagnostics, Anaheim, CA) following the modification used by Walmsly et al, 1992.[2] The iron in EDTA plasma was dissociated from its Fe (III)-Transferrin complex by the addition of an acidic buffer containing hydroxylamine and 7.7mmol/l zinc sulphate heptahydrate (AR grade, BDH Laboratory Chemicals). The colored Fe (II) complex with the chromogenic reagent Ferene was measured at 560 nm in a semi automated biochemistry analyzer, (Chem 7, ERBA Mannheim, Transasia).

Plasma Transferrin Measurement:

Plasma transferrin (mg/dl) was measured in an automated clinical chemistry analyser (AU480, Beckman Coulter, USA) using the commercial Immuno turbidimetry kit (OSR 6152, Beckman Coulter, USA).

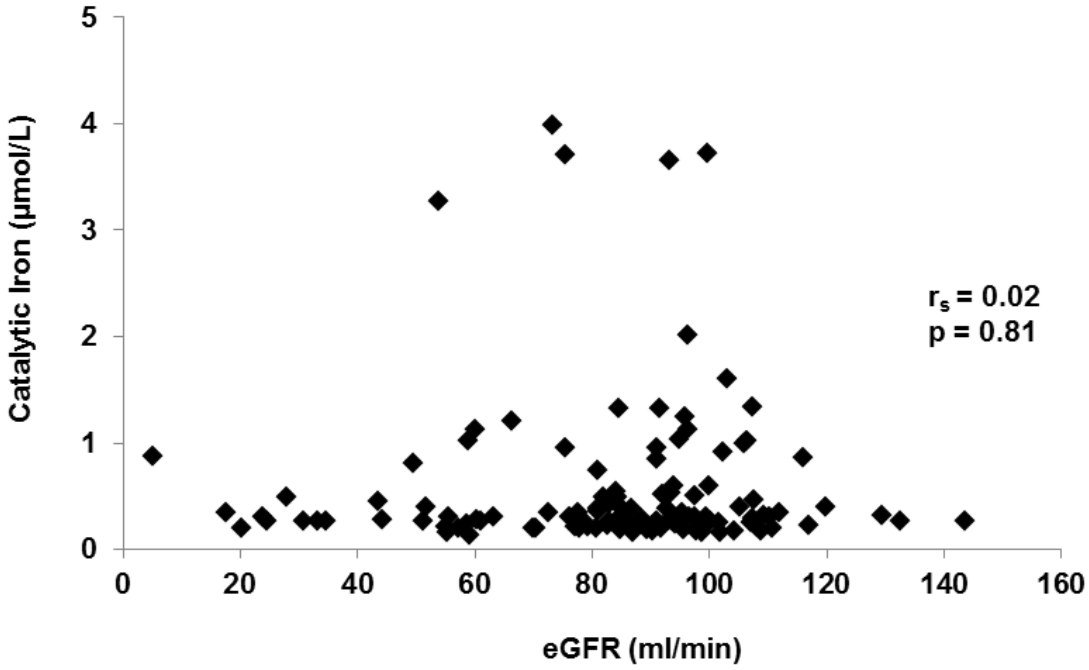
Plasma Total Iron Binding Capacity (TIBC) Estimation:

Plasma TIBC (µg/dL) was estimated from the plasma transferrin concentration, since TIBC cannot be measured directly in EDTA plasma. The estimation is based on the molecular weight of transferrin (80kD) and the known ratio of TIBC to transferrin.[3]

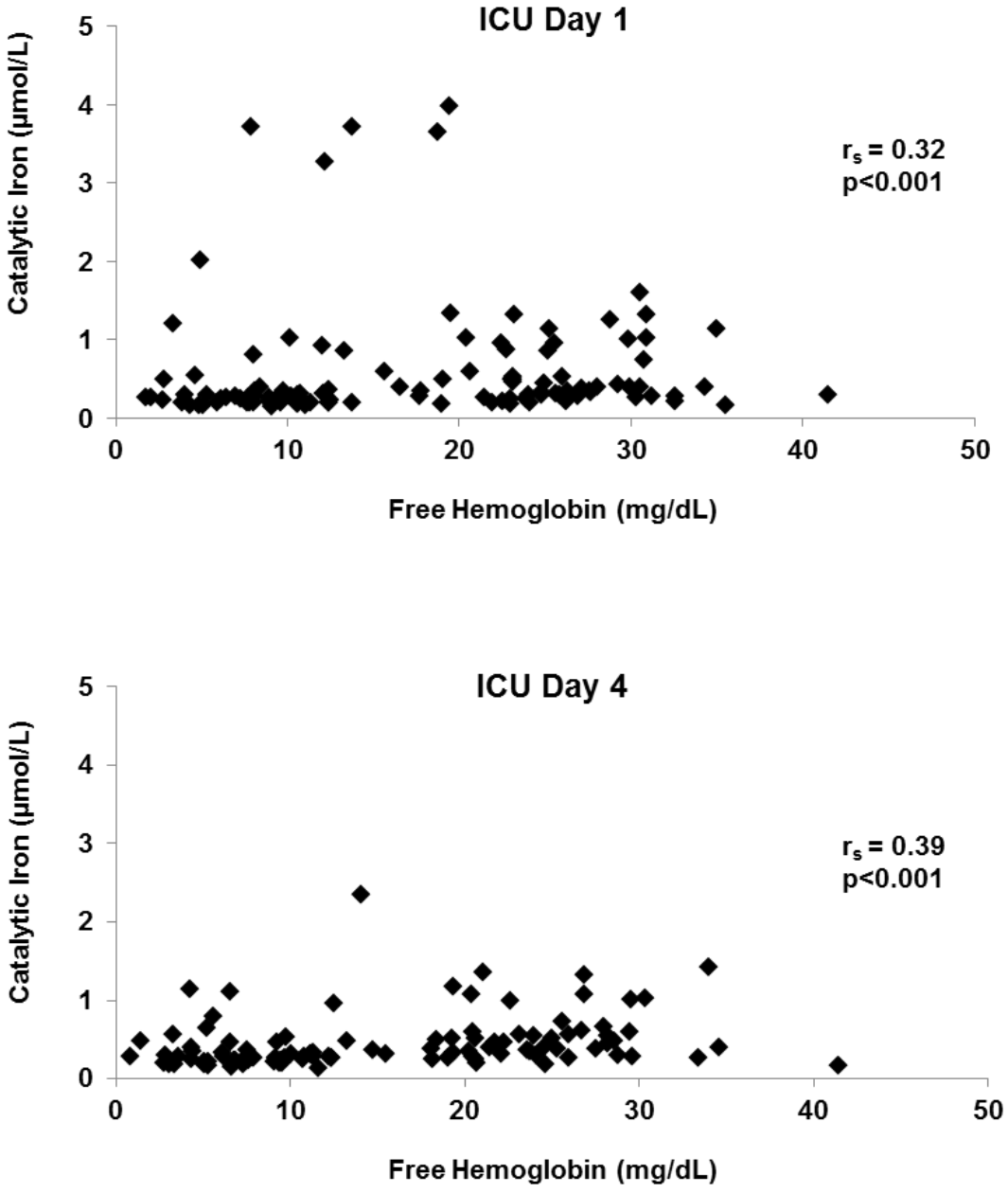
Plasma Ferritin Measurement:

Plasma ferritin (ng/ml) was measured by a two site immunoenzymometric assay kit (Tosoh Corporation, Japan) using the automate Tosoh AIA 360 analyzer. Ferritin present in the test sample was bound with the monoclonal antibody immobilized on a magnetic solid phase and enzyme labeled monoclonal antibody in the AIA Pack test cups. The magnetic beads were washed to remove the unbound enzyme labeled monoclonal antibody and were incubated with a fluorogenic substrate. The amount of enzyme labeled monoclonal antibody that binds to the beads is directly proportional to the ferritin level in the plasma sample.

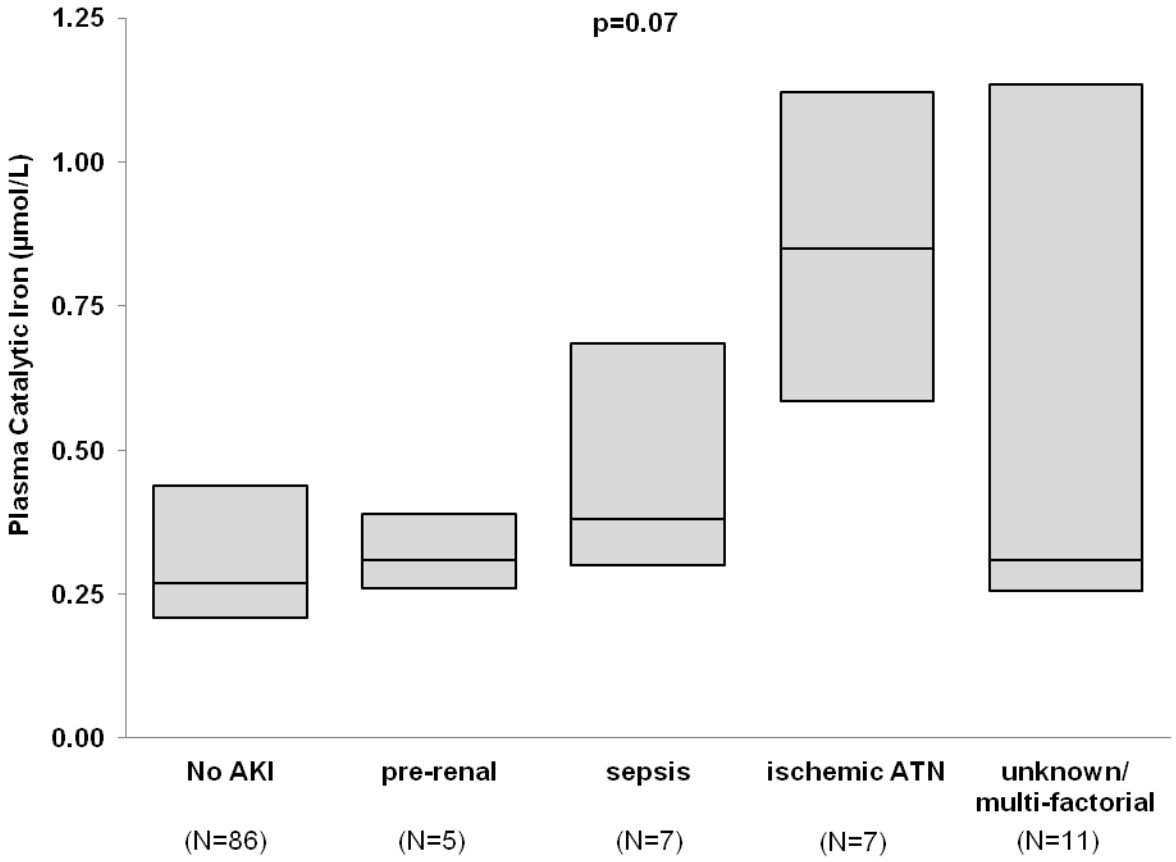
Supplemental Figures



Supplemental Figure 1. Lack of association between eGFR and plasma catalytic iron levels on ICU Day 1.



Supplemental Figure 2. Correlations between plasma catalytic iron and free hemoglobin levels on ICU Days 1 and 4



Supplemental Figure 3. ICU Day 1 plasma catalytic iron levels by AKI etiology.

Supplemental Table

	N=33
Unknown / multi-factorial	11 (33.3)
Ischemic acute tubular necrosis	7 (21.2)
Sepsis	7 (21.2)
Pre-renal azotemia	5 (15.2)
Abdominal compartment syndrome	1 (3.0)
Acute interstitial nephritis	1 (3.0)
Contrast nephropathy	1 (3.0)

Supplemental Table. Etiology of incident AKI. Data are presented as *n* (%).

Supplemental References

1. Lele S, McCullough PA, Rajapurkar M. Seum catalytic iron as a novel biomarker of vascular injury in acute coronary syndromes. EuroIntervention 2009; 5:336-342.

2. Walmsely TA, George PM, Fowler RT. Colorimetric measurement of iron in plasma samples anticoagulated with EDTA. J Clin Pathol 1992; 45:151-154.

3. Gambino R, Desvarieux E, Orth M, Matan H, Ackattupathil T, Lijoi E, Wimmer C, Bower J, Gunter E. The relation between chemically measured total iron-binding capacity concentrations and immunologically measured transferrin concentrations in human serum. Clin Chem 1997; 43(12):2408-2412.