Serial Measurement of Cell-cycle Arrest Biomarkers [TIMP-2]•[IGFBP7] and Risk for Progression to Death, Dialysis or Severe Acute Kidney Injury in Patients with Septic Shock

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ONLINE DATA SUPPLEMENT

METHODS DETAILS

Study design

The ProCESS trial investigated three resuscitation strategies (two experimental protocols versus usual care) in patients with septic shock (1). Patients admitted to the emergency department in whom sepsis was suspected according to the treating physician, who were at least 18 years of age, who met two or more criteria for systemic inflammatory response syndrome (temperature >38° C or <36° C; heart rate >90 beats per minute; respiratory rate >20 breaths per minute or PaCO2 <32 mm Hg; white blood cell count >12,000/mm3, <4,000/mm3, or >10% immature (band) forms) and who had refractory hypotension or a serum lactate level of 4 mmol per liter or higher were recruited in this trial. The “early goal-directed therapy” (EGDT) protocol used specific amounts of fluids, vasoactive medication and blood transfusions based on hemodynamic and central venous oxygen saturation targets (2). The “protocol-based standard care” (PSC) used a simpler structured fluid and vasopressor approach that targeted blood pressure, heart rate, and the simple bedside clinical assessment. In the “usual care” arm, the clinical providers performed the resuscitation strategy without any input by the study team. All three groups received aggressive, early care, albeit in different ways. The trial showed that protocolized-based resuscitation strategies did not improve outcomes in patients with septic shock as compared to usual care.

Urinary [TIMP-2]•[IGFBP7] analyses

We obtained urine samples at the time of enrollment and after 6 hours of resuscitation. We immediately centrifuged the samples and stored the supernatant at -80C, thawing

immediately prior to analysis. The [TIMP-2]•[IGFBP7] measurement used a clinical immunoassay (NephroCheckTM Test, Astute Medical, San Diego, CA, USA). Urinary TIMP-2 and IGFBP7 are simultaneously measured and the product [TIMP-2]•[IGFBP7] is automatically calculated by the ASTUTE140 Meter, divided by 1,000 to report a single numerical test result with a unit of $(ng/ml)^2/1000$ (the unit for all [TIMP-2]•[IGFBP7] tests and cutoff value in this study). [TIMP-2]•[IGFBP7] measurements occurred at hour 0 and 6 hours after enrollment and we reported them in a categorical way (positive or negative) using previously validated cutoffs (3, 4). We used the "high-sensitivity" cutoff (≤ 0.3 $(ng/ml)^2/1000$) in the primary analysis and the "high-specificity" cutoff ($>2.0(ng/ml)^2/1000$) in sensitivity analyses.

Outcome measures

The indication to start RRT was left to the clinicians at each site, according to clinical judgment.

We classified AKI according to Kidney Disease Improving Global Outcomes (KDIGO) guideline using both sCr and UO criteria (5). We assessed AKI in the first 6 hours after enrollment and patients were classified as having AKI when sCr and/or UO met KDIGO criteria for at least AKI stage 1 (maximum sCr in the first 6 hours >1.5–1.9 times baseline creatinine; urine output < 0.5 ml/kg/h for 6-12 hours). We also determined AKI stage within the first week for outcome assessment, based on maximum severity by either sCr or UO criteria. We obtained baseline (the lowest value among the most recent pre-hospital sCr values up to one year prior to the hospital admission), and then measured admission and reference (the lower of baseline and admission) sCr as

described in previous studies (6, 7). For patients with no known prehospital sCr and no known medical history of chronic kidney disease, premorbid sCr was estimated using the Modification of Diet in Renal Disease (MDRD) equation (8). We selected the lower SCr value from either hospital admission creatinine or the MDRD creatinine as the baseline value. Chronic kidney disease (CKD) was defined by GFR <60 mL/min/1.73m^2 for ≥3 months.

Statistical analysis

We reported the proportion of events among 4 subgroups of patients with different biomarkers trajectories (subgroup -/-, both hour 0 and 6 negative; subgroup -/+, negative at hour 0 and positive at hour 6; subgroup +/-, positive at hour 0 and negative at hour 6; subgroup +/+, positive at hour 0 and 6). We also performed sensitivity analyses by using the high-specificity cutoff for [TIMP-2]•[IGFBP7] (>2.0(ng/ml)^2/1000) and using different outcomes (excluding death from the primary endpoint).

Finally, we described the effect of resuscitation protocols and individual interventions on biomarker profiles and outcomes. We compared the proportion of patients who received each specific intervention among the biomarker trajectories. Fisher's exact test was used for testing intravenous antibiotics. Two sample t-test was used for testing intravenous fluids with square root transformation. Wald test was used for testing the remaining interventions. The survival analysis was conducted by Stata 14, the rest of the analysis were conducted by R version 3.5.3.

Table E1. Baseline characteristics comparison between study population and patients excluded for missing biomarkers data

	All study patients (n=688)	**Excluded patients with no available [TIMP-2]*[IGFBP7] (n=293)**	**p-value**
Age, years, median (IQR)	62 (50-74)	63 (51-75)	0.19
Male sex	367 (53.3%)	164 (56.0%)	0.49
Race			0.19
White	491 (71.4%)	219 (74.7%)	
Black or African American	153 (22.2%)	51 (17.4%)	
Other	44 (6.4%)	23 (7.8%)	
Ethnicity			0.43
Hispanic	78 (11.3%)	28 (9.6%)	
Non-Hispanic	610 (88.7%)	265 (90.4%)	
APACHE II score	19 (15-23)	19 (14-24)	0.47
SOFA score	6 (4-9)	6 (4-9)	0.35
Comorbidities			
Charlson comorbidity index	2 (1-4)	2 (1-4)	0.58
Hypertension	406 (59.0%)	164 (56.0%)	0.40
Chronic respiratory disease	155 (22.5%)	67 (22.9%)	0.98
Cancer	134 (19.5%)	59 (20.1%)	0.90
Renal impairment	50 (7.3%)	27 (9.2%)	0.36
Acute congestive heart failure	70 (10.2%)	37 (12.6%)	0.31
Prior myocardial infarction	75 (10.9%)	33 (11.3%)	0.96
Cerebral vascular disease	75 (10.9%)	19 (6.5%)	0.04
Peripheral vascular disease	58 (8.4%)	21 (7.2%)	0.59
Chronic dementia	56 (8.1%)	20 (6.8%)	0.56
Hepatic cirrhosis	40 (5.8%)	21 (7.2%)	0.52
Peptic ulcer disease	34 (4.9%)	18 (6.1%)	0.54
AIDS and related syndromes	15 (2.2%)	10 (3.4%)	0.37
Enrollment criteria			
Hyperlactatemia	399 (58.0%)	172 (58.7%)	0.91
Refractory hypotension	373 (54.2%)	156 (53.2%)	0.82
Physiologic variables			
Serum lactate, mmol/L	2.00 (1.14-3.48)	2.10 (1.11-4.26)	0.11
Systolic blood pressure, mmHg	99 (84-120)	93 (81-112)	0.002
Anemia	104 (15.12%)	43 (14.7%)	0.96
MAP	70 (60-87)	67 (58-82)	0.02
Stage 3 AKI, RRT or death within 7 days	113 (16.4%)	80 (27.3%)	<0.001
Stage 3 AKI within 7 days	80 (11.6%)	49 (16.7%)	0.01
RRT within 7 days	2 (0.3%)	7 (2.4%)	0.004
Death within 7 days	57 (8.3%)	45 (15.4%)	0.001

Reasons for biomarkers missing in the 293 patients. Deviation reasons were reported in 155 patients for hour 0 sample and 113 patients for hour 6 sample. Hour 0 sample was not obtained for the following reasons: anuria (55 patients), technical problems (3 patients), no foley placement (15 patients), subject refused (2 patients), quantity not sufficient (80 patients). Hour 6 sample was not obtained for 113 of 293 patients for the following reasons: anuria (31 patients), no foley placement (7 pts), death before hour 6 (4 pts), quantity not sufficient (54 patients), technical issues (8 patients), subject in OR (2 patients), subject refused (7 patients).

Table E2. Breakdown of the composite endpoint among the different biomarker trajectories

	AKI stage 3	RRT	Death	Composite endpoint
Subgroup -/-	13 (7.3%)	0	4 (2.2%)	15 (8.5%)
Subgroup -/+	9 (16.3%)	0	5 (9%)	12 (21.8%)
Subgroup +/-	12 (7.3%)	0	8 (4.9%)	16 (9.8%)
Subgroup +/+	46 (15.6%)	2 (0.6%)	40 (13.6%)	70 (23.8%)

Legend

AKI, Acute Kidney Injury; RRT, Renal Replecement Therapy

Table E3. Multiple comparison using Benjamini-Hochberg procedure

	Generalized Wilcoxon test	Generalized Wilcoxon test with BH procedure	Tarone-Ware test	Tarone-Ware test with BH procedure	Peto-Peto test	Peto-Peto test with BH procedure
7 days						
Grp 1 vs 2	0.0397	0.0794	0.0387	0.0774	0.0396	0.0792
Grp 1 vs 3	0.2690	0.2739	0.2662	0.2732	0.2679	0.2745
Grp 1 vs 4	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Grp 2 vs 3	0.2739	0.2739	0.2732	0.2732	0.2745	0.2745
Grp 2 vs 4	0.1936	0.2739	0.2002	0.2732	0.1932	0.2745
Grp 3 vs 4	0.0009	0.0027	0.0010	0.0030	0.0009	0.0027
30 days						
Grp 1 vs 2	0.0088	0.0222	0.0089	0.0267	0.0087	0.0238
Grp 1 vs 3	0.2194	0.2633	0.2164	0.2597	0.2180	0.2616
Grp 1 vs 4	0.0002	0.0012	0.0002	0.0012	0.0002	0.0012
Grp 2 vs 3	0.1085	0.1628	0.1115	0.1673	0.1092	0.1638
Grp 2 vs 4	0.7779	0.7779	0.8419	0.8419	0.7952	0.7952
Grp 3 vs 4	0.0111	0.0222	0.0142	0.0284	0.0119	0.0238
60 days						
Grp 1 vs 2	0.0205	0.0540	0.0222	0.0666	0.0204	0.0580
Grp 1 vs 3	0.0939	0.1409	0.0902	0.1353	0.0929	0.1394
Grp 1 vs 4	0.0001	0.0006	0.0001	0.0006	0.0001	0.0006
Grp 2 vs 3	0.2926	0.3511	0.3212	0.3854	0.2956	0.3547
Grp 2 vs 4	0.6005	0.6005	0.6382	0.6382	0.6121	0.6121
Grp 3 vs 4	0.0270	0.0540	0.0371	0.0742	0.0290	0.0580
90 days						
Grp 1 vs 2	0.0870	0.1740	0.1013	0.2026	0.0870	0.1740

Grp 1 vs 3	0.1799	0.2699	0.1823	0.2735	0.1788	0.2682
Grp 1 vs 4	0.0003	0.0018	0.0005	0.0030	0.0004	0.0024
Grp 2 vs 3	0.4474	0.4474	0.4993	0.4993	0.4516	0.4516
Grp 2 vs 4	0.4297	0.4474	0.4439	0.4993	0.4369	0.4516
Grp 3 vs 4	0.0254	0.0762	0.0352	0.1056	0.0272	0.0816
1 year						
Grp 1 vs 2	0.0498	0.0996	0.0582	0.1164	0.0498	0.0996
Grp 1 vs 3	0.2511	0.3130	0.2695	0.3382	0.2503	0.3160
Grp 1 vs 4	0.0003	0.0018	0.0005	0.0030	0.0003	0.0018
Grp 2 vs 3	0.2608	0.3130	0.2818	0.3382	0.2633	0.3160
Grp 2 vs 4	0.5673	0.5673	0.6036	0.6036	0.5773	0.5773
Grp 3 vs 4	0.0158	0.0474	0.0211	0.0633	0.0169	0.0507

Table E4. Differences in intervention treatments between different [TIMP-2]•[IGFBP7] trajectories among patients with negative biomarker at hour 0 (3a) and patients with positive biomarker at hour 6 (3b)

3a)

Intervention	Subgroup -/- n=176	Subgroup -/+ n=55	p-value*
Resuscitation			
Central venous catheterization	116 (65.9%)	44 (80.0%)	0.05
Central venous oximeter catheterization	60 (34.1%)	26 (47.3%)	0.08
Intravenous fluids, L, mean	2.6	3.3	0.047
Vasopressor use	68 (38.6%)	31 (56.4%)	0.02
Dobutamine use	5 (2.8%)	7 (12.7%)	0.009
Blood transfusion	17 (9.7%)	11 (20.0%)	0.04
Ancillary Care			
Mechanical ventilation	20 (11.4%)	15 (27.3%)	0.005
Intravenous antibiotics	174 (98.9%)	55 (100.0%)	-
Corticosteroids	41 (23.3%)	7 (12.7%)	0.1

3b)

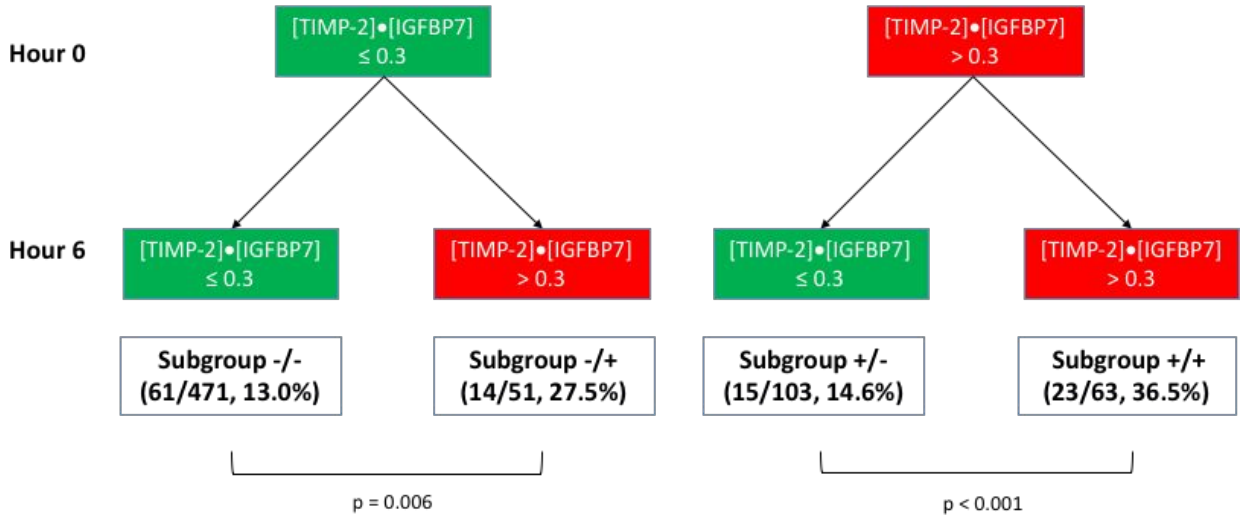
Intervention	Subgroup +/- n=163	Subgroup +/+ n=293	p-value*
Resuscitation			
Central venous catheterization	108 (66.3%)	207 (70.6%)	0.33
Central venous oximeter catheterization	50 (30.7%)	95 (32.4%)	0.7
Intravenous fluids, L, mean	2.9	2.8	0.76
Vasopressor use	66 (40.5%)	163 (55.6%)	0.002
Dobutamine use	8 (4.9%)	6 (2.0%)	0.1
Blood transfusion	11 (6.7%)	38 (13.0%)	0.04
Ancillary Care			
Mechanical ventilation	29 (17.8%)	92 (31.4%)	0.002
Intravenous antibiotics	159 (97.5%)	287 (98.0%)	0.75
Corticosteroids	29 (17.8%)	54 (18.4%)	0.87

Mechanical ventilation, central venous catheterization, and ancillary care (antibiotics, corticosteroids, and activated protein C) were counted from arrival at the emergency department to 6 hours. Resuscitation therapies (intravenous fluids, vasopressor, and dobutamine infusions, and blood product administration) were counted from randomization to 6 hours.

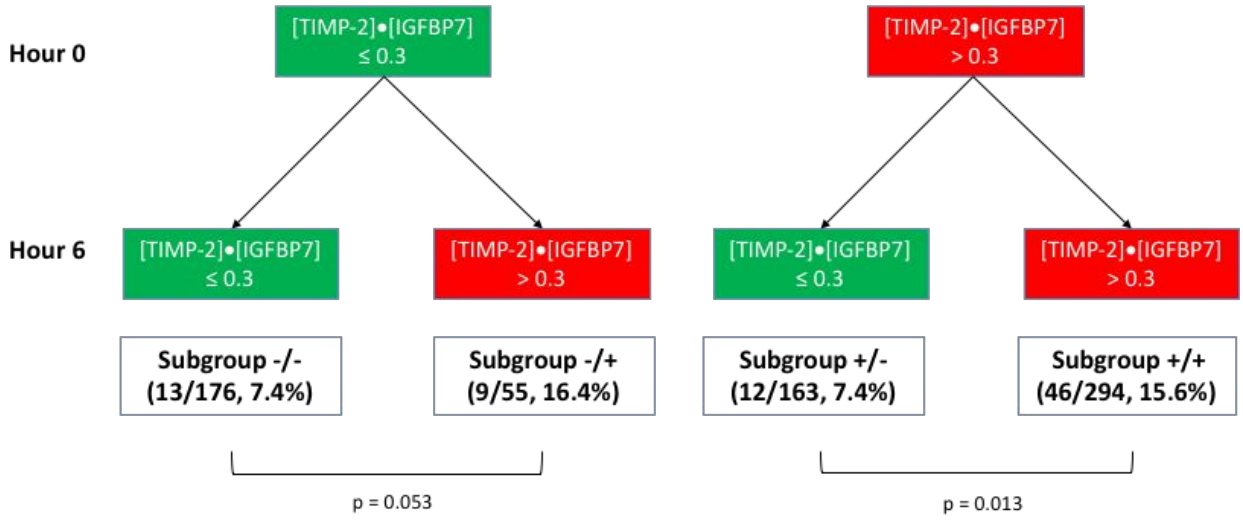
* *P* values are shown for two-way comparison. Fisher's exact test was used for testing intravenous antibiotics. Two sample t-test was used for testing intravenous fluids with square root transformation. Wald test was used for testing the rest of interventions.

Figure E1. Risk assessment flow diagram combining [TIMP-2]•[IGFBP7] at hour 0 and 6 using the high-specificity cutoff (>2.0 $(ng/ml)^2/1000$ (E1a) and excluding death from the endpoint (E2b)

E1a



E1b



REFERENCES

1. Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, Terndrup T, Wang HE, Hou PC, LoVecchio F, Filbin MR, Shapiro NI, Angus DC. A randomized trial of protocol-based care for early septic shock. *N Engl J Med*, 2014/03/19. 2014;370:1683–1693.

2. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E, Tomlanovich M. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med*, 2002/01/17. 2001;345:1368–1377.

3. Honore PM, Nguyen HB, Gong M, Chawla LS, Bagshaw SM, Artigas A, Shi J, Joannes-Boyau O, Vincent JL, Kellum JA. Urinary Tissue Inhibitor of Metalloproteinase-2 and Insulin-Like Growth Factor-Binding Protein 7 for Risk Stratification of Acute Kidney Injury in Patients With Sepsis. *Crit Care Med*, 2016/06/30. 2016;44:1851–1860.

4. Hoste EA, McCullough PA, Kashani K, Chawla LS, Joannidis M, Shaw AD, Feldkamp T, Uettwiller-Geiger DL, McCarthy P, Shi J, Walker MG, Kellum JA. Derivation and validation of cutoffs for clinical use of cell cycle arrest biomarkers. *Nephrol Dial Transpl*, 2014/09/23. 2014;29:2054–2061.

5. Outcomes KDIG. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Inter* 2012;Suppl. 2:1–138.

6. Kellum JA, Chawla LS, Keener C, Singbartl K, Palevsky PM, Pike FL, Yealy DM, Huang DT, Angus DC. The Effects of Alternative Resuscitation Strategies on Acute Kidney Injury in Patients with Septic Shock. *Am J Respir Crit Care Med*,

2015/09/24. 2016;193:281–287.

7. Kellum JA, Sileanu FE, Murugan R, Lucko N, Shaw AD, Clermont G. Classifying AKI by Urine Output versus Serum Creatinine Level. *J Am Soc Nephrol*, 2015/01/09. 2015;26:2231–2238.

8. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care*, 2004/08/18. 2004;8:R204-12.