Role of Tissue Disorder Markers in the Evaluation of Disease Progress and Outcome Prediction: A Prospective Cohort Study in Non-Cardiac Critically ill Patients

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Background: Acute Physiology and Chronic Health Evaluation (APACHE) has a remarkable association with clinical syndrome, life expectancy, and length of ward stay. But the defects are obvious. It is crucial to detect an effective and convenient evaluation method to monitor disease progress and predict outcome. **Objective:** To determine whether myoglobin (Mb) and other five tissue disorder biomarkers, troponin-I, creatine-kinase, creatine kinase-muscle brain, aspartate aminotransferase, and lactate dehydrogenase are independent predictors of disease progress and mortality in non-cardiac critical illness. **Methods:** A prospective study with 179 patients admitted to the Intensive Care Unit was conducted. All serum tissue disorder markers and APACHE-II score were measured within 24 hr of admission. **Results:** All the six biomarkers were significantly correlated with disease severity stratified by APACHE-II and outcome. Serial blood samples were taken from 17

patients on detection of two new organs failure. The occurrence of organs failure was significantly associated with the elevation of Mb, troponin-I, and APACHE-II. Multivariate analysis demonstrated that elevated Mb was the principal risk factor related to mortality either during hospitalization or 180-day followup. Kaplan–Meier method and log-rank test also showed that patients with elevated Mb levels had significantly shorter survival. The mortality was higher in patients with both Mb > 500 ng/ml and APACHE-II > 20 than in those with only Mb > 500 ng/ml or APACHE-II > 20. **Conclusion:** All the six tissue disorder markers are predictors of disease severity, organ failure, and outcome in non-cardiac critically illness. Among them, Mb plays a pivotable role. The combined use of Mb and APACHE-II suggest an effective method to determine the outcome of critical ill syndrome. *J. Clin. Lab. Anal.* 24:376–384, 2010.

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INTRODUCTION

Acute Physiology and Chronic Health Evaluation (APACHE) is one of the most widely used assessing systems in the world. It helps to collect a set of quality information on patients in the Intensive Care Unit (ICU) and use that information to improve predicting disease severity and clinical outcomes. Although APACHE score has a remarkable association with clinical syndrome, predicting hospital mortality, ICU mortality, length of ward stay, life expectancy, and resource allocation, the defects are also obvious. These include the complicated calculation of values, the lack of

evidence-based analysis needed to prove the efficiency of APACHE score stratification in the origin of various diseases, as well as the uncertainty of its role in the

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monitor of new organic dysfunction (1–3). Therefore, it develops a universal tendency to establish novel and simple quantitative measurements and the tissue disorder biomarkers are a new direction.

Since 1997, a number of research finding revealed that cardiac troponin I (c-TnI), the specific protein related to myocardial injury, is a strong predictor of underlying disease progress and hospital mortality (4,5). Compared with APACHE-II, which predominately evaluates hemodynamic changes rather than heart function, c-TnI could provide a direct marker for myocardial injury, even if clinically unrecognized (6,7). Ammann et al. has estimated that as many as 15% of ICU admissions are complicated by some degree of myocardial injury and as many as 85% of patients with sepsis may have raised cardiac c-TnI (8).

However, limited information is available concerning other cardiac markers, for example creatine kinase–muscle brain (CK-MB), as well as other tissue disorder biomarkers, such as myoglobin (Mb), creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), contribute to the outcome of organ failure and disease severity or to the correlation with APACHE (9). We hypothesize that if elevated c-TnI is correlated with poor outcome in non-cardiac critical illness, as a potential indicator of unrecognized myocardial injury, Mb and other markers might be either more effective than or at least as effective as c-TnI. The reason is that some of them are more sensitive, although less specific than c-TnI in detecting underlying myocardial injury and they also reflect other organ injury (e.g., renal failure, liver dysfunction, myonecrosis, etc.) (10,11). According to our clinical observation in the ICU, we noted several disease situations (e.g., hypoxia, acidosis, severe inflammation, multiple organ dysfunction, etc.) affected the expression of these proteins (especially Mb) in serum in addition to c-TnI. Moreover, tissue breakdown elevates levels of LDH, and therefore a measure of it indicates disorders including meningitis, encephalitis, acute pancreatitis, and HIV, etc. AST is elevated as a result of acute liver damage and other tissue injury, including cardiac muscle, skeletal muscle, kidney, and brain tissue. CK is already determined routinely in emergency patients, particularly in patients with chest pain or if acute renal failure is suspected.

Therefore, we conducted a prospective cohort study to determine whether cardiac markers, including Mb, CK, CK-MB, AST, and LDH, along with c-TnI are independent and simple quantitative predictors of disease progress and outcome in non-cardiac critically ill patients.

MATERIALS AND METHODS

Our prospective study was conducted in the emergency ICU (EICU, having 12 beds for resuscitation

and four beds for rehabilitation) within the emergency department in Ruijin Hospital, Shanghai Jiaotong University, School of Medicine, China. The hospital is one of the largest and is typical of its type in China. The study protocol was approved by the Ethics Committee of Shanghai Jiaotong University, School of Medicine before its initiation. A written informed consent was obtained from relatives of the critically ill patients.

Our study considered a total of 179 non-cardiac critically ill patients who were admitted consecutively to the EICU during a 12-month period (from April 2005 to April 2007, including all the patients' followup). The nursing staffs and the physicians who provided care for the patients in the EICU were totally unaware of the results of our study. One of the investigators who did not participate in routine patient care made daily rounds in the ICU, recording relevant data from patients' medical records. Patients diagnosed with acute coronary artery syndrome, defined as unstable angina, typical chest pain, ischemic electrocardiography changes, or cardiogenic pulmonary edema was excluded. Furthermore, patients having rhabdomyolysis, acute renal failure or those requiring chronic hemodialysis or those who underwent major surgery during the month preceding admission (in order to exclude peri-operative myocardial injury) were also excluded. All the 179 patients underwent an electrocardiography examination and transthoracic cardiac ultrasonography (US) at the time of admission. No typical signs of acute coronary insufficiency on serial electrocardiography or any regional wall abnormality or low left ventricular ejection fraction on US examination were detected at that time. During the stay in the ICU and during followup, cardiac US was performed according to clinical indication.

A variety of clinical and laboratory variables were measured and recorded within 24 hr of admission, including values in APACHE-II score, six tissue damage biomarkers, routine biochemical examinations, and underlying diseases, etc. Clinical evaluation also included the development of new organ dysfunction, records of mechanical ventilation, length of stay in the ICU, and mortality. APACHE-II score was calculated based on the value obtained from the series of physiologic measurements during the first 24 hr after admission to the ICU. All the values were measured again in those of 17 cases when two new organs failure appeared. We used ICU discharge as the initial time point because most of the overall mortality analysis was based on in-hospital mortality. Secondary end point was at the 180-day followup.

The definition used for systemic inflammatory response syndrome, sepsis, and septic shock were those of

the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference. Individual organ system dysfunction and failure were defined by the criteria of Rubin et al. Serum Mb, c-TnI, CK, CK-MB, AST, and LDH levels in healthy subjects were below the minimal concentration (70 ng/ml, 0.5 ng/ml, 269 IU/L, 0.40 ng/ml, 10–64 IU/L, 91–192 IU/L) detectable by the assay.

The disease severity was stratified as “ill”(APACHE-II ≤ 10), “severe ill”(10 < APACHE-II ≤ 20), and “critically severe ill”(APACHE-II > 20), on the basis of their APACHE-II scores taken at admission. Mb level under 140.00 ng/ml was defined in normal group, above 140.00 ng/ml but under 1,000.00 ng/ml in positive or elevated group, and above 1,000.00 ng/ml in prominently elevated group.

Serum tissue disorder biomarkers were measured by the Access Immunoassay Suplan kit (BECKMAN Coulter /ACCESS-DXI800 Accu, rat polyclonal antibodies). Blood samples were collected in test tubes containing no anticoagulant or preservative and the serum was separated from the clot within 60 min of blood collection. The method was highly sensitive due to the use of a quantitative chemiluminescent assay, combined with the two-site immunoradiometric assay enzyme immunoassay. First step was the sandwich technique, including antibody Mab alkaline phosphatase conjugated and paramagnetic particles coated with antibody Mab. After the sandwich formation, substrate Lumi-Phos*530 dioxetane-P was added. Signal was produced directly proportional to the protein concentration in the sample and was detected by immunoassay analysis system (BECKMAN Coulter/ACCESS-DXI800).

Statistical Analysis

According to the results of univariate test, continuous normal variables were expressed as mean value $\pm$ SD, and abnormal distribution data were expressed as quartile. Categorical variables were expressed as percentage; comparisons between these groups were made using the χ^2 test. Abnormal distribution data were analyzed in the following manner by non-parametric tests: Kruskal–Wallis test for completely randomized data, signed rank sum test for paired design, and Spearman test for rank correlation. As age of the patient, as a variable, is an important physiological marker in APACHE-II score and in the evaluation of prognosis, which is also directly correlated with tissue damage biomarkers in rank correlation analysis, covariance analysis was used to remove the distortion of the age factor to assure the precise comparison of these markers among groups stratified by prognosis,

APACHE-II, or Mb levels. Before the covariance analysis, all the abnormal distribution variables were processed by rank transformation.

Multivariate analysis of prognostic factors was performed by Cox regression method. Cumulative survival curves were constructed by Kaplan–Meier method and compared with the log-rank test. Results were considered significant at $P < 0.05$. Statistical analysis was performed with the SAS 8.2 statistical package (SAS Institute Inc., Cary, NC).

RESULTS

Population Demographics

The study population consisted of 179 patients admitted during the 12-month period. It consisted of 108 (62.5%) men and 71 (37.5%) women, ranging in age from 20 to 98 years (66.02 ± 16.5). The reasons for admission to the ICU were as follows: pneumonia or other inflammation with sepsis or septic shock ($n = 62$), chronic obstructive pulmonary disease with respiratory failure ($n = 27$), stroke ($n = 31$), acute pancreatitis ($n = 27$), and other diseases ($n = 33$), including gastrointestinal bleeding with hypovolemic shock, cancer, heat shock, and intoxication. The distribution of the above-mentioned diseases and sex ratio was insignificant among patients in groups stratified by APACHE-II score and Mb levels (See Tables 1–3).

The Relationship Between APACHE-II Score and Tissue Disorder Biomarkers

Among the three groups of “ill”(APACHE-II ≤ 10), “severe ill”(10 < APACHE-II ≤ 20), and “critically severe ill”(APACHE-II > 20), all the tissue disorder bioenzymes, the number of patients treated with mechanical ventilation, the length of stay, and the mortality increased dramatically in patients of higher APACHE group ($P < 0.05$), which indicates these parameters in non-cardiac critically ill patients are significantly correlated with severe clinical assessments ($P < 0.05$, Table 1). After repeated statistical analysis using covariant analysis to remove distortion caused by age, these elevated variables were also significantly correlated with elevated APACHE-II. Among all the cardiac markers, c-TnI, Mb, CK, and CK-MB even more effective in accordance with disease progress, because they were significantly different in every two compared groups (Table 1). Moreover, the fluctuation of Mb and CK covered a more broad extension than AST, LDH, CK-Mb, and c-TnI, and they increased more sharply when APACHE-II increased and clinical situation

TABLE 1. Comparison of Characteristic Parameters in Three Groups Stratified by APACHE II

	APACHE-II ≤ 10 III	11 ≤ APACHE-II ≤ 20 Critical ill	APACHE-II ≥ 20 Severely critical ill	P-value
Number	72	59	48	
Age (years) ^a	54 (43.5–66.5)	74 (67–80)	77.0 (70–82.5)	
Male	49 (68.06%)	29 (49.15%)	30 (62.50%)	0.083
Cause of admission ^b				
Infection	18 (29.03%)	23 (37.10%)	21 (33.87%)	0.074
COPD	8 (29.63%)	10 (37.04%)	9 (27.00%)	0.46
Stroke	14 (45.16%)	10 (32.26%)	7 (22.58%)	0.78
AP	16 (59.26%)	8 (29.63%)	3 (11.11%)	0.052
Others	17 (51.52%)	8 (24.24%)	8 (24.24%)	0.31
Parameters of disease severity				
APACHE-II	4 (2–6)	13 (11–16)	25.5 (23.0–32.5)	<0.0001 ^c
Length of stay (day)	10 (7–16)	14 (7–24)	9.0 (4.0–28.5)	0.04
Ventilation	1 (1.39%)	19 (32.20%)	25 (52.08%)	<0.0001
Mortality in ICU	2 (2.78%)	24 (40.68%)	32 (66.67%)	<0.0001 ^c
Cardiac markers on admission				
Mb (ng/ml)	30.30 (18.35–52.50)	146.8 (58.0–311.9)	558.25 (111.10–1,459.45)	<0.0001 ^c
CK (IU/L)	67 (35.5–133)	121 (62–479)	192.0 (94.0–1,211.5)	<0.0001 ^c
AST (IU/L)	26.5 (19.0–43.5)	36 (22–65)	44.5 (31.0–77.0)	0.0016
CK-MB (ng/ml)	1.5 (0.9–2.5)	3.40 (1.70–5.60)	4.20 (1.95–11.85)	0.0002 ^c
LDH (IU/L)	182 (142–260.5)	325 (216–412)	344.5 (248.5–448.5)	<0.0001
c-TnI (ng/ml)	0.03 (0.01–0.05)	0.06 (0.03–0.18)	0.19 (0.055–1.030)	<0.0001 ^c

Values are expressed as median (interquartile range) or number (percentage). APACHE, Acute Physiology and Chronic Health Evaluation; CK-MB, creatine kinase–muscle brain; Mb, myoglobin; CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; c-TnI, cardiac troponin I; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; AP, acute pancreatitis.

^aAs the variable, age, is an important marker either in the APACHE II score or in the evaluation of prognosis, all the statistics were analyzed by covariant analysis to avoid the age interference; therefore, the *P* value was not presented yet for age (The coefficient correlation between age and APACHE II, Mb, LDH, AST, CK-MB, CK, and c-TnI are *r* = 0.867, 0.397, 0.286, 0.389, 0.332, 0.289, and 0.174 respectively, *P* < 0.01).

^bInfection: also included systemic inflammatory response syndrome, sepsis, septic and shock; COPD: the group of which also included respiratory failure. Others: included gastrointestinal bleeding with hypovolemic shock, cancer, heat shock, and intoxication. Ventilation: the number of patients of mechanical ventilation.

^cValues were also significantly different between each of two groups (*P* < 0.05) after covariant analysis among the three groups.

TABLE 2. Comparison of Tissue Disorder Biomarkers in 179 Patients Stratified By Outcome

	Survival group	Non-survival group	P-value
N	121	58	
Age (years) ^a	61 (48–75)	78 (72–82)	
Male	33 (56.90)	75 (61.98)	0.51
APACHE-II score	7 (4–12)	22 (15–26)	<0.0001
Mb (ng/ml)	39.5 (22.8–98.8)	341.9 (154.3–1,046)	<0.0001
LDH (IU/L)	230 (156–338)	247 (241–516)	<0.0001
CK (IU/L)	75 (40–186)	181 (83–1.56)	<0.0001
AST (IU/L)	28 (21–44)	58 (32–100)	0.0002
CK-MB (ng/ml)	1.80 (1.0–3.5)	3.7 (2.4–9.1)	0.0003
c-TnI (ng/ml)	0.03 (0.02–0.06)	0.17 (0.06–0.64)	<0.0001

Values are expressed in median (interquartile range). APACHE, Acute Physiology and Chronic Health Evaluation; CK-MB, creatine kinase–muscle brain; Mb, myoglobin; CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; c-TnI, cardiac troponin I. All the markers were measured on admission day.

^aThe same as Table 1.

became worse. This demonstrates that they might be more sensitive in monitoring underlying disease progression (Table 1).

Pathological Release of Tissue Disorder Biomarkers During New Organ Failure

A total of 17 patients were confirmed for having more than two new organs failure during hospitalization. Serial blood samples were taken from them at admission and on detection of two more failures of organs. The occurrence of two more organs failure was significantly associated with elevation of Mb, LDH, c-TnI, and APACHE-II (Table 4).

The Relationship Between Outcome and Tissue Disorder Biomarkers

APACHE-II and all cardiac markers are significantly higher in the non-survival group than in the survival group; this suggests that these markers might have the capability of predicting outcome of critical ill syndrome (See Table 3).

The results of multivariate analysis (Cox regression model) were shown in Table 5. Four variables were included in the final model. Both in the first and in the secondary end-point, elevated Mb level (*P* < 0.0001) is chosen as the principle high risk factor for prognosis,

TABLE 3. Comparison of All the Markers in Three Groups Stratified by Myoglobin Level, Expressed as Median (Interquartile Range) or Number (Percentage)

	Mb < 140 (Normal group)	140 ≤ Mb < 1,000 (Elevated group)	Mb ≥ 1,000 (Prominently elevated group)	P-value
N	106	55	18	
Age ^a	62.5 (48.0–74.0)	75 (68–82)	77.5 (72.5–83.0)	
Male	62 (58.94)	31 (56.32)	15 (83.33)	0.10
Cause of admission ^b				
Infection	36 (58.06)	17 (27.42)	9 (14.52)	0.34
COPD	13 (48.15)	10 (37.04)	4 (14.81)	0.38
Stroke	18 (58.06)	11 (35.48)	2 (6.45)	0.75
Acute pancreatitis	21 (77.78)	6 (22.22)	0	0.058
Others	20 (60.61)	11 (33.33)	2 (6.06)	0.75
Summary of LIFETEST procedure—(Log rank test χ^2 51.5971, $P < 0.0001$)				
Censored	96	24	2	<0.0001
Death rate ^c	10 (9.43)	31 (56.36)	16 (88.89)	<0.0001
Survival time	54 (38-)	38 (9–46)	7 (3–12)	<0.0001
Parameters of disease severity				
APACHE II ^c	4 (2–6)	15 (12–22)	25.5 (23.0–35)	<0.0001
Length of hospital stay (days)	12 (7–18)	10 (7–24)	7 (3.0–12)	0.12
Patients with mechanical ventilation	8 (7.55)	23 (41.82)	14 (77.78)	<0.0001
Tissue disorder biomarkers on admission				
Mb (ng/ml) ^c	31.25 (19.60–54.00)	284.3 (172–604.5)	1,880.95 (1,269.30–4,000)	<0.0001
CK (IU/L) ^c	62 (35–97)	373 (123–644)	1,496.5 (535–1,918.0)	<0.0001
AST (IU/L) ^c	25.5 (19.0–40.0)	43 (29–91)	73.5 (63.0–469.0)	<0.0001
CK-MB (ng/ml) ^c	1.65 (0.9–3.00)	3.8 (2.1–7.2)	14.55 (4.50–32.80)	<0.0001
LDH (IU/L) ^c	202 (150–276)	345 (246–399)	442 (332–1,130)	<0.0001
c-TnI (ng/ml) ^c	0.035 (0.020–0.060)	0.07 (0.04–0.27)	1.01 (0.06–6.36)	<0.0001

APACHE, Acute Physiology and Chronic Health Evaluation; CK-MB, creatine kinase–muscle brain; Mb, myoglobin; CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; c-TnI, cardiac troponin I; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease.

^{a,b,c}The same as Table 1.

TABLE 4. Comparison of Tissue Disorder Biomarkers Detected on Admission and on Appearance of Two More New Organs Failure

	Admission	Two more new organ failure	Signed rank	P-value
APACHE-II score	13 (7–14)	22 (14–31)	60	<0.0001
Mb (ng/ml)	138.1 (51.5–325.8)	831.3 (125.0–1,598.3)	58.5	0.0038
c-TnI (ng/ml)	0.04 (0.03–0.21)	0.18 (0.05–1.01)	56	0.058
AST (IU/L)	32 (19–63)	61 (36–70)	15	0.46
LDH (IU/L)	239 (182–399)	391 (299–581)	43.5	0.039
CK (IU/L)	113 (62–264)	322 (201–1,301)	3.05	0.16
CK-MB (ng/ml)	3.4 (2.3–4.8)	4.5 (2.5–8.0)	19.5	0.33
Albumin	23 (20–31)	23 (19–26)	8	0.64

Value are listed as median (interquartile range).

even more effective than the APACHE-II. The variable, c-TnI, was not included in the final model.

Mb Level Specifically Associated with Disease Severity and Mortality

Mb expression is more significantly consistent with the APACHE-II in the above results than c-TnI or other variables. Furthermore, we analyzed whether Mb alone

is independently associated with disease severity and mortality, which could be hopefully used as a sole physiological measurements compared with APACHE II (Table 3). Among three groups stratified by serum Mb level, the number of patients treated with mechanical ventilation, APACHE-II, and death rate were significantly elevated in parallel with Mb elevation. Mb was also associated with other tissue damage markers. We also performed a combination analysis using

TABLE 5. Summary of Stepwise OrocEDURE of Multivariate Analysis using Cox Regression in 179 Non-Cardiac Critically Ill Patients

Step	Value entered	Parameter estimate	Standard error	Score χ^2	Pr> χ^2	Hazard ratio	95% Hazard ratio confidence limits
The first time point—the initial hospitalization							
1	Mb	0.0006609	0.000151	19.0976	<0.0001	1.001	1.000–1.001
2	APACHE	0.04316	0.01398	9.539	0.0017	1.044	1.016–1.073
3	AST	0.00116	0.000438	76.9721	0.0052	1.001	1.001–1.002
4	CK-MB	−0.01383	0.0054	6.5522	0.0083	0.986	0.976–0.997
The second time point—the initial hospitalization							
1	Mb	0.0006909	0.000149	21.5144	<0.0001	1.001	1.000–1.001
2	APACHE	0.06514	0.01218	28.5983	<0.0001	1.067	1.042–1.093
3	AST	0.00108	0.000421	6.5559	0.0105	1.001	1.000–1.002
4	CK-MB	0.01699	0.0055	9.5356	0.002	0.983	0.973–0.994

APACHE, Acute Physiology and Chronic Health Evaluation; CK-MB, creatine kinase–muscle brain; Mb, myoglobin; AST, aspartate aminotransferase.

Fig. 1. Cumulative survival rates in the severely elevated, elevated and normal Mb groups (Kaplan–Meier method, $P < 0.0001$, log-rank). (A) the first time point–discharge; (B) 180-day followup.

Kaplan–Meier method (Fig. 1) and log-rank test (Table 3), both of which showed that patients with elevated Mb levels had a significantly shorter survival ($P < 0.0001$). These results are consistent with the

prognosis prediction both during the hospitalization observation and during 180-day followup, indicating that Mb is a promising measurement in the survival detection.

Possible Combination of Mb and APACHE-II in the Better Prediction of Disease Outcome

After clinical analysis of all the 179 cases, we found that although acute hemodialysis was performed as soon as possible for those whose Mb levels were detected higher than 1,000 ng/ml to avoid acute renal failure, the mortality was also high (88.89%, 16/18. One of the only two survivals had experienced amputation of both femurs in the earlier stage because of complete occlusion of both femoral veins); this suggests that patients faced with Mb > 1,000 ng/ml are almost nearing the end of the life or are in poor health if they survive the treatment. We also detected that the mortality was 77%(28/36) for patients whose Mb levels were detected higher than 500 ng/ml; 66%(32/48) for patients whose APACHE-II were detected higher than 20; and 83%(20/24) for those who measured up both conditions. The combined use of Mb and APACHE-II suggests a possible earlier and effective method to determine the outcome prediction.

DISCUSSION

APACHE II hospital mortality predictions are based on the prognostic impact of the deviation from normality for 12 physiologic values (Acute Physiology Score or APS), age, chronic health status, and one of 56 disease groups. These variables were used to obtain risk-adjusted predictions of mortality that could be used for outcome comparisons among ICUs that were adjusted for differing patient case-mix. Since 1991, these outcome predictions were re-evaluated and updated, and the new version of the set of physiological measurements is called APACHE IV, which now has 116 disease groups and 27 possible variables. The advantage of APACHE IV critical care series is that it has employed a carryover rule for using the data with defaulted values; the second advantage is that it more precisely determines the impact of neurologic derangement. We carried forward the APACHE II as before instead of using the new equation of APACHE IV for this study, because all the severe diseases of the patients recruited to our ICU was included in the 56 disease groups of APACHE II, also because we didn't have patients with neurologic disorder, and because we analyzed more than twice or even three times of the physiological values to avoid the possibility of missing values due to the clinical signs of the critical ill patients changing extremely quick in order to well follow the disease change from the first 24 hr of the admission.

C-TnI, as Well as Other Tissue Damage Biomarkers Predicts Disease Severity and Outcome in Non-Cardiac Critical Illness

In our study, we found out not only c-TnI but also Mb, AST, LDH, CK, and CK-MB levels were all

prominently elevated in more severe patients stratified by APACHE-II and prognosis (Tables 1 and 2), which suggests that all of these markers correlate with disease severity and prognosis. All the tissue damage markers were also significantly correlated with some of the important values in the APACHE-II, such as white blood cell count or neutrophil, etc (Data not show), which suggests that they might correlate with infection. These results are consistent with recent studies that have reported unexpectedly elevated c-TnI levels are significantly associated with a higher APACHE-II score in ICU patients without coronary syndrome. The possible interpretation is that the c-TnI value indicates the chronic hypoxia, appearance of new organ failure, increased ICU mortality, occurrence of bacteremia and shorter cumulative survival during the 180-day followup (12–20).

In our study, Mb and CK were especially effective because their fluctuation covered a broader extension and increased sharply to higher levels in accordance with APACHE-II increase, thus demonstrates Mb and CK might be more sensitive in monitoring disease progression by quantified stratification of disease severity when compared with c-TnI etc. This might be due to the fact that Mb is an essential protein that facilitates oxygen delivery from the intracapillary erythrocyte to the mitochondria in order to maintain oxidative phosphorylation for muscle contractility (21,22); thus, the Mb expression might be extremely sensitive to the condition of hypoxia or any disease inducing organic hypoxia. Another interpretation could be that the expression of c-TnI is in micro-quantity in the serum, which is hundred times less than Mb in the serum; therefore, the variation of c-TnI is less significant compared with Mb. Further studies of serial measurements of these markers are necessary to observe the sensitivity of these markers in detecting disease stages and might give additional value for early risk stratification assessment.

Changed Value of Tissue Disorder Biomarkers in Serial Measurements Due to the Occurrence of New Organ Failure

Serial biochemical markers measurements are necessary for ICU doctors to evaluate patient condition and observe treatment efficiency in early stage. Mb, LDH, c-TnI, and APACHE-II were found significantly greater in 17 of the 179 patients when two more organ failures were discovered than were detected at the time of admission (Table 4). This suggests that serial measurements of Mb, LDH, c-TnI, and APACHE might give additional value of early forecast of new organ failure and contribute to adverse prognosis in non-cardiac critical illness. However, because of the limited number

of patients participating in our study, further studies of a larger patient population are warranted to confirm the value of their serial measurements on outcome prediction. The nutrition marker, albumin, did not decrease significantly when new organ failure appeared.

The Prominent Role of Mb in Evaluation of Disease Severity and Outcome Prediction

In agreement with Kollef et al., who considered it biologically plausible that c-TnI independently contributed to the prediction of hospital mortality, our multivariate analysis of Cox regression demonstrated that c-TnI was not identified as a significant risk factor for death (Table 5), whereas Mb is the main risk factor in relationship with death, during a short period of hospitalization or in the longer period of the 180-day followup, thus indicating elevated Mb level is likely to reflect not only on organ failure but also on predicting the outcome with non-cardiac critical illness (4).

Mb is known to become nephrotoxic when releasing in greater amounts into the general circulation and is not specific in detection of myocardial injury (23). Nevertheless, Emig et al. discovered that Mb induced increase in vascular tone additionally contributes to the detrimental role of this protein in hypovolemic shock in anesthetized rats; this suggests that pathological release of Mb results in increase in vascular resistance of vital organs that may contribute to decompensation of tissue supply when occurring in hypovolemic shock (24). Other basic research has already confirmed that Mb is an important cytoplasmic hemoprotein in facilitating oxygen transport in striated muscle, and also has a functional role (e.g., as a reservoir for oxygen, as a cytoprotective protein against reactive oxygen species, and as a modulator of the signaling molecular nitric oxide) after research on the zebra fish and Mb-null mice to chronic hypoxia (25–27). Therefore, it is estimated that Mb might elevate whenever organic hypoxia or shortage of other energy supply occurs. However, little is known about the role of Mb in the clinical investigation of hypoxia, multiple organ failure, disease severity, and prognosis in non-cardiac critical illness. The reason why researchers emphasize on c-TnI rather than Mb is that they might consider Mb as a sensitive non-specific marker to myocardium (6). Nevertheless, in our findings, the sensitivity is more important than specificity in early evaluation of disease progress and outcome. Moreover, the unspecificity will provide assistance to our detecting earlier information related to other organs dysfunction.

Accordingly, we performed further research based on the stratification of Mb level to support this hypothesis. Based on our results, elevated APACHE-II and

mortality rate are significantly correlated with Mb increase, particularly considering that Kaplan–Meier analysis finds that the discriminative effect of Mb elevation is evident ($P < 0.0001$) from the first day. Combining with statistical analysis of Cox regression, our findings demonstrate that Mb elevation is a principal risk factor in association with illness severity and high mortality rate. Moreover, the combined use of Mb and APACHE-II score might play an additional role in the earlier outcome prediction (Table 5, Fig. 1).

One of the limitations of our study is that our patient population was too small to perform further study, such as the association between pathological expression of cardiac markers and the outcome of various disease origins. Secondly, there is a possibility to find more detailed levels of Mb stratification in assessment of disease progress because of its remarkable elevation range and its sensitivity to multiple organ failure. We also conclude that further analysis is necessary to evaluate the stratification of other cardiac markers, as some of them we also have confirmed to be significant when compared with either APACHE or outcome.

Our study nonetheless emphasizes that the cardiac markers we studied play a pivotable role in the evaluation of disease severity and outcome prediction. Among the roles, Mb contributes to more accurate and reasonable evaluation provided by the multivariable APACHE-II score and prognosis. The study also acts to confirm our hypothesis that Mb might be more diversified and sensitive indicators in detecting disease progress not only for its sensitive monitor of myocardial injury but also for reflecting other organs faced with decompensation of tissue supply including hypoxia.

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