Clinical Research

Role of the mTOR Signalling Pathway in Human Sepsis-Induced Myocardial Dysfunction

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See editorial by Walley, pages 809−812 of this issue.

ABSTRACT

Background: Sepsis-induced myocardial dysfunction (SIMD) is a life-threatening complication of sepsis. Mammalian target of rapamycin (mTOR) signalling pathway is significantly associated with SIMD in an animal model; however, there have been no clinical studies of the association in humans.

Methods: We enrolled 88 patients with sepsis who were admitted to the intensive care unit (ICU) between April 2017, and April 2018. Biochemical indexes, hemodynamic parameters, and bedside echocardiographic parameters were recorded. Serum levels of mTOR, phosphorylated ribosome S6 protein kinase (PS6K), microtubule-associated protein light chain 3 type II (LC3B), Bcl-2-interacting mediator of cell death (BIM), interleukin 6, interleukin 10, and interferon-γ were examined.

RÉSUMÉ

Contexte : La dysfonction myocardique induite par le sepsis (DMIS) est une complication du sepsis pouvant être mortelle. Dans un modèle animal, une association significative a été observée entre la cible de la voie de signalisation de la rapamycine chez les mammifères (mTOR) et la DMIS; toutefois, aucune étude clinique n'a été effectuée au sujet d'une telle association chez l'humain.

Méthodologie : Nous avons recruté 88 patients atteints de sepsis qui avaient été admis au service des soins intensifs (SSI) entre avril 2017 et avril 2018. Les indices biochimiques, les paramètres hémodynamiques et les paramètres des échocardiographies réalisées au chevet des patients ont été consignés. Les taux sériques de mTOR, de protéine kinase ribosomale S6 phosphorylée (PS6K), de chaîne légère 3 de la protéine associée aux microtubules de type II (LC3B), de

The appearance of cardiomyocyte damage and cardiac function depression in the early phase of sepsis is an important factor that negatively affects patient prognosis and increases the mortality rate by up to 50%.[1-4] Sepsis-induced myocardial dysfunction (SIMD) is characterized by left ventricular dilatation and depressed ejection fraction.[4] Although the precise pathogenesis of SIMD is unknown, we previously showed that inhibition of the mammalian target of rapamycin (mTOR) pathway is cardioprotective in a rat model of sepsis-associated myocardial dysfunction and that the effect may be mediated by acceleration of autophagy.[5]

mTOR is an atypical serine/threonine protein kinase that functions as 2 distinct complexes—mTORC1 and mTORC2—and plays an important regulatory role in cell proliferation, metabolism, regeneration, and differentiation.[6] The balance between mTORC1 and mTORC2 activity is important for the regulation of ischemic damage and cardiac

remodelling after myocardial infarction.[7] The mTOR pathway also regulates cardiomyocyte metabolism, and deregulated cardiac mTORC1 activation is a pathophysiologic mediator of cardiac hypertrophy and dysfunction in different types of cardiomyopathy.[8] Previous preclinical studies showed that suppression of mTORC1 activity occurs as an adaptive cellular response to various forms of heart disease and promotes cell survival through activation of autophagy and inhibition of proteasome activity.[7-9] However, to our knowledge, there have been no clinical investigations of the activation status of the mTOR signalling pathway during the development of SIMD.

Here, we aimed to test our hypothesis that activation of mTOR and its associated effector pathways may play a crucial role in patients with sepsis and SIMD through deactivation of autophagy.

Materials and Methods

Patients and study design

This prospective study was performed at the Peking Union Medical College Hospital (PUMCH) between April 2017, and April 2018 and was approved by the PUMCH

Results: Compared with non-SIMD patients, patients with SIMD had higher ICU and 28-day mortality, PS6K and BIM levels, but lower LC3B levels. Serum PS6K levels in patients with SIMD were significantly negatively and positively correlated with LC3B and BIM, respectively. Multivariate regression analysis revealed that PS6K concentration at admission was an independent predictor of 28-day mortality. Receiver operating characteristic curve analysis indicated that a PS6K concentration cutoff of 42.43 pg/mL at ICU admission could predict the incidence of SIMD with a sensitivity and specificity of 91.7% and 96.2%, whereas a cutoff concentration of 41.17 pg/mL PS6K could predict 28-day mortality with a sensitivity and specificity of 83.3% and 54.3%, respectively.

Conclusions: Patients with sepsis and SIMD had higher ICU and 28-day mortality. Higher serum PS6K concentrations were significantly associated with SIMD incidence and 28-day mortality, suggesting that activation of the mTOR pathway may play a major role in SIMD.

médiateur de mort cellulaire interagissant avec Bcl-2 (BIM), d'interleukine-6, d'interleukine-10 et d'interféron-γ ont été examinés.

Résultats : Les patients atteints de DMIS, comparativement à ceux qui n'étaient pas atteints, présentaient une mortalité pendant leur séjour au SSI et après 28 jours et des taux de PS6K et de BIM plus élevés, mais des taux de LC3B plus faibles. Chez les patients atteints de DIMS, les taux sériques de PS6K étaient, de façon significative, corrélés négativement avec les taux de LC3B et positivement avec ceux de BIM. Une analyse de régression multivariée a révélé que la concentration de PS6K à l'admission était un facteur prédictif indépendant de la mortalité après 28 jours. L'analyse de la courbe ROC (*receiving operating characteristic*) a montré qu'un seuil de coupure de 42,43 pg/ml de PS6K à l'admission au SSI permettait de prédire l'incidence de la DMIS avec une sensibilité de 91,7 % et une spécificité de 96,2 %, tandis qu'un seuil de coupure de 41,17 pg/ml de PS6K permettait de prédire la mortalité après 28 jours avec une sensibilité de 83,3 % et une spécificité de 54,3 %.

Conclusions : La mortalité, que ce soit au SSI ou après 28 jours, était plus élevée chez les patients atteints de sepsis et de DMIS. Les concentrations sériques de PS6K plus élevées étaient associées de façon significative à l'incidence de la DMIS et à la mortalité après 28 jours, ce qui laisse croire que l'activation de la voie du mTOR pourrait jouer un rôle clé dans la DMIS.

institutional review board (approval number JS-1170). Informed consent was obtained from all the enrolled patients. The study was registered at the Chinese Clinical Trial Registry (chictr.org.cn; identifier ChiCTR-OOB-17014129).

Inclusion criteria were (1) age ≥ 18 years, (2) intensive care unit (ICU) stay of more than 48 hours, and (3) diagnosis of sepsis (see below). Exclusion criteria were (1) age < 18 years, (2) heart attack or acute exacerbation of previous heart disease in the last week; (3) heart surgery in the last week, with results that might affect the interpretation of SIMD; and (4) previous abnormal echocardiography and no recent evaluation (within 6 months), with results that might affect the interpretation of the results.

Sepsis was defined according to consensus international guidelines[1] as life-threatening organ dysfunction caused by a dysregulated host response to infection. Organ dysfunction was defined as an acute change in total Sequential Organ Failure Assessment (SOFA) score of ≥ 2 points consequent to the infection. As there is no universally accepted definition of SIMD, we integrated past references[10-13] to obtain the following diagnostic criteria: (1) a diagnosis of sepsis and (2) either typical ultrasonic manifestations of septic cardiomyopathy (left ventricle ejection fraction [LVEF]) ≤ 50% or (left ventricle end diastolic diameter [LVEDD] >50 mm) or at least 2 of the following: cTnI (cardiac troponin I) > 3-fold the upper limit of normal with no sign of acute coronary syndrome (no elevation of ST segment or depression; no complaint of chest pain/discomfort); N terminal natriuretic peptide (NT-ProBNP) > 3-fold the upper limit of normal with no sign of fluid overload (central venous pressure [CVP] < 12 mm Hg or inferior vena cava [IVC] < 2.0 cm); manifestations of low cardiac output (low stroke volume/velocity time integral, prolonged Pv-aCO$_2$, and low ScvO$_2$); and a requirement for inotropes (epinephrine, dobutamine, milrinone, levosimendan, or deslanoside).

Vital sign tests, laboratory tests, echocardiography, and analysis of serum concentrations of mTOR-related molecules were all carried out early in the morning of the first full day after admission to ICU. Baseline clinical and laboratory characteristics included age, sex, comorbidities, hemodynamic parameters, blood chemistry, arterial blood gas analysis, Acute Physiology and Chronic Health Evaluation (APACHE) II score, and SOFA score. Life-sustaining treatments included mechanical ventilation, vasopressors, and renal replacement. Follow-up data included duration of ICU and in-hospital stays, ICU and in-hospital mortality rate, and 28-day mortality rate. Peripheral blood samples were collected and centrifuged immediately, and the serum was withdrawn and stored at −80° refrigeration until assessment of mTOR, phosphorylated ribosome S6 protein kinase (PS6K), microtubule-associated protein light chain 3 type II (LC3B), Bcl-2-interacting mediator of cell death (BIM), interleukin 6 (IL-6), interleukin 10 (IL-10) and interferon-γ (IFN-γ) concentrations by enzyme-linked immunosorbent assay (ELISA) (mTOR: Abcam ab45996 Shanghai, China; PS6K: Abcam ab9366 Shanghai, China; LC3B: Affinity AF4650 CST China; BIM: Abcam ab112387 Shanghai China; IFN-γ: Abcam ab56070 Shanghai China; IL-6: Abcam ab6672 Shanghai, China; IL-10: Abcam ab34843 Shanghai, China).

Echocardiographic examinations were performed by 2 experienced intensivists. An ultrasonic machine (M-Turbo Sonosite, Highland Heights, Ohio) equipped with a 15 MHz transducer was used for noninvasive transthoracic echocardiography. LVEDD and LVEF were assessed using the modified biplane Simpson equation in the apical 4- and 2-chamber views according to the American Society of Echocardiography's guidelines.[14] Data from 3 consecutive cardiac cycles were analyzed and averaged. Two cardiologists assisted in the interpretation of clinical indicators to rule out acute coronary syndrome. If they disagreed, a third, more experienced intensivist resolved the disagreement. An independent clinical

research organization monitored the conduct of the trial according to Good Clinical Practice and Standard Operating Procedures in compliance with Chinese government regulations.

Statistical analysis

Normally distributed data are expressed as the mean and standard deviation and were compared using Students' t-test. Non-normally distributed data are expressed as the median and interquartile range, and differences were analyzed with the Mann−Whitney U test. Categorical variables are presented were recorded as proportions and were compared using the χ^2 or Fisher's exact tests. The results are expressed as the odds ratio (OR) with 95% confidence intervals (CIs). The significance level was set at a 2-sided P value of 0.05. Univariate and multivariate logistic regression analyses were performed to identify mTOR-associated parameters with predictive value for 28-day mortality. The results are expressed as the Wald index, OR, and 95% CIs. The discriminatory ability of mTOR-related parameters for predicting the incidence of SIMD and 28-day mortality were determined by receiver operating characteristic (ROC) curve analysis. The reliabilities and consistencies of the diagnostic tests were assessed by calculating their sensitivity, specificity, positive predictive value, and negative predictive value. Statistical analysis was performed using SPSS version 22.0 (SPSS, Chicago, IL).

Results

Patient characteristics

Within the 12-month period of this study, 143 patients with sepsis were admitted to our ICU. Of these, 44 satisfied at least 1 of the exclusion criteria (5 were aged < 18 years, 33 had heart attacks or operations in the preceding week, and 6 had unclear echocardiography results), 3 patients refused to sign the consent form, 4 patients had incomplete data, and 4 were lost to follow-up. A total of 88 patients were enrolled in the study, 36 of whom met the SIMD criteria (Fig. 1). There were no significant differences in age, sex, comorbidities, APACHE II score, or SOFA score between the SIMD and non-SIMD groups (Table 1).

Clinical characteristics

The SIMD and non-SIMD groups displayed no significant differences in CVP (8.6 ± 2.4 and 8.3 ± 2.9 mm Hg, respectively, $P = 0.68$) or IVC (1.5 ± 0.5 and 1.5 ± 0.6 cm, respectively, $P = 0.41$). Compared with non-SIMD patients, the SIMD group had significantly lower cardiac output, which manifested as longer Pv-aCO$_2$ (5.1 ± 2.2 vs 2.6 ± 1.6 mm Hg, $P < 0.001$), lower ScvO$_2$ (66.7% ± 6.2% vs 76.4% ± 6.3%, $P < 0.001$), greater requirement for inotropes (30.6% vs 7.7%, $P = 0.006$), and higher serum concentrations of cTnI (0.61 ± 0.67 vs 0.14 ± 0.28 μg/L, $P < 0.001$) and NT-ProBNP (1338 ± 1762 vs 4915 ± 5038 pg/mL, $P = 0.004$). Patients with SIMD also had lower LVEF (55% ± 8% vs 51% ± 9%, $P < 0.001$) and larger LVEDD (48.9 ± 0.3 vs 45.7 ± 0.6 cm, $P = 0.046$) than the non-SIMD group. SIMD patients had significantly longer ICU stays than the non-SIMD patients (14 ± 9.9 vs 10 ± 5.2 days, $P = 0.028$) and significantly higher ICU mortality rate (30.6% vs 13.5%, $P = 0.047$) and 28-day mortality rate (30.6% vs 13.5%, $P = 0.047$) (Table 2), which is consistent with previous studies.

Comparison of mTOR pathway-associated parameters in SIMD and non-SIMD patient groups

Although there was no significantly difference in mTOR levels between the 2 groups, patients in the SIMD group had higher levels of PS6K (60.9 [50.8-70.0] vs 36.0 [32.9-40.7]
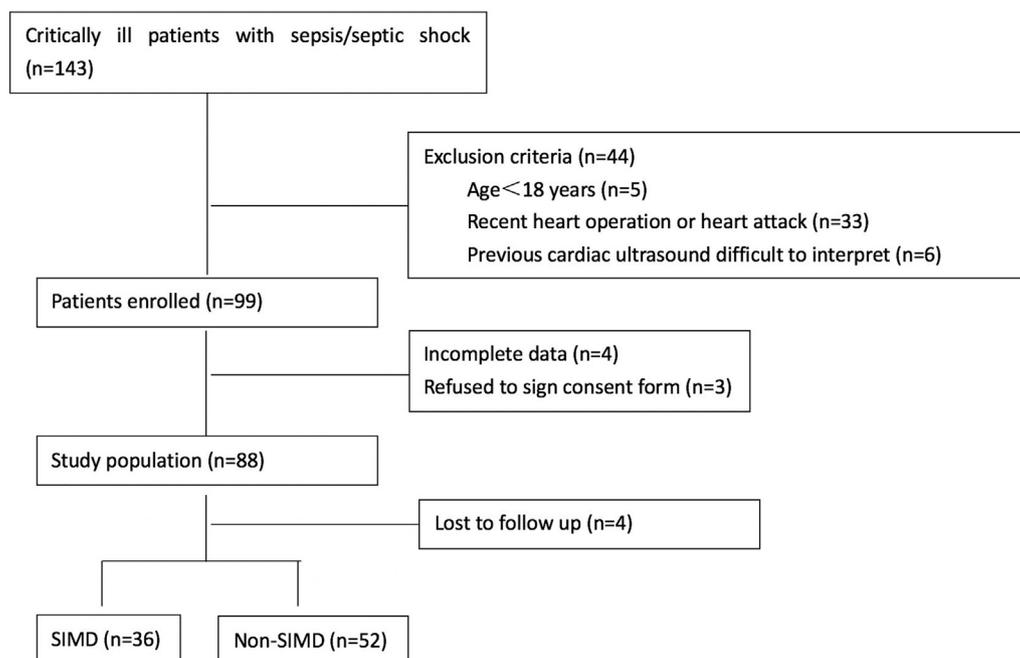

Figure 1. Enrollment chart. SIMD, sepsis-induced myocardial dysfunction.

Table 1. Characteristics of patients at ICU admission

	All patients N = 88	SIMD N = 36	Non-SIMD N = 52	P value*
Mean age (years)	64.5 ± 14.5	62.7 ± 14.7	65.7 ± 14.4	0.348
Gender (male, %)	55 (62.5%)	19 (52.8%)	36 (69.2%)	0.117
Chronic heart disease	19 (21.6%)	10 (27.7%)	9 (17.3%)	0.241
APACHE II score	18.1 ± 6.4	17.4 ± 6.0	18.6 ± 6.7	0.364
SOFA score	8.8 ± 4.5	10 ± 4.2	8.0 ± 4.6	0.075
Life-support				
MV	70 (79.5%)	27 (75%)	43 (82.7%)	0.519
NE (μg/kg/min)	0.19 ± 0.20	0.23 ± 0.19	0.17 ± 0.20	0.198
Inotropes (n, %)	15 (17.0%)	11 (30.6%)	4 (7.7%)	0.006
CRRT (n, %)	25 (28.4%)	9 (25%)	16 (30.7%)	0.628
HR (beats per min)	101 ± 21	101 ± 20	101 ± 23	0.913
MAP (mm Hg)	84 ± 10	85 ± 11	83 ± 9	0.506

Data are presented as the mean ± standard deviation or number (%) of patients.

APACHE II score, Acute Physiology and Chronic Health Evaluation II score; CRRT, continuous renal replacement therapy; HR, heart rate; MAP, mean arterial blood pressure; MV, mechanical ventilation; NE, norepinephrine; SOFA, sequential organ failure assessment score.

*P value for the comparison between SIMD and non-SIMD.

pg/mL, $P < 0.0001$), a downstream target of the mTOR pathway, as were levels of the apoptosis marker BIM (509.1 [378.5-1155.2] vs 401.0 [315.5-537.8] pg/mL, $P = 0.008$); in contrast, levels of the autophagy protein LC3B were lower in the SIMD group than the non-SIMD group (27.3 [19.1-48.1] vs 43.0 [20.7-111.0] pg/mL, $P = 0.016$) (Table 3). When concentrations of these molecules were evaluated according to the 28-day mortality of patients within the SIMD and non-SIMD groups, the same trends were observed (Fig. 2A-D).

To determine whether levels of mTOR pathway-related molecules could predict the incidence of SIMD among patients with sepsis, we performed ROC curve analysis of the parameters that differed significantly between the SIMD and non-SIMD patient groups. PS6K levels had the greatest discriminatory ability compared with the other parameters, with an area under the curve (AUC) value of 0.974. A cutoff value of 45.3 pg/mL PS6K at admission to ICU was able to

predict the incidence of SIMD with a sensitivity of 94.4% and specificity of 90.4% (Fig. 3A, Table 4).

Comparison of mTOR pathway-associated parameters in survivors and nonsurvivors

Next, we compared the features of patients with sepsis after stratification by 28-day mortality. The mTOR pathway-associated molecules were significantly different (Fig. 4). To assess their ability to predict mortality, we performed ROC curve analysis of the molecules that differed significantly between the survivors and nonsurvivors. As we observed for the prediction of SIMD incidence, PS6K had the greatest discriminatory ability (AUC 0.742), and a cutoff value of 41.17 pg/mL PS6K at admission to ICU predicted mortality with a sensitivity of 83.3% and specificity of 54.3% (Table 4, Fig. 3B). To further assess the power of mTOR pathway-related molecules and clinicopathological parameters to

Table 2. Clinical characteristics of patients

	All patients N = 88	SIMD N = 36	Non-SIMD N = 52	P value*
CVP (mm Hg)	8.5 ± 2.6	8.6 ± 2.4	8.3 ± 2.9	0.678
Lac (mmol/L)	1.5 ± 0.6	1.5 ± 0.5	1.4 ± 0.6	0.41
ScvO$_2$ (%)	71.6 ± 8.1	66.7 ± 6.2	76.4 ± 6.3	< 0.001
Pv-aCO$_2$ (mm Hg)	3.8 ± 2.3	5.1 ± 2.2	2.6 ± 1.6	< 0.001
cTnI (μg/l)	0.40 ± 0.58	0.61 ± 0.67	0.14 ± 0.28	< 0.001
NT-ProBNP (pg/mL)	3590 ± 4468	4915 ± 5038	1338 ± 1762	0.004
Echocardiographic parameters				
IVC (cm)	1.6 ± 0.33	1.6 ± 0.34	1.5 ± 0.31	0.144
LVEF	55% ± 8%	51% ± 9%	61% ± 5%	< 0.001
LVEDD (mm)	45.7 ± 0.6	48.9 ± 0.3	43.1 ± 0.7	0.046
Prognostic parameters				
ICU stay (days)	11.9 ± 7.8	14 ± 9.9	10 ± 5.2	0.028
Hospital stay (days)	24.2 ± 21.5	23.3 ± 17.1	24.9 ± 24.6	0.127
ICU mortality (n, %)	18 (20.5%)	11 (30.6%)	7 (13.5%)	0.047
In-hospital mortality (n, %)	20 (22.7%)	11 (30.6%)	9 (17.3%)	0.116
28-day mortality (n, %)	18 (20.5%)	11 (30.6%)	7 (13.5%)	0.047

Data are presented as the mean ± standard deviation or number (%) of patients.

CVP, central venous pressure; cTnI, cardiac troponin I; IVC, inferior vena cava width; Lac, lactate; LVEDD, left ventricle end diastolic diameter; LVEF, left ventricle ejection fraction; NT-ProBNP, N-terminal pro-brain natriuretic peptide; Pv-aCO$_2$, arteriovenous carbon dioxide partial pressure difference; ScvO$_2$, central venous blood oxygen saturation.

*P value for the comparison between SIMD and non-SIMD.

Table 3. mTOR pathway-associated parameters in sepsis patients with or without SIMD

	SIMD N = 36	Non-SIMD N = 52	P value*
mTOR	641.9 (518.4-792.3)	679.4 (528.4-878.8)	0.248
PS6K	60.9 (50.8-70.0)	36.0 (32.9-40.7)	< 0.0001
LC3B	27.3 (19.1-48.1)	43.0 (20.7-111.0)	0.016
BIM	509.1 (378.5-1155.2)	401.0 (315.5-537.8)	0.008
IL-6	67.1 (55.2-91.1)	78.1 (62.2-103.3)	0.34
IL-10	11.5 (8.72-13.5)	13.1 (10.1-16.2)	0.23
IFN-γ	21.6 (17.3-32.6)	18.8 (14.9-24.7)	0.046

Data are presented as the median and interquartile range in pg/mL.

BIM, Bcl-2 interacting mediator of cell death; IFN-γ, interferon γ; IL-6, interleukin 6; IL-10, interleukin 10; LC3B, microtubule associated protein light chain 3 type II; PS6K, phosphorylated ribosome S6 protein kinase.

*P value for the comparison between SIMD and non-SIMD.

predict 28-day mortality in patients with sepsis, we performed multivariate logistic regression analysis of all parameters that were significantly different in the univariate analysis. Among them, only PS6K showed a significant association with 28-day mortality ($\beta = -0.082$, 95% CI, 0.908-0.999, $P = 0.002$; Table 5). Thus, a high concentration of PS6K, which reflects abnormal activation of the mTOR pathway, was an independent risk factor for 28-day mortality in this patient cohort (Table 5).

Correlations between mTOR, PS6K, LC3B, and BIM

We hypothesized that inhibition of mTOR and its associated effector pathways played a similarly protective role in patients with sepsis and SIMD. Consistent with this, Pearson's analysis identified significant negative correlations between levels of PS6K and LC3B ($r = -0.547$, $P < 0.0001$) and levels of mTOR and BIM ($r = -0.488$, $P < 0.0001$), as well as significant positive correlations between levels of PS6K and BIM ($r = 0.755$, $P < 0.0001$) and levels of mTOR and LC3B ($r = 0.8922$, $P < 0.0001$) (Fig. 5).
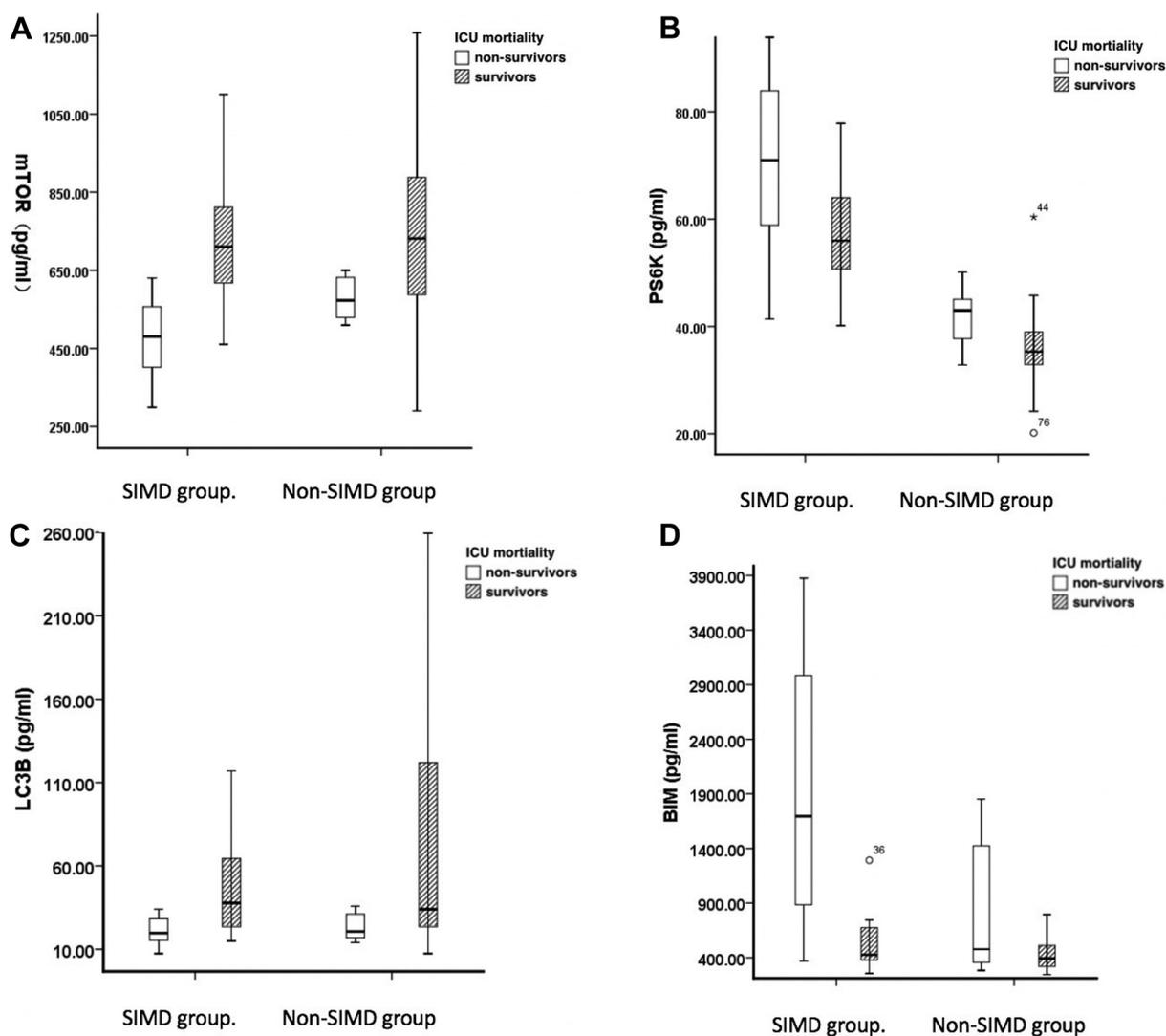

Figure 2. Serum mTOR (**A**), PS6K (**B**), LC3B (**C**), and BIM (**D**) levels in sepsis-induced cardiomyopathy (SIMD) and non-SIMD patients stratified by 28-day mortality. **Boxes and whiskers** represent the median, Q1–Q3. **Asterisks with numbers** represent data beyond the boxes.
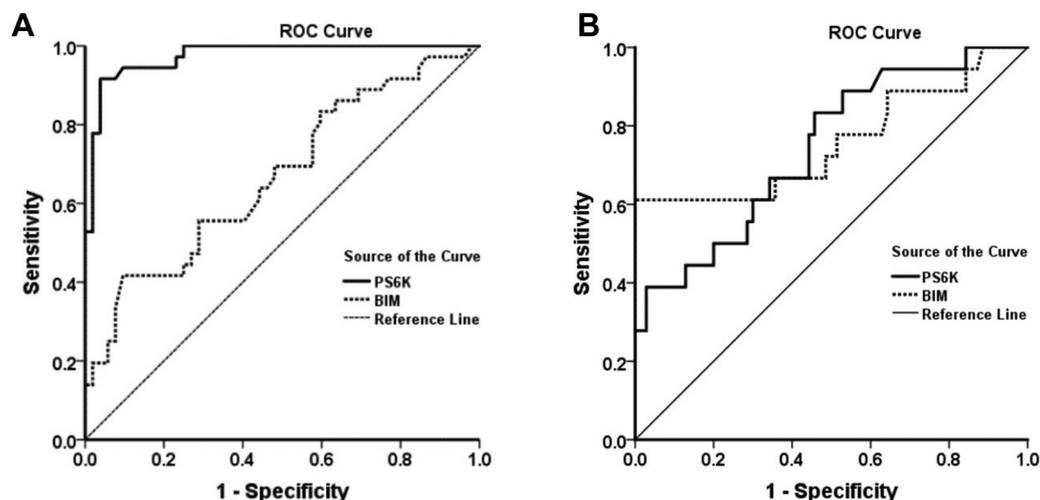
Figure 3. Receiver operating characteristic curve analysis of the ability of PS6K and BIM to predict the incidence of SIMD (**A**) or 28-day mortality (**B**).

Discussion

To our knowledge, this is the first prospective clinical study of the involvement of the mTOR pathway in patients with sepsis and SIMD. Among our cohort of 88 patients, the 36 patients with SIMD had higher ICU and 28-day mortality rates, higher serum PS6K and BIM levels, and lower serum LC3B levels than the 52 patients without SIMD. Notably, of the molecules examined, concentrations of PS6K showed the best ability to predict the incidence of SIMD and 28-day mortality, as reflected by AUC. Higher PS6K concentrations were associated with greater incidence of SIMD and lower survival probability, suggesting a potential role for the mTOR pathway in the incidence of SIMD and rate of 28-day mortality in patients with sepsis, possibly via inactivation of autophagy and enhancement of apoptosis.

Patients in the SIMD group had significantly higher 28-day mortality than the non-SIMD patients, which is consistent with the current consensus and clinical findings.[4] However, some authors contend that SIMD is a self-protective mechanism for severely infected patients. Vieillard et al.[15] demonstrated that patients in a hyperkinetic state had significantly higher mortality than patients in either a hyperkinetic or normal-output state. However, Repesse et al.[16] suggested that the results of that seminal study should be revisited. LVEF reflects the coupling between LV afterload and LV contractility, and a normal LVEF may be observed even when arterial tone is severely depressed. Thus, rather than patients with low EF showing better survival, the increase in mortality may have been due to persistent profound

vasoplegia.[16] A recent meta-analysis also suggested that ventricular dysfunction or dilatation in patients with sepsis was not associated with lower mortality.[17]

The mTOR signalling pathway plays an important regulatory role in many cell functions, including proliferation, metabolism, regeneration, and differentiation. One study[18] has shown that mTOR was the key signalling pathway for regulation of autophagy in cardiomyocytes, whereas more recent work[6] confirmed that autophagy imbalance mediated by the mTOR pathway was associated with aggravation of ischemic cardiomyocyte necrosis. Our previous study[5] demonstrated that rats with cecal ligation and puncture-induced sepsis showed reduced mTOR pathway activation compared with sham-operated rats, an effect that may have been mediated by increased autophagy. In the current study, although there was no significant different in serum mTOR levels between the 2 groups, serum PS6K level, a downstream target of mTORC1, was significantly higher. In addition, serum levels of LC3B, a major autophagy protein, were lower in SIMD than non-SIMD patients, whereas the BCL-2 family member BIM, which promotes apoptosis, was significantly higher. Therefore, this study has confirmed in humans the results of our earlier animal experiments that the mTOR pathway plays a role in cardiac health through the regulation of autophagy and apoptosis. We speculate that in patients with SIMD, the mTOR signalling pathway is abnormally activated, leading to inhibition of autophagy, enhancement of apoptosis, and myocardial damage and necrosis, a major mechanism of cardiac dysfunction in SIMD. Our findings

Table 4. ROC curve analysis for the prediction of SIMD and mortality in sepsis patients

Parameter	Cutoff value (pg/mL)	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	P value
ROC curve for the prediction of SIMD incidence					
PS6K	45.3	0.974 (0.9466-1.002)	94.44 (81.34-99.32)	90.38 (78.97-96.8)	< 0.0001
BIM	506.2	0.6683 (0.5521-0.7844)	55.56 (38.1-72.06)	71.15 (56.92-82.87)	0.0075
ROC curve for the prediction of 28-day mortality					
PS6K	41.17	0.742 (0.6138-0.8703)	0.8333 (0.5858-0.9642)	0.5429 (0.4194-0.6626)	0.0016
BIM	1327	0.7579 (0.6046-0.9113)	0.6111 (0.3575-0.827)	1 (0.9487-1.000)	0.0008

AUC, area under the curve; BIM, Bcl-2-interacting mediator of cell death; CI, confidence interval; PS6K, phosphorylated ribosome S6 protein kinase; ROC, receiver operating characteristic.
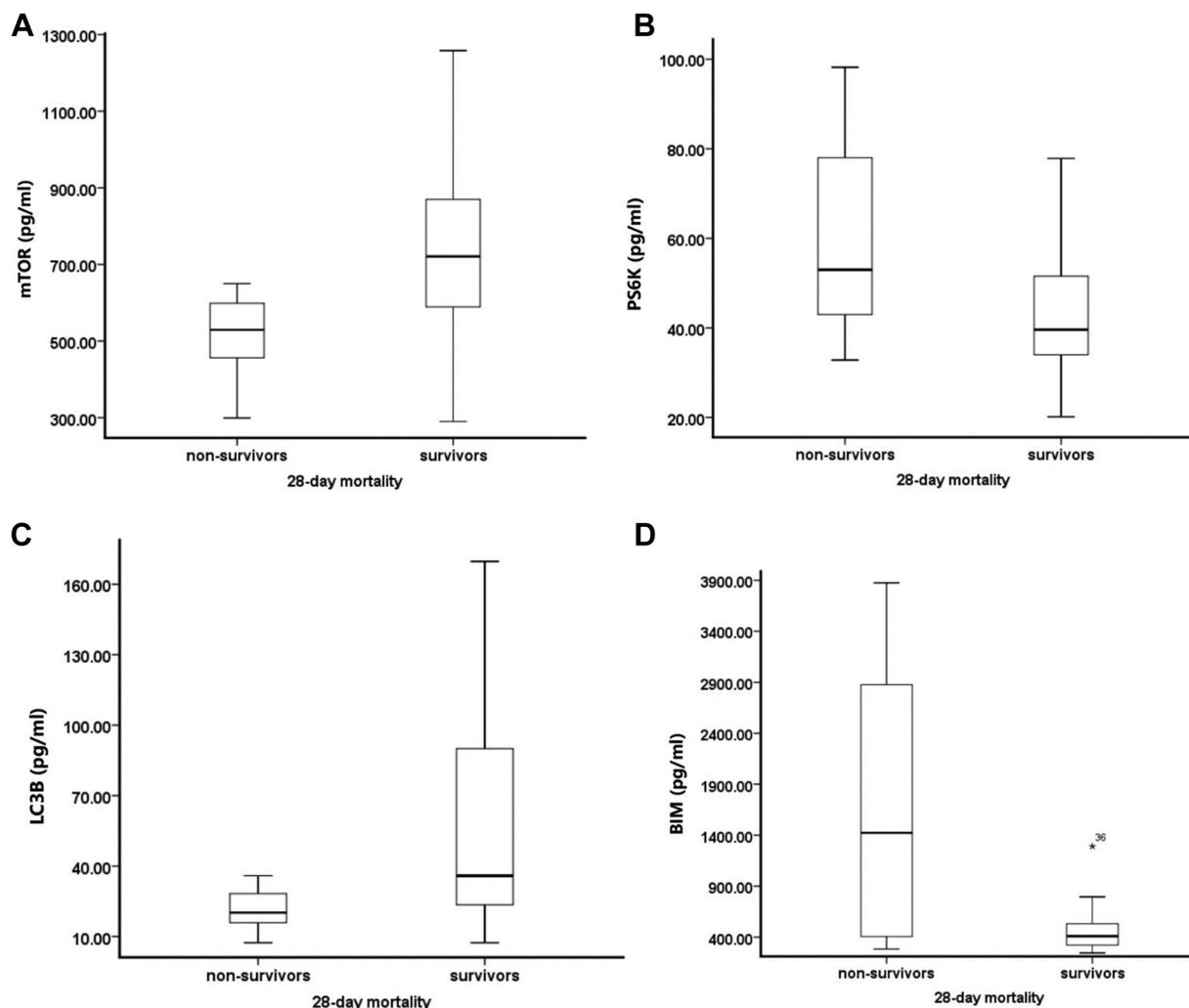
Figure 4. Serum mTOR (**A**), PS6K (**B**), LC3B (**C**), and BIM (**D**) levels in patients with sepsis stratified by 28-day mortality. **Boxes and whiskers** represent the median, Q1–Q3. **Asterisks with numbers** represent data beyond the boxes.

that PS6K and LC3B concentrations are negatively correlated, whereas PS6K and BIM concentrations are positively correlated support this hypothesis. In addition, our results are also consistent with the study by Zhang et al.[19] However, our findings in patients differ from our previous basic research using a rat model of sepsis, which showed lower mTOR expression in the cardiomyocytes of rats with SIMD. It is possible that the differences are due to examining different stages of sepsis, when the myocardium has specific energy states or when mTOR pathway-associated effector levels in

serum differ from those in tissue. Regardless of the precise reason for the discrepancy, this topic clearly requires further exploration.

Paradoxically, we found that the serum concentration of mTOR was reduced in patients with SIMD compared with non-SIMD patients, although there was no statistical significance. We propose the following explanations for our findings.[20-22] First, the ELISA method employed measured total mTOR concentrations in serum, whereas the active intracellular form is phosphorylated mTOR. Second, mTOR activity is mediated and regulated via binding of adaptor proteins to form mTORC1 and mTORC2, which may play different roles at different stages of SIMD. The balance between mTORC1 and mTORC2 activity may thus be crucial for its involvement in SIMD, and their ratio in serum remains to be investigated. Finally, additional mechanisms may be involved in mTOR pathway activation in SIMD. The precise reason for the low serum mTOR levels in our SIMD patient group will be the subject of further studies.

Despite intense research on autophagy and apoptosis, the translation of basic research findings to clinical application continues to be difficult. Since its discovery in the 1970s,

Table 5. Multivariate logistic regression analysis of factors predicting 28-day mortality in sepsis patients

	β	SE	Wald	OR	P value
APACHEII score	−0.061	0.068	0.820	0.940	0.365
Vasopressor	0.436	2.120	0.042	1.547	0.837
LVEF	1.381	4.224	0.107	3.979	0.744
PS6K	−0.082	0.027	9.483	0.921	0.002
Constant	6.263	3.499	3.205	524.933	0.073

LVEF, left ventricle ejection fraction; OR, odds ratio; PS6K, phosphorylated ribosome S6 protein kinase; SE, standard error of the mean.
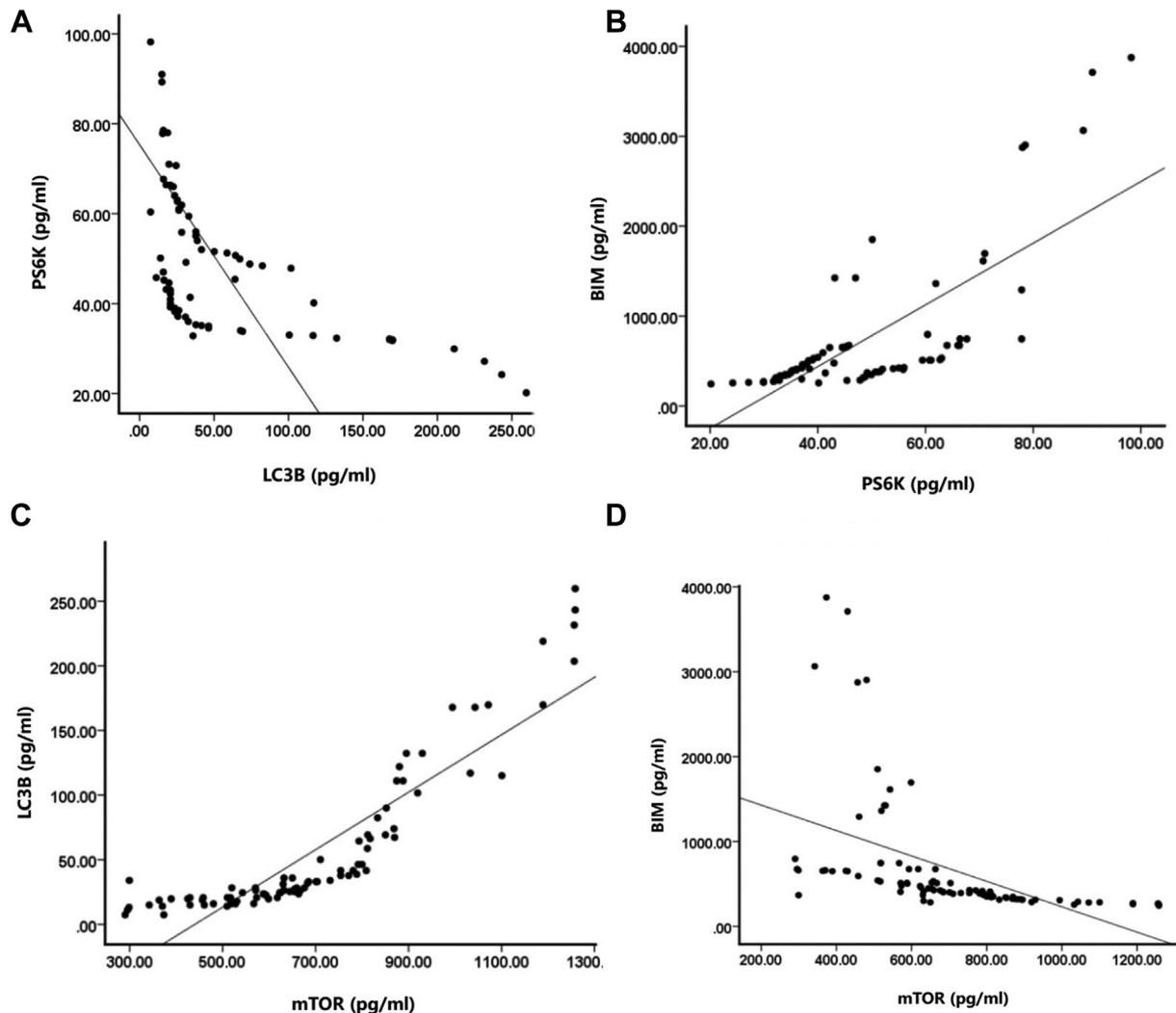
Figure 5. Correlations between serum PS6K and LC3B level (**A**), PS6K and BIM levels (**B**), mTOR and LC3B levels (**C**), and mTOR and BIM levels (**D**) in patients with sepsis.

rapamycin has been widely used as an immunosuppressant in organ transplantation. Many studies have demonstrated that rapamycin-mediated inhibition of mTOR regulates autophagy and apoptosis in various cell types, but few studies have examined the effects of rapamycin on sepsis-related organ dysfunction. Our recent study[5] showed that treatment of SIMD mice with rapamycin inhibited mTOR signalling, enhanced autophagy, and significantly improved cardiac function. As rapamycin is a well-studied drug with a large amount of clinical trial and postmarketing data, it should be relatively straightforward to test its therapeutic utility for patients with SIMD. In our study, multivariate regression analysis and ROC curve analysis demonstrated that 28-day mortality was significantly correlated with PS6K, LC3B, and BIM in both SIMD and non-SIMD patient groups and that PS6K could predict 28-day mortality in all patients with sepsis. These findings confirm that mTOR signalling and an imbalance between autophagy and apoptosis play a role in the prognosis of patients with SIMD. Moreover, inhibiting the mTOR signalling pathway could be a novel therapy not only

for reducing the incidence of SIMD but also for improving the prognosis, which we are currently exploring.

There are several limitations to this study. First, the results are not entirely consistent with our previous nonclinical research, which may be due to differences in myocardial tissues and plasma contents at different stages of sepsis. Indeed, a recent study demonstrated that autophagy in the liver is initially activated during sepsis, followed by a subsequent phase in which autophagy fails to proceed to completion.[23] Further animal experiments and clinical trials will be necessary to determine whether similar events are occurring in the heart. Second, the precise mechanism by which mTOR signalling is involved in SIMD needs to be studied further. For example, experiments in knockout mice and selective inhibition of mTORC1 and mTORC2 will be helpful in determining the specific roles. Third, the patient sample size in this study was small, and the results will need to be verified with larger cohorts in multicentre studies. Nevertheless, this study breaks new ground in several ways: It is the first study to determine the mTOR signalling pathway status in patients

with SIMD, and it is the first to provide preliminary evidence that an imbalance between autophagy and apoptosis mediated by the mTOR pathway might be an important molecular mechanism underlying SIMD.

Conclusion

Patients with sepsis and SIMD have higher ICU and 28-day mortality rates than non-SIMD patients. Activation of the mTOR pathway may play a major role in SIMD. All raw data used to construct the figures and tables and to support the findings of this study are available from the corresponding author upon request.

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Disclosures

The authors have no conflicts of interest to disclose.

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