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ORIGINAL RESEARCH

Soluble ST2 Is a Sensitive and Specific Biomarker for Fulminant Myocarditis

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BACKGROUND: The aim of the study was to identify biomarkers that can facilitate early diagnosis and treatment of fulminant myocarditis (FM) in order to reduce mortality.

METHODS AND RESULTS: First, the expression profiles of circulating cytokines were determined in the plasma samples from 4 patients with FM and 4 controls using human cytokine arrays. The results showed that 39 cytokines from patients with FM were changed at admission. Among them, 8 cytokines returned to normal levels at discharge, including soluble ST2 (sST2), which showed the most marked dynamic changes from disease onset to resolution. Then, in a cohort of 76 patients with FM, 57 patients with acute hemodynamic dysfunction attributable to other causes, and 56 patients with non-FM, receiver operating characteristic curve analyses suggested that plasma sST2 level was able to differentiate FM from non-FM or other FM-unrelated acute heart failure more robustly N-terminal pro-B-type natriuretic peptide or cardiac troponin I. Moreover, longitudinal analysis of plasma sST2 was performed in 10 patients with FM during hospitalization and 16 patients with FM during follow-up. Finally, the diagnostic value was validated in an additional 26 patients with acute onset of unstable hemodynamics. The cutoff value of plasma sST2 for optimal diagnosis of FM was established at 58.39 ng/mL, where a sensitivity of 85.7% and specificity of 94.7% were achieved.

CONCLUSIONS: Elevated sST2 level was associated with mechanical stress or inflammation. Especially, sST2 might be used as a potential biomarker for the rapid diagnosis of FM, which was characterized by strong mechanical stretch stimulation and severe inflammatory response.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT03268642.

Key Words: adult ■ biomarker ■ fulminant myocarditis ■ inflammatory ■ soluble ST2 (sST2)

Myocarditis is a rare disease, with a prevalence of ≈22 per 100 000 people.[1] Acute myocarditis is generally considered a mild and self-limited condition caused by infection, autoimmune disorders, poisoning, or toxic drug effects, although some cases can progress to chronic heart failure (HF).[2–4] While multiple pharmacological drugs have been associated with myocarditis, temporal trends and overall mortality have not been reported. Systematic analysis of drug-associated myocarditis reported in the World Health Organization pharmacovigilance database promoted trials on the use of steroids and interleukin (IL) 1β inhibitors, which might change therapeutic perspectives (ClinicalTrials.gov Identifier: NCT05150704 and

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CLINICAL PERSPECTIVE

What Is New?

- In the current study, we found a significant induction of a broad spectrum of inflammatory cytokines in patients' plasma at the onset of fulminant myocarditis, supporting the presence of so-called inflammatory storm as a part of the pathogenic features of the disease.

What Are the Clinical Implications?

- The induction and normalization of plasma soluble ST2 were significantly and specifically correlated with the onset and clinical improvement of fulminant myocarditis, implicating its potential diagnosis role in the disease.

Nonstandard Abbreviations and Acronyms

cTnI	cardiac troponin I
FM	fulminant myocarditis
NFM	nonfulminant myocarditis
sST2	soluble ST2

NCT03018834).[5] Fulminant myocarditis (FM), however, is the most severe type of myocarditis characterized by its sudden occurrence and rapid deterioration.[6] Patients with FM quickly develop hemodynamic dysfunction and require immediate mechanical circulatory support. Moreover, respiratory, liver, or kidney failure may occur simultaneously.[7,8] In a study based on an international multicenter registry, Ammirati et al[9] found that patients with FM had significantly higher rates of cardiac death and heart transplantation than those with non-FM (NFM) at both 60-day (28.0% versus 1.8%) and 7-year (47.7% versus 10.4%) follow-up. Therefore, developing an effective diagnostic and treatment regimen for FM remains to be a significant clinic challenge with major unmet needs.

It is emphasized by both the American Heart Association (AHA) and the Chinese Society of Cardiology that early diagnosis and appropriate treatment will benefit patients with FM.[6,10] However, one of the main challenges in realizing a definitive diagnosis for FM is to distinguish it from other cardiac conditions with similar clinical features of sudden-onset of hemodynamic dysfunctions, especially from acute HF caused by unknown preexisting cardiomyopathies. Patients with FM and unrelated acute HF often present with similar patterns of clinical symptoms and laboratory results but require different therapies. Endomyocardial biopsy could help distinguish the different pathological types of acute HF attributable to different causes. However, this invasive examination was not routinely performed[11] and the sensitivity was limited.[12] Although contrast enhancement cardiac magnetic resonance imaging is a more sensitive technique to detect areas of myocardial damage, it may be limited in access and not readily available under emergent circumstances.[13] Both endomyocardial biopsy and cardiac magnetic resonance imaging have their limitations, and most of cannot obtain results in the first moments after patients with FM are admitted to the hospital. Considering that the condition of patients with FM changes and progresses rapidly, a robust and specific marker for quick results to differentiate these conditions would be critical. Unfortunately, specific diagnostic biomarkers for suspected myocarditis are limited. The current diagnosis for myocarditis relies partially on circulating troponin level and echocardiography features (class of recommendation 1, level of evidence C), which lack sensitivity.[4,11] NT-proBNP (N-terminal pro-B-type natriuretic peptide) and cardiac troponin I (cTnI) are frequently used as biomarkers for FM. However, they are also generic biomarkers for diverse forms of HF or myocardial injury attributed to different causes. Therefore, new reliable, sensitive, and specific biomarkers for FM are urgently needed. Recently, COVID-19 and the messenger RNA vaccine gained further attention on myocarditis.[14,15]

Recently, a circular RNA, hsa-circ-0071542, was reported as a promising biomarker for pediatric FM.[16] However, the detection method is technically complex and time-consuming. Considering the fact that FM is a severe inflammatory disease of the heart, we deduce that inflammation-associated cytokines may serve as potential biomarkers for FM.

Motivated by the need to identify a robust biomarker for the differential diagnosis of FM and a significant association of this disease with inflammation, we performed a comprehensive profiling of 122 inflammatory cytokines in plasma from a cohort of 4 patients with FM at their admission and discharge. Compared with age- and sex-matched controls, we observed significant changes for 39 cytokines in the FM samples at admission, supporting a state of so-called inflammatory storm. A subset of these cytokines was found to be normalized at discharge, with soluble ST2 (sST2) displaying the most significant changes during the onset and resolution of FM. In a separate cohort of patients with FM and patients with FM-unrelated acute hemodynamic dysfunction, plasma sST2 was found to be able to differentiate FM with higher specificity and sensitivity than the currently established biomarkers. Furthermore, longitudinal pattern of plasma sST2 was characterized in patients with FM during hospitalization and postdischarge follow-up. Finally, in a prospective

cohort study from an independent cohort, a threshold value of 58.39 ng/mL for plasma sST2 led to FM diagnosis with an accuracy of 92.3% and a positive likelihood ratio of 16.2. These results support that sST2 is a highly specific and sensitive biomarker for FM during the acute onset of the disease.

METHODS

The raw data that support the findings of this study are available from the corresponding authors on request. The authors declare that all data supporting the findings of this study are available within the article and its supplementary information files or from the corresponding author on reasonable request.

Study Population

The overall study design is shown in Figure 1. All patients and controls were recruited at Tongji Hospital, Wuhan, China, between April 2017 and March 2021. The study was approved by the ethics review board of Tongji Hospital and Tongji Medical College (ID: TJ-C20160202), and conformed to the principles of the Declaration of Helsinki. Informed consent was obtained from the patients directly or from their immediate family members in the case of incapacity.

FM and NFM (patients with myocarditis without severe hemodynamic compromise) were diagnosed according to the 2013 European Society of Cardiology position statement, 2017 Chinese Society of Cardiology expert consensus statement, and 2009 International Consensus Group on Cardiovascular Magnetic Resonance in Myocarditis statement.[4,17,18] Specifically, the diagnostic criteria for FM included the following: (1) the onset of acute HF symptoms was shorter than 2 weeks; (2) inotropic support or mechanical circulatory support caused by hemodynamical instability was administered; and (3) the presence of myocarditis was confirmed by cardiac magnetic resonance imaging before discharge. Only patients with cardiac magnetic resonance imaging–confirmed FM were enrolled in this study (Figure S1). Coronary angiography was performed in patients aged >25 years to rule out acute myocardial infarction (AMI). The exclusion criteria were: (1) patients aged <11 years; (2) patients with possible acute coronary syndrome, but without coronary angiography to distinguish acute coronary syndrome versus FM; (3) patients with myocardial injury caused by sepsis; and (4) patients with unstable hemodynamics or shock caused by hypovolemia.[17] During hospitalization, 11 patients were diagnosed as having lymphocytic myocarditis by endocardium myocardial biopsy. All patients enrolled after December 2019 were negative for severe acute respiratory syndrome coronavirus 2.

Acute HF was diagnosed according to the 2013 American College of Cardiology Foundation/AHA guideline for the management of HF,[19] as well as the 2016 European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic HF.[20]

For initial plasma cytokine profiling, 4 healthy controls and 4 sex- and age-matched patients with FM were enrolled. Samples were collected at admission before any treatment and at discharge for the patients with FM (Table S1).

For retrospective analysis, the study cohort included 76 patients with FM, 56 patients with NFM, 57 patients with hemodynamic unstable acute HF with various causes (including 21 acute myocardial infarction, 15 dilated cardiomyopathy, 10 valvular heart diseases, and 11 arrhythmias or congenital heart diseases), and 8 control individuals. Blood samples from the study cohort were collected at admission before any treatment, and their clinical characteristics are presented in Table and Table S2.

For longitudinal measurement during hospitalization, blood samples were collected from 10 patients with FM every 1 or 2 days in the morning from admission to discharge (Table S3).

For follow-up analysis, 16 patients with FM were recruited. By January 31, 2021, their plasma samples were collected at 6 months, 1 year, and 2 years post-discharge (Table S4).

In a prospective study, 26 patients with evidence initially suggestive of FM were enrolled between December 2020 and March 2021. Patients with confirmed AMI or angina and those whose symptoms were clearly not related to FM when presenting were excluded (Figure 1, Table S5).

For all participants, whole blood from different cohorts was drawn into sodium heparin tubes and processed immediately to obtain plasma, which was then stored at –80 °C before measurements. Baseline characteristics and therapeutic information of patients were collected from medical records and confirmed by the study physicians.

Human Cytokine Arrays

Plasma samples from 4 controls and 4 patients with FM both at admission before any treatment and at discharge underwent human cytokine arrays analyses (Cat#: QAH-CYT-8-1, QAH-IMR-1-1, QAH-CYT-4-1, and QAH-TH17-1-1), including 122 inflammation-associated cytokines (RayBiotech). Data were analyzed as previously described.[21] In brief, after normalizing the value of cytokines, volcano plots and expression heatmaps were generated using Prism 8 software (GraphPad Software). A different color code (right) represented a normalized level of cytokines log2 (fold change). When the control group was compared with patients with FM,

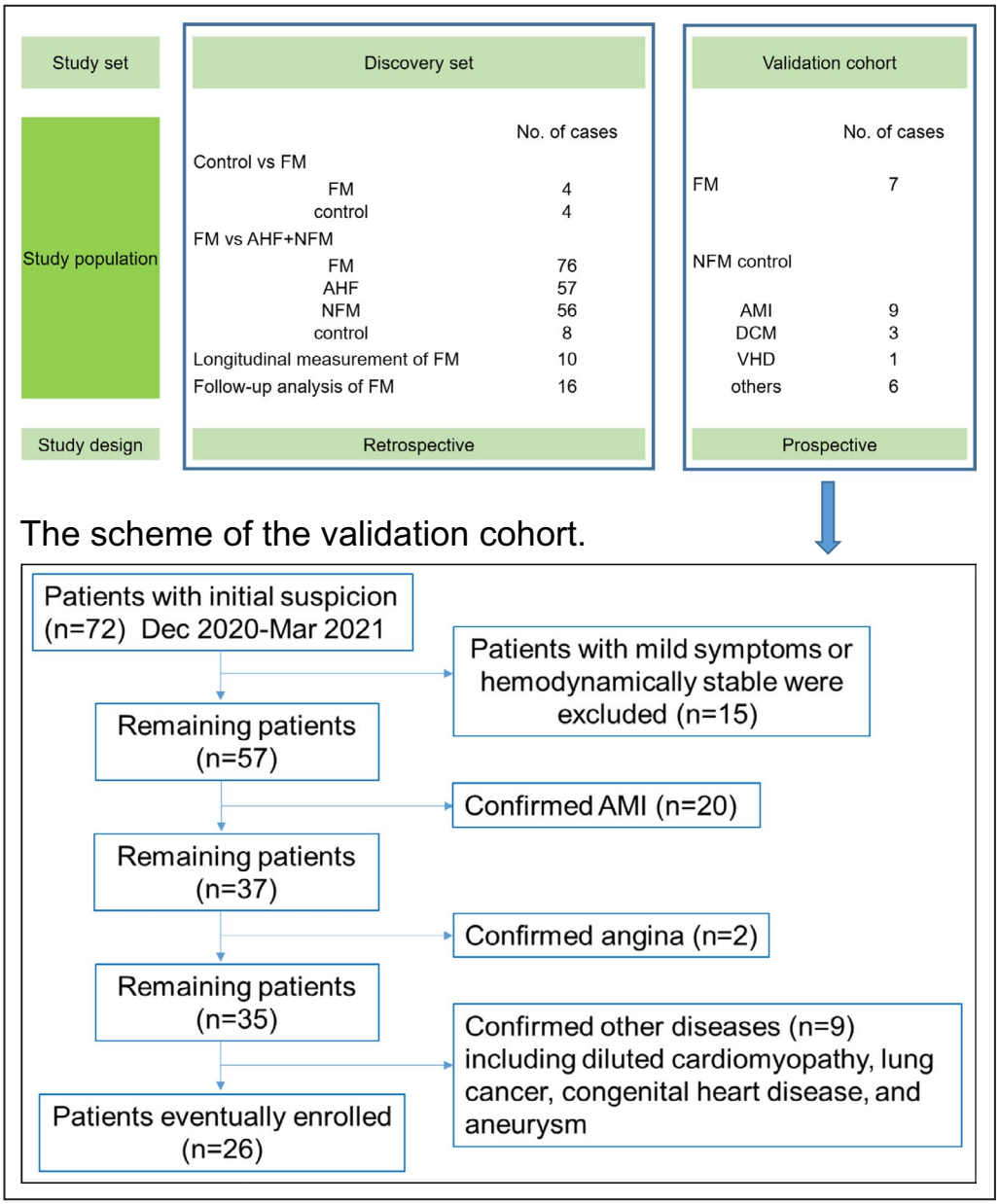

Figure 1. Overall study design and the scheme of the validation cohort.
Detailed population information and the corresponding objectives are shown in the Supplemental Tables. AHF indicates acute heart failure; AMI, acute myocardial infarction; DCM, dilated cardiomyopathy; FM, fulminant myocarditis; NFM, nonfulminant myocarditis; and VHD, valvular heart diseases.

the horizontal and vertical dotted lines in the volcano plots represented the threshold value for the significance used to define upregulation or downregulation of cytokines was a fold change >2 (or <0.5), as well as with an adjusted *P* value of <0.05, respectively. Statistical differentially expressed cytokines between 2 groups were identified through fold change and adjusted *P* value.

Measurement of Circulating sST2 Levels

Circulating levels of human sST2 were measured using a Human ST2/interleukin-33 receptor Quantikine ELISA kit (Cat#: DST200) from R&D System according to the manufacturer's instruction.

Statistical Analysis

Data are shown as scatter plots. A Kolmogorov-Smirnov test was performed to determine the normal distribution of continuous data. Continuous values are shown as mean±SD if normally distributed, or medians and first to third quartile (quartile 1–quartile 3) if not normally distributed. Student *t* test was used to compare the differences in normally distributed continuous

Table. Baseline Clinical Characteristics of Patients With NFM and Those With FM in the Validation Cohort

	NFM (n=56)	FM (n=76)	*P* value
Age, y	29.5 (20.3–47.0)	33.0 (23.3–49.0)	0.3159
Men/women, n	41/15	37/39	<0.0001
Asian race, n (%)	55 (99.1)	76 (100)	0.424
Presenting symptoms, n (%)			
Chest pain and/or tightness	49 (87.5)	57 (75.0)	0.082
Dyspnea	8 (14.3)	15 (19.7)	0.49
Syncope	2/56 (3.6)	9 (11.8)	0.116
Prodromal symptoms, n (%)			
Fever	19 (34.0)	43 (56.6)	0.012
Gastrointestinal symptoms	12 (21.4)	24 (31.6)	0.442
Respiratory symptoms	9 (16.1)	14 (18.4)	0.818
Fatigue	3 (5.4)	19 (25.0)	0.004
Duration of presenting symptoms <2 wk	56 (100)	76 (100)	…
Echocardiography at admission			
LVED, cm	4.9±0.6	4.7±0.6	0.3321
LVEF, %	57.0 (50.0–62.0)	30.0 (20.0–41.0)	<0.0001
Admission laboratory tests			
NT-proBNP, pg/mL	821.0 (292.5–3365.3)	8528.0 (3484.8–19 116.0)	<0.0001
cTnI, pg/mL	5474.7 (1755.0–17 341.0)	38 817.8 (19 256.3–50 000.0)	<0.0001
Increased hs-CRP, n (%)	42 (79.2)	66 (92.1)	0.045
Leukocyte $\times 10^9$/L	8.8 (6.8–10.8)	11.5 (8.8–14.9)	0.0004
ALT, U/L	37.5 (23.0–66.8)	53.5 (39.3–212.8)	0.0009
AST, U/L	60.0 (34.0–97.5)	181.5 (103.0–359.0)	<0.0001
Creatinine, μmol/L	68.0 (58.0–81.0)	85.5 (66.3–117.0)	0.0003
Urea, mmol/L	4.1 (3.0–5.5)	6.2 (4.0–11.3)	<0.0001
Potassium, mmol/L	4.0 (3.6–4.2)	4.1 (3.7–4.6)	0.034
ECG findings at admission, no./total no. (%)			
Normal	4/49 (8.2)	0/72 (0)	0.025
ST-T segment abnormalities	31/49 (63.3)	47/72 (65.3)	0.848
Atrioventricular block	2/49 (4.1)	8/72 (11.1)	0.199
Arrhythmia	1/49 (2.0)	5/72 (6.9)	0.399
Supraventricular tachycardia	2/49 (4.1)	2/72 (2.8)	1
Ventricular tachycardia	0/49 (0)	6/72 (8.3)	0.08

Data are presented as mean±SD if normally distributed or median (quartile 1–quartile 3) if not normally distributed. $P<0.05$ vs nonfulminant myocarditis (NFM) (Student *t* and Mann-Whitney tests were used to calculate the significance). Categorical variables were compared with the chi-square or Fisher exact tests. ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; cTnI, cardiac troponin I; FM, fulminant myocarditis; hs-CRP, high-sensitivity C-reactive protein; LVED, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

values, and Mann-Whitney and Kruskal-Wallis tests were used to evaluate the differences in non-normally distributed continuous values. Paired Student *t* and Wilcoxon signed rank tests were used to calculate the significance between paired samples. Categorical variables were compared with the chi-square test or Fisher exact test. Associations were analyzed using Spearman correlation and adjusted for multiple sites using linear regression analysis for clinical data. Receiver operating characteristic (ROC) curves were constructed and the area under the curve (AUC) was calculated to evaluate the diagnostic performance of sST2. A Delong test was used to calculate the statistical significance among AUCs.[22] The optimal cutoff values for sST2 and cTnI from the retrospective analysis were established based on the highest Youden index as a summation of maximum sensitivity and specificity. Patients with cTnI or NT-proBNP concentrations below the lower limit of detection were assigned a value of half the lower limit of detection. Patients with cTnI concentrations above the upper detection limit were assigned a value of the upper detection limit. The sensitivity, specificity, accuracy, positive and negative predictive values, and positive and negative likelihood ratios were calculated following Choi[23] for positive and negative test results. All diagrams were drawn using Prism 8 software,

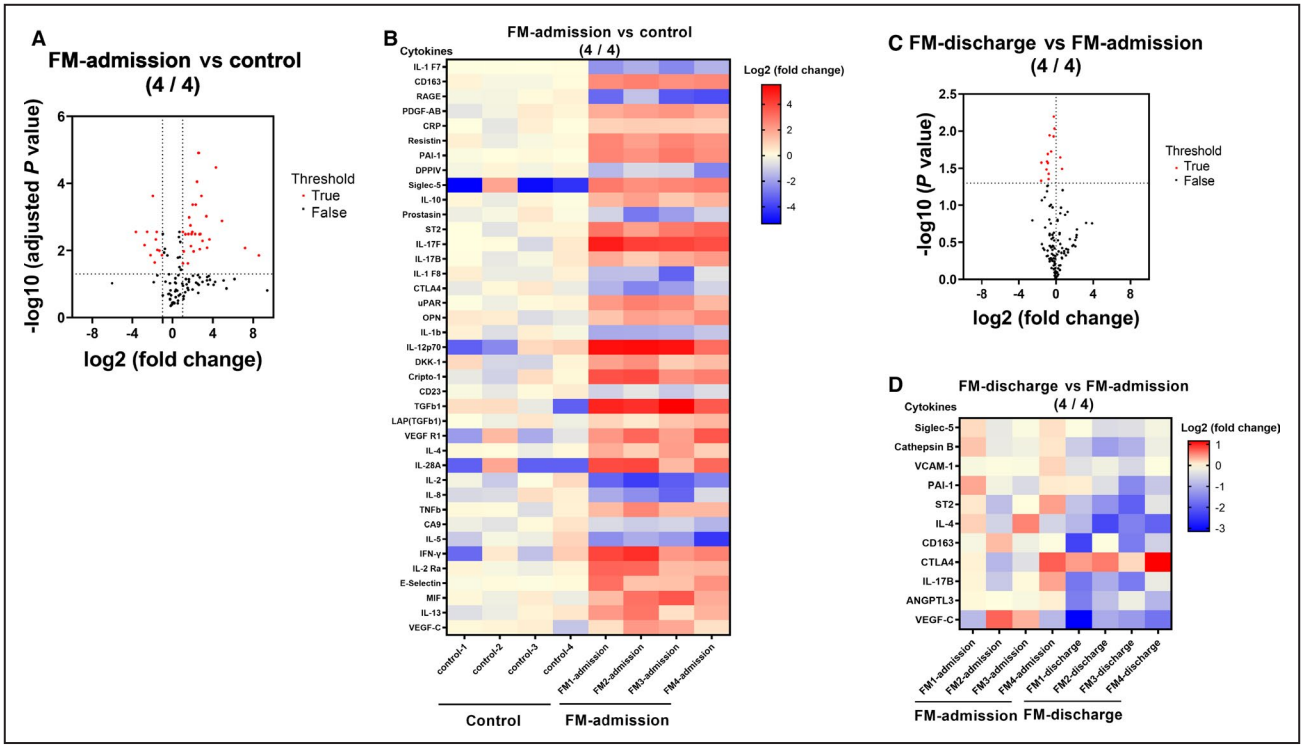

Figure 2. Levels of 122 human cytokines in the patients with fulminant myocarditis (FM) and controls.
A, Volcano plot of the expression of human cytokines in 4 controls and 4 patients with FM at admission. LIMMA test was used to calculate the significance. The red plots represent differentially expressed proteins with an adjusted *P* value <0.05, whereas the black plots represent insignificant changes. The horizontal and vertical dotted lines in volcano plots represented the threshold value for the significance used to define upregulation or downregulation of cytokines was a fold change >2 (or <0.5), as well as with an adjusted *P* value of <0.05. **B**, Expression heatmap of cytokines that significantly changed in 4 patients with FM at admission compared with 4 controls, which correspond to the red plots in Figure 2A. The normalized levels of cytokines log2 (fold change) were indicated by a different color code (right). **C**, Volcano plot of the expression of human cytokines in 4 patients with FM at admission and 4 patients with FM at discharge. The red plots represent differentially expressed proteins with a *P*<0.05, whereas the black plots represent insignificant changes. The horizontal and vertical dotted lines in volcano plots represent the threshold value for the significance used to define upregulation or downregulation of cytokines was a fold change >1, as well as with a *P* value of <0.05. **D**, Expression heatmap of cytokines that significantly changed in 4 patients with FM at discharge vs 4 patients with FM at admission. Those cytokines corresponded to the red plots in Figure 2C. A different color code (right) represented normalized level of cytokines log2 (fold change). CRP indicates C-reactive protein; DKK, Dickkopf protein; DPPIV, dipeptidyl peptidase IV; IFN-γ, interferon γ; IL, interleukin; MIF, macrophage migration inhibitory factor; OPN, osteopontin; PAI-1, plasminogen activator inhibitor 1; PDGF, platelet-derived growth factor; TGFb, transforming growth factor β; TNFb, tumor necrosis factor β; uPAR, urokinase plasminogen activator receptor; and VEFG-C, vascular endothelial growth factor C.

SPSS 22 (IBM), or R software (The R Foundation). The differences with *P*<0.05 were considered significant.

RESULTS

Plasma Profile of Inflammation-Associated Cytokines in Patients With FM

To identify inflammation-associated biomarkers for FM, plasma samples from 4 patients with FM and 4 age-/sex-matched controls were collected for analysis of cytokine profiles using a human cytokine array (Table S1).

Differentially expressed human cytokines between the controls and patients with FM at admission were identified and illustrated as a volcano plot (Figure 2A) and a heatmap (Figure 2B). Among the 122 detected cytokines, 39 showed significant changes (28 increase and 11 decrease) in the patients with FM at admission, indicative of an inflammatory cytokine storm at the onset of FM. Somewhat unexpected, comparing the FM samples on admission versus discharge, only 11 cytokines were found to be significantly altered (Figure 2C and 2D). Among them, 8 cytokines were also significantly altered (7 increase and 1 decrease) at admission compared with those of the controls (Table S6). Among these 8 cytokines, plasma sST2 showed the most robust induction in the FM samples at admission according to fold changes and demonstrated a significant reduction at discharge.

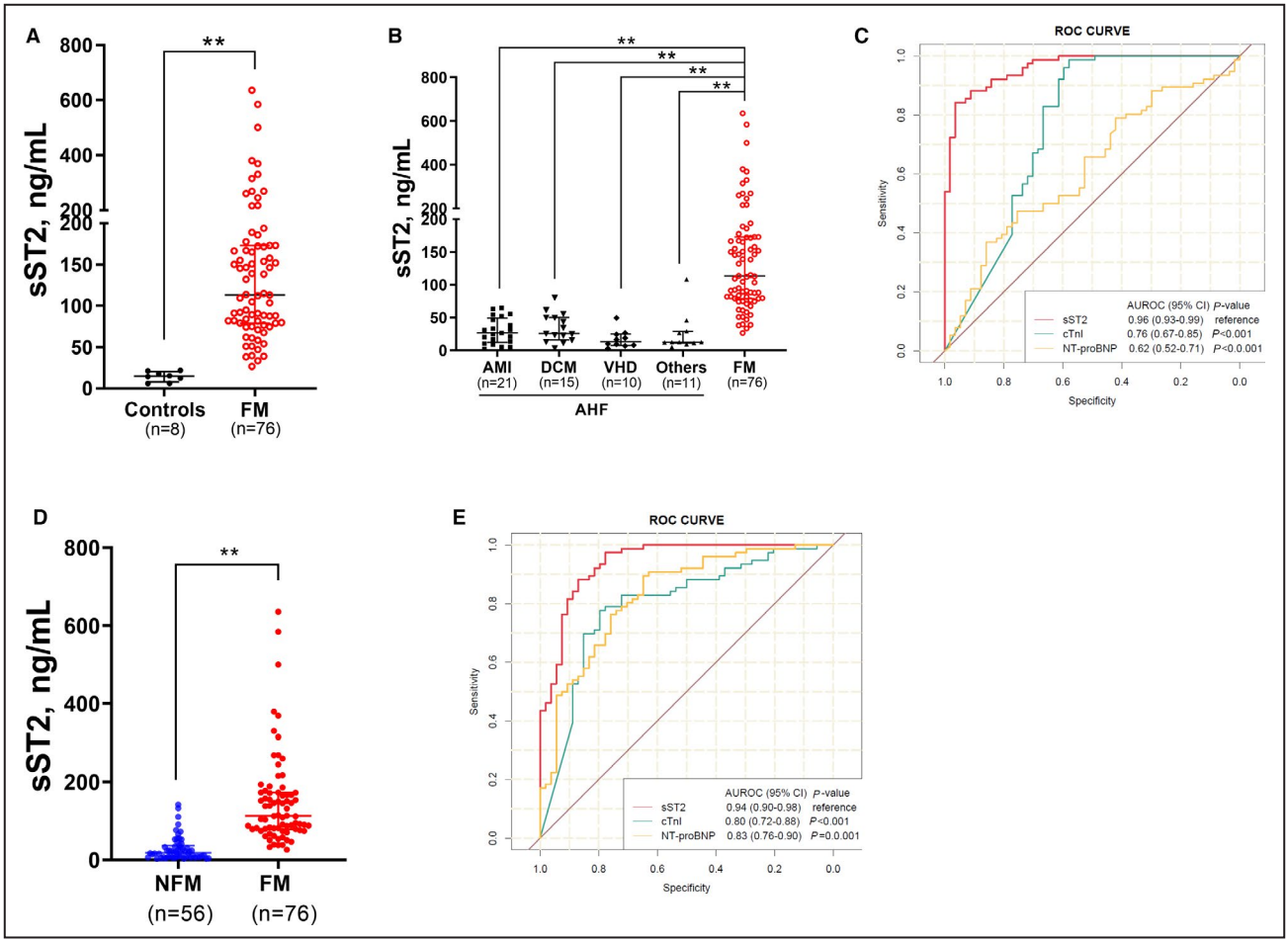

Figure 3. Diagnostic performance of plasma soluble ST2 (sST2) for the detection of fulminant myocarditis (FM).
A, Circulating concentrations of sST2 in 76 patients with 8 control individuals (data are presented as medians and quartile 1 to quartile 3 [Q1–Q3], and Mann-Whitney test was used to elevate the differences, $^{**}P<0.05$). **B**, Circulating concentrations of sST2 in 76 patients with FM and 57 patients with acute heart failure (AHF; data are presented as medians and Q1 to Q3, and Kruskal-Wallis test was used to elevate the differences, $^{**}P<0.05$). **C**, Receiver operating characteristic (ROC) curves of plasma sST2, NT-proBNP (N-terminal pro-B-type natriuretic peptide), and cardiac troponin I (cTnI) in 76 patients with FM and 57 patients with AHF (the Delong test was used to calculate significance). **D**, Circulating concentrations of sST2 in 56 patients with nonfulminant myocarditis (NFM) and 76 patients with FM (data are presented as medians and Q1 to Q3, and Mann-Whitney test was used to elevate the differences, $^{**}P<0.05$). **E**, ROC curves of plasma sST2, NT-proBNP, and cTnI in 56 patients with NFM and 76 patients with FM (the Delong test was used to calculate significance). AMI indicates acute myocardial infarction; AUROC, area under the receiver operating characteristic; DCM, dilated cardiomyopathy; and VHD, valvular heart disease.

Diagnostic Performance of Plasma sST2 Level for the Differential Diagnosis of FM

To validate the data obtained from the array analysis, we performed a retrospective analysis on a cohort of patients with FM with control individuals and patients with NFM by targeted measurement of plasma sST2 levels (Table, Table S2, and Table S7). All of the patients with FM received a life support–based comprehensive treatment regimen, including intra-aortic balloon pump, extracorporeal membrane oxygenation, continuous renal replacement therapy, immunomodulation therapy (intravenous immunoglobulin and corticosteroids), and antivirus treatment (oseltamivir or penciclovir), as soon as FM was diagnosed. Most of the patients with FM (74 of 76) recovered at discharge based on cardiac performance (Table S8).[17] Obviously, the patients with FM showed much higher plasma sST2 levels than the controls (Figure 3A), and further ROC curve analysis showed that sST2 demonstrated a perfect performance (Figure S2). Compared with the patients with hemodynamic unstable acute HF attributable to other causes, such as AMI, dilated cardiomyopathy, and valvular heart diseases, the patients with FM showed much higher plasma sST2 levels at admission (Figure 3B). More relevantly, ROC curve analyses showed that sST2 demonstrated a statistically better performance than cTnI and NT-proBNP in distinguishing FM from hemodynamic unstable acute HF attributable to other causes

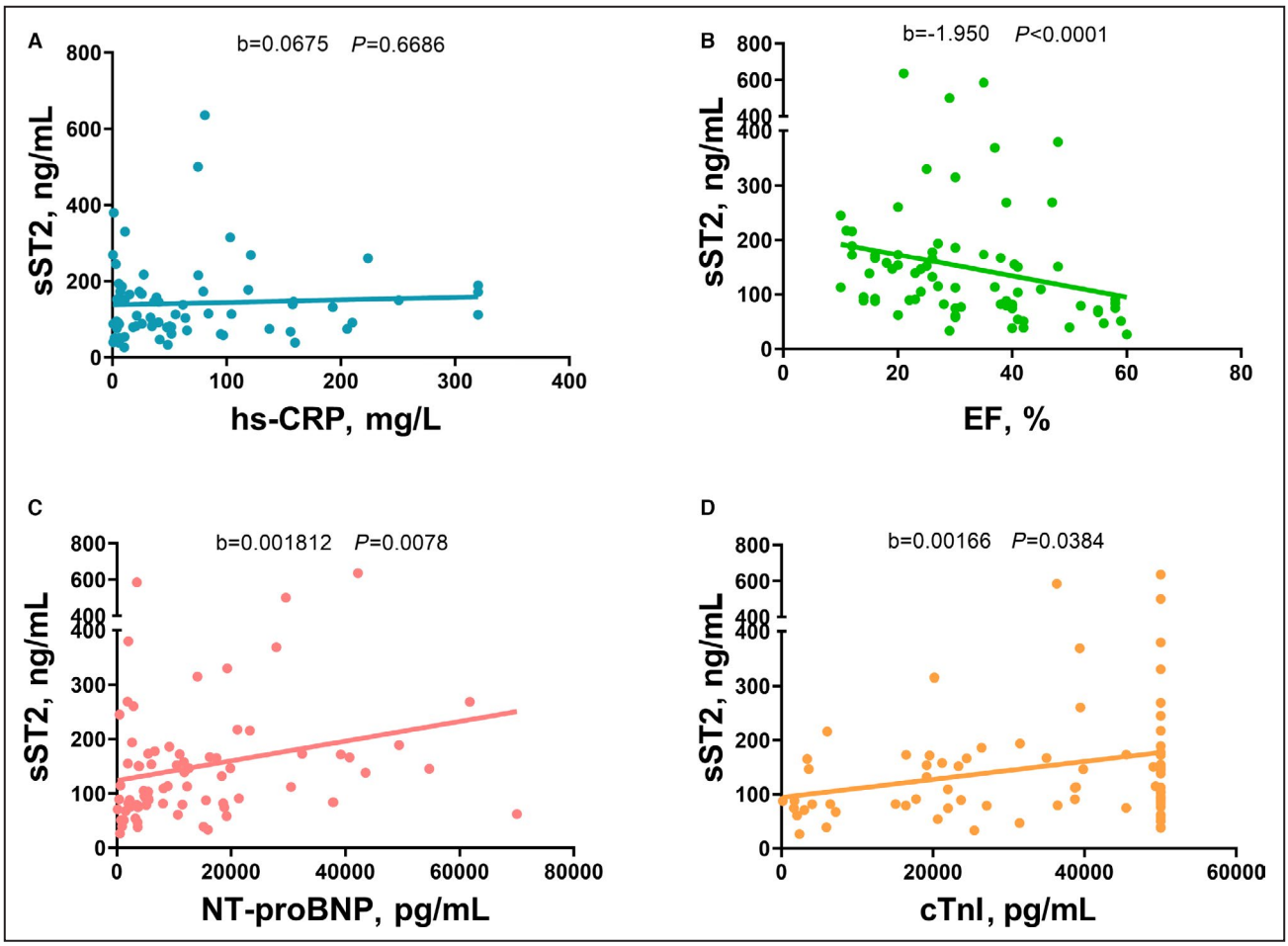

Figure 4. Correlations between plasma soluble ST2 (sST2) level and heart damage.
Correlation analysis of plasma sST2 with high-sensitivity C-reactive protein (hs-CRP) (**A**), ejection fraction (EF) (**B**), NT-proBNP (N-terminal pro-B-type natriuretic peptide) (**C**), and cardiac troponin I (cTnI) (**D**) value in 76 patients with fulminant myocarditis (FM; Spearman correlation and linear regression analysis were used to calculate significance). b indicates regression coefficient.

(Figure 3C). As shown in Figure 3D, compared with the NFM group, the plasma sST2 concentrations in the FM group were significantly elevated. Most important, the ROC curve analyses revealed that sST2 values distinguished FM from NFM with an AUC of >0.90 (AUC_{sST2}=0.94). Compared with cTnI (AUC_{cTnI}=0.80) and NT-proBNP ($AUC_{NT\text{-}proBNP}$=0.83), sST2 yielded the highest AUC for FM detection (Figure 3E). The optimal cutoff value of sST2 to distinguish FM from other conditions was determined to be 58.39 ng/mL, where the highest Youden index was achieved with the maximal summation of sensitivity and specificity (Table S9). At this threshold, an 87.5% specificity and 88.2% sensitivity were accomplished to distinguish FM from NFM in this cohort, leading to an overall accuracy of 87.9% in FM diagnosis from the discovery cohort (Table S9). The positive predictive value was 90.5% and the negative predictive value was 84.5%. These results indicate that sST2 has superior sensitivity and specificity over current standard biomarkers for the diagnosis of FM, and plasma sST2 at admission is a robust inflammation-associated biomarker for differential diagnosis of FM.

Correlations Between Plasma sST2 Level and Heart Damage

There was no significant difference in plasma sST2 concentrations between the sexes in patients with FM (Figure S3A). Plasma sST2 concentrations were slightly increased with age (Figure S3B). There was no significant difference in sST2 level in patients with FM and NFM aged younger or older than 50 years (Figure S3C and S3D). Similarly, plasma level of sST2 did not correlate with the concentrations of high-sensitivity C-reactive protein (CRP) (Figure 4A). In contrast, the plasma concentration of sST2 was positively correlated with plasma NT-proBNP and cTnI levels but negatively correlated with cardiac systolic function, indicated by the ejection fraction values (Figure 4B through 4D). These results

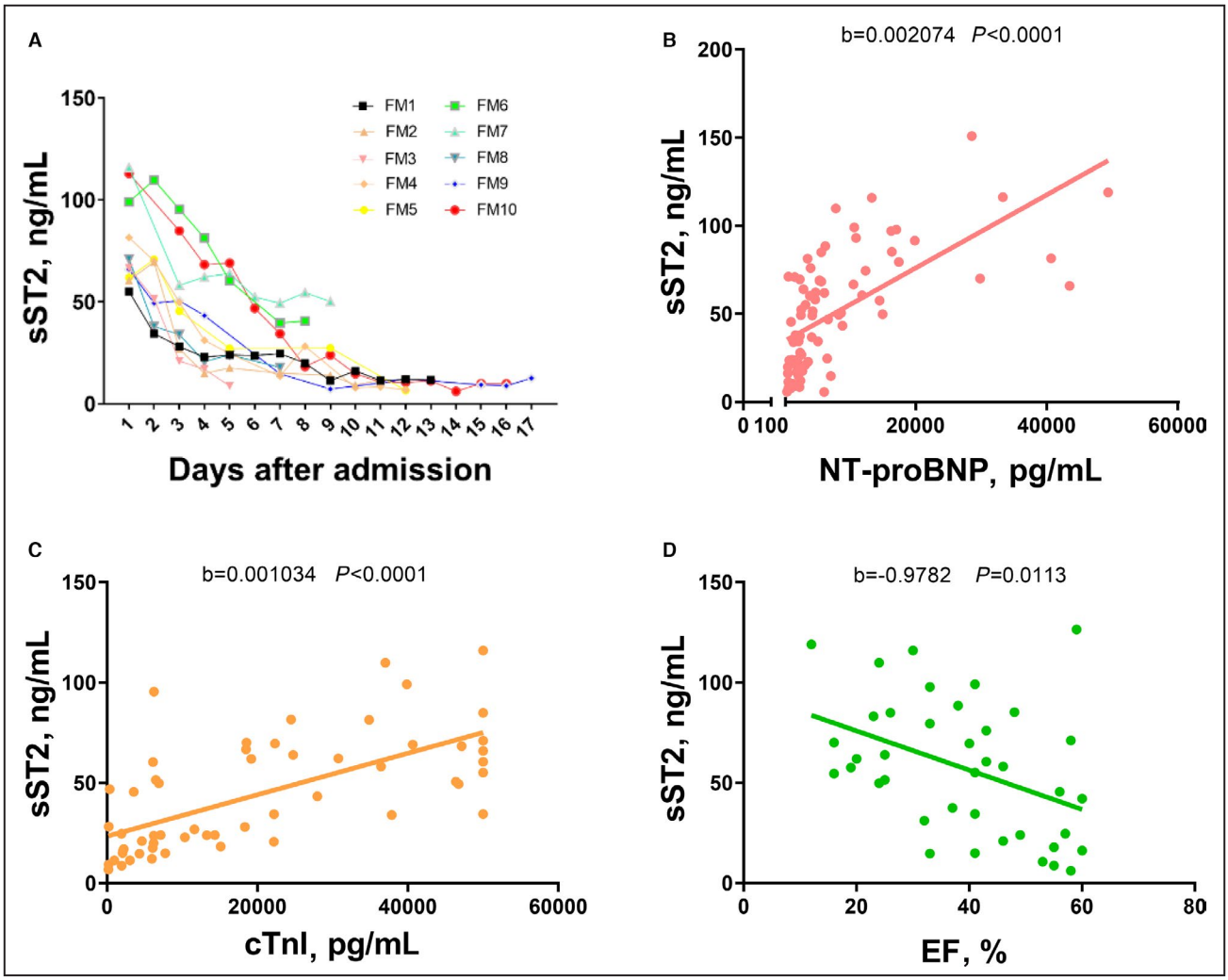

Figure 5. Plasma levels of soluble ST2 (sST2) in patients with fulminant myocarditis (FM) during hospitalization.
A, Circulating concentrations of sST2 in 10 patients with FM during hospitalization. Correlation analysis of plasma sST2 with NT-proBNP (N-terminal pro-B-type natriuretic peptide) (**B**), cardiac troponin I (cTnI) (**C**), and ejection fraction (EF) (**D**) values during hospitalization (Spearman correlation and linear regression analysis were used to calculate significance). b indicates regression coefficient.

suggest that sST2 might be induced not only as part of the systemic inflammatory response but also in association with specific cardiac stress.

Expression Pattern of Plasma sST2 in Patients With FM During Hospitalization and Follow-Up

The time-dependent expression pattern of plasma sST2 in the acute phase of FM was characterized in 10 hospitalized patients with FM from admission to discharge (Table S3). All of these patients received a life support–based comprehensive treatment regimen as soon as FM was diagnosed, and all recovered at discharge.[17] The average hospitalization time was 13 days. As shown in Figure 5A, circulating sST2 levels were gradually decreased in these patients with FM during the course of recovery. Consistent with the results at admission, the plasma concentration of sST2 was positively correlated with plasma NT-proBNP and cTnI levels, but negatively correlated with cardiac systolic function throughout the period of hospitalization (Figure 5B through 5D). These data suggest that plasma sST2 is a dynamic indicator for the severity of FM during the acute phase of the disease.

Considering that FM might cause adverse sequelae after the acute phase, we followed up with 16 patients with FM for 2 years (Table S4). Although plasma sST2 concentrations were highly elevated at admission for all of these patients, they became rapidly normalized following discharge (Figure S4A). Furthermore, the plasma concentrations of sST2 were no longer associated with cardiac systolic function in the recovered patient with FM during the follow-up period

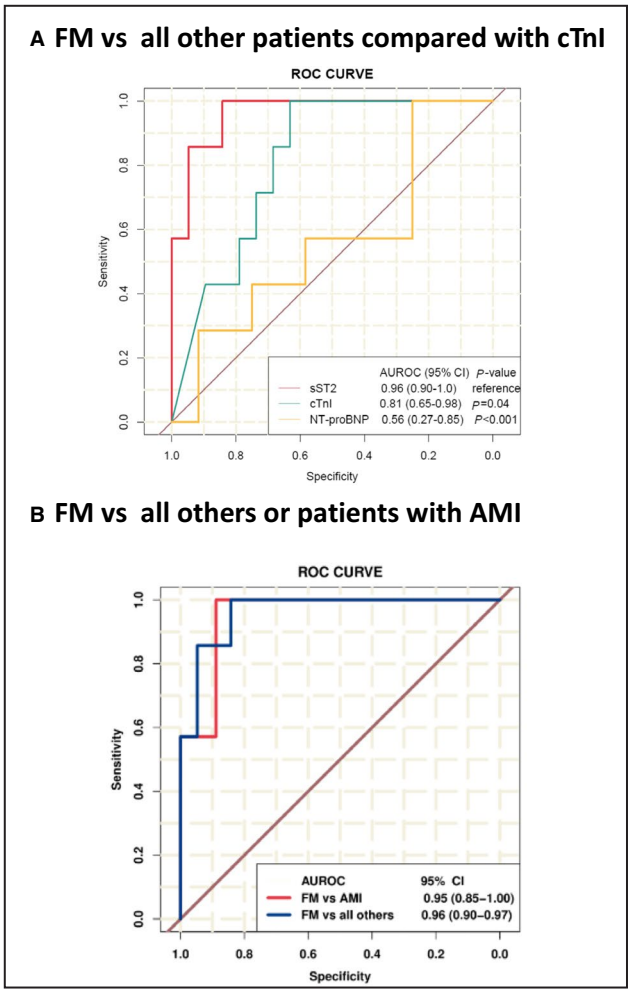

Figure 6. Receiver operating characteristic (ROC) curves of circulating soluble ST2 (sST2) in the validation cohort.
A, Patients with fulminant myocarditis (FM) vs all other patients compared with NT-proBNP (N-terminal pro-B-type natriuretic peptide), cardiac troponin I (cTnI), and sST2. **B**, Patients with FM vs all other patients or those with acute myocardial infarction (AMI) using sST2. AUROC indicates area under the receiver operating characteristic.

(Figure S4B) nor with ventricular chamber diameters (Figure S4C).

These data indicate that plasma sST2 was mainly a marker for the acute phase rather than the chronic post-recovery phase of FM.

Differential Diagnosis of Patients With FM Using Plasma Levels of sST2

To validate the diagnostic performance for FM using plasma sST2, we conducted a prospective study where the plasma sST2 was measured at admission from a total of 26 patients with acute onset of unstable hemodynamics but undiagnosed causes. Following the final diagnosis, we found that, when sST2 was used, the AUC from the ROC curve to distinguish the 7 patients with FM from all other controls was 0.96 (0.9–1.0) (Figure 6A). In contrast, when cTnI was used, the AUC for FM versus all other patients was 0.81 (0.65–0.97), and, when NT-proBNP was used, the AUC was 0.56 (0.27–0.85). Furthermore, the AUC for sST2 to distinguish patients with FM (n=7) from patients with AMI (n=9) was 0.95 (0.85–1.0) (Figure 6B). Moreover, at the cutoff level of 58.39 ng/mL set from the retrospective cohort (Figure 6 and Table S10), the diagnosis of FM was achieved with 85.7% sensitivity and 94.7% specificity, leading to an overall accuracy of 92.3% in FM diagnosis from the prospective cohort. In contrast, a 1194.75 pg/mL cutoff value of cTnI was able to achieve 100% sensitivity but only 63.2% specificity, resulting in a 73.1% accuracy in FM diagnosis. Positive and negative likelihood ratios for sST2 were 16.2 and 0.15, respectively. Positive likelihood ratio was >10 and positive predictive value was 85.7%, indicating that sST2 at 58.39 ng/mL was a robust diagnostic tool for FM.

Thus, the diagnostic performance of sST2 at 58.39 ng/mL was significantly superior to cTnI. In addition, to explore whether the level of sST2 had potential value for follow-up prognosis assessment of patients with FM, we found that the patients with high levels of sST2 at admission showed a trend toward a higher risk of cardiovascular rehospitalization (Table S11). Moreover, there were statistical differences in left ventricular ejection fraction value and NT-proBNP levels between the 2 subgroups (Table S12), which was consistent with the previous data that the level of sST2 correlated with the 2 indicators. The results suggested that the higher hazard ratios in the $sST2_{high}$ group might be attributable to the lower left ventricular ejection fraction and higher B-type natriuretic peptide levels at admission. The data imply that the sST2 level was of potential value for the prognosis assessment in patients with FM.

DISCUSSION

Accurate and early diagnosis of FM can effectively reduce its associated morbidity and mortality. However, the current cardiac injury–based biomarkers for FM have low specificity for accurate diagnosis. In this report, we focused on cytokines involved in inflammatory response, a well-established pathophysiological mechanism underlying myocarditis.[4]

In general, myocarditis is recognized as an inflammatory disease of cardiomyocytes.[24] The autoimmune/inflammatory response rather than the ongoing trigger, such as viral infection, is the key pathophysiology of myocarditis.[25] However, the landscape of systemic inflammatory response in patients with FM is largely unknown. To our knowledge, the current

study is the first to provide comprehensive profiles of circulating inflammation-associated cytokines in adult patients with FM. Our finding revealed that the expression of nearly one third of the detected cytokines (39 of 122 [32.0%]) were significantly changed in patients with FM, compared with that in controls. This overwhelming scale of cytokine alterations from different inflammatory players at the onset of the disease is consistent with the status of so-called inflammatory storm caused by myocarditis-related pathogens at the onset of FM. Unexpectedly, among these cytokines provoked by the disease, only a small subset of 8 cytokines were also normalized or further changed when FM was resolved. Among them, the plasma sST2 concentration showed the highest increase in fold at the onset of FM.

Interestingly, sST2 concentration was not associated with plasma CRP level, a commonly used biomarker of systemic inflammation, supporting a specific association of its release with FM. In fact, mechanical stress and inflammation were 2 main contributors to the increased sST2 level.[26] ST2 expression could be induced by biomechanical strain in cardiomyocytes.[27] Recently, alveolar epithelium was found to be an important noncardiac origin of elevated sST2.[28] It is well-known that CRP is an acute-phase protein, which is synthesized by liver cells and released into the blood when the body experiences an acute bacterial infection, malignant tumor, ischemia, or tissue injury. Apart from inflammation, a slightly elevated CRP level might also reflect distressed or injured cells homeostasis maintenance in daily life.[29] Until now, the correlation between CRP and mechanical stress was unclear. The different origins and inducers might account for the weak correlation between sST2 and CRP in the current study. In addition, unlike other biomarkers for systemic inflammation, such as IL-2, IL-10, and IL-17, the plasma sST2 level rapidly decreased along with the clinical improvement in patients with FM. Therefore, sST2 is a unique and dynamic player in the inflammatory storm associated with FM. Recently, Blanco-Domínguez et al[30] identified mmu-miR-721, and its human homologue hsa-miR-Chr8:96, which was synthesized by type 17 helper T cells, could be used to distinguish patients with myocarditis from those with myocardial infarction. We also identified that circulating miR-4763-3p was a novel potential biomarker candidate for human adult FM.[31] These data urgently suggest that the underlying inflammatory-related mechanisms during myocarditis need further investigation.

From an extensive clinical cohort of patients with FM and NFM, we found that the level of circulating sST2 in patients with FM was much higher than that in patients with NFM. This is consistent with earlier histological findings that adult patients with FM generally have a higher degree of inflammatory infiltration in the heart section than patients with NFM.[32] Previously, it was found that sST2 levels were elevated in patients with myocarditis and New York Heart Association class III to IV HF, predominantly in men <50 years.[33] However, our results showed that the circulating sST2 levels were not correlated with patients' sex and age. Compared with the study conducted by Coronado et al,[33] the patients with myocarditis they included were those with symptoms lasting <6 months, among which 65% were patients with New York Heart Association class I or II HF, similar to our patients with NFM, while we enrolled patients with acute myocarditis, whose symptoms lasted <2 weeks. All of the patients with FM in our study were administered inotropic support or mechanical circulatory support for acute hemodynamical instability (with hypotension and cardiogenic shock, similar to New York Heart Association class III or IV). Variations in the baseline characteristics of the cohorts may contribute to the different observations. In addition, another study found no significant difference in circulating sST2 levels among 5301 patients with chronic HF of different ages <80 years.[34] Since the majority of patients and average age in the current study was <50 years, more cohorts of patients with myocarditis should be conducted to reveal the impact of age and sex on circulating sST2 levels in the future.

Most relevant to the potential utility of sST2 as a biomarker for FM diagnosis, plasma sST2 at admission showed remarkable sensitivity (88.2%) and specificity (87.5%) for the differential diagnosis of FM from NFM. It significantly outperformed 2 current biomarkers for cardiac injury, cTnI, and NT-proBNP. This is consistent with previous studies showing that cTnI was induced in patients with FM and those with AMI-induced acute HF, compared with patients with other causes induced by hemodynamically unstable acute HF.[35,36] Therefore, the plasma sST2 induction observed in patients with FM may not only be caused by a generic systemic inflammatory response but also by local cardiac stress. From longitudinal examination, we further demonstrated that the induction of plasma sST2 was observed at the onset of FM, then dynamically normalized along with the resolution of the disease during hospitalization and remained stable during post-FM period.

ST2 is located at a conserved locus on human chromosome 2 and mouse chromosome 1.[37] The sST2 protein is generated from a truncated messenger RNA transcript lacking the 3' three exons, thus the transmembrane and cytoplasmic domains.[38] Generally, sST2 is believed to act as a decoy receptor to sequester free IL-33, thus preventing ST2/IL-33 signaling.[39] ST2/IL-33 possesses beneficial effects against hypertension and HF via specific targets.[40] sST2 is upregulated after mechanical or IL-1β stimulation in cardiomyocytes.[27] Previous studies show that

circulating sST2 level was positively correlated with IL-1β in acutely decompensated HF.[41] An earlier study found that increased ST2 was induced by IL-1β in cardiomyocytes.[27] Consistently, IL-1β administration in mice with viral myocarditis increased the plasma sST2 level.[33] In particular, inflammation and adverse cardiac remodeling were alleviated in chronic coxsackievirus B3–induced mice with myocarditis treated with an IL-1β inhibitor, canakinumab.[42] In addition, a case report revealed that an IL-1 blocker successfully treated a 17-year-old patient with myocarditis caused by adult-onset Still disease.[43] Moreover, in a human endotoxin model, increased plasma sST2 was observed in healthy donors injected with lipopolysaccharide (2 ng/kg) within 24 hours.[44] Together, elevated sST2 might indicate some immunomodulating pharmacological treatments, eg, IL-1 modulators. Meanwhile, early in 1997, it was suggested that continuous renal replacement therapy lowered the plasma levels of some mediators, especially cytokines and complement by a combination of membrane convection and adsorption.[45] Recently, Zhai et al[46] found that oXiris-endotoxin adsorption technology-based continuous renal replacement therapy effectively reduced the level of inflammation such as IL-6 and IL-10 in patients with sepsis. This suggested that continuous renal replacement therapy could also be used to modulate the inflammatory pattern.

sST2 has been found to be associated with HF attributed to different causes other than myocarditis. Studies found that combination of sST2 and NT-proBNP offered improvement in assessing the risk of death or transplantation in 1141 outpatients with chronic HF, and implied that sST2 has an independent prognostic value in patients with chronic HF,[47] patients with ischemic HF,[48] and in the elderly population with HF.[49] A meta-analysis including 7 follow-up studies found that the risk of all-cause death in patients with chronic HF with reduced ejection fraction was positively correlated with logST2 level (hazard ratio, 1.75; 95% CI, 1.37–2.22).[50] Similarly, sST2 levels were significantly increased in symptomatic patients with HF with preserved ejection fraction compared with asymptomatic patients (30.2±14.1 versus 42.8±29.0 ng/mL, P=0.04).[51] Recently, circulating sST2 was recognized as a valuable biomarker for prognostication and monitoring patients with acute HF.[52] Importantly, sST2 was listed as a category IIb recommendation for the diagnosis of HF in the 2017 American College of Cardiology/AHA focused update of guidelines for the management of HF.[53]

In addition, sST2 levels were also significantly increased in patients with acute aortic dissection characterized by smooth muscle cell extension and extensive vascular injury, and the results of a prospective validation cohort suggested that it might be a potential novel biomarker and contributed to the early diagnosis of acute aortic dissection.[54] As reported by Pascual-Figal et al,[55] ST2 concentrations significantly increased after heart transplantations when acute rejection occurred (odds ratio, 4.9; 95% CI, 1.7–14.5 [P=0.004]). Moreover, a study that enrolled 41 children who underwent heart transplantation described that sST2 levels significantly increased during incidences of heart transplantation rejection.[56] In addition, a recent study showed that ST2 deficiency markedly alleviated the thickened artery intima, complicated vascular stenosis and infiltration of inflammatory cells in allograft after heart transplantation in mice.[57] These findings indicate that serum sST2 levels could predict rejection in heart transplantation.

To our knowledge, this study provided the first evidence indicating sST2 as a robust biomarker for FM with high specificity and sensitivity. While the initial detection of sST2 was first discovered from cytokine profiling in a small cohort of patients with FM, its diagnostic performance was demonstrated in both retrospective and prospective analysis from 2 independent cohorts of FM, NFM, and FM-unrelated patients with acute HF. However, there were some limitations in this study. Although this investigation included a relatively large number of patients with FM, it was a single-center study. Considering the short follow-up time, cardiovascular events may not have occurred yet since hazard ratios for primary and secondary outcomes did not reach statistical significance. In addition, compared with another study,[9] there was no active cancers or serious autoimmune diseases in our study cohort. Therefore, we need to continue follow-up studies in the FM cohort. Moreover, we might have underestimated other cytokines that were not included in the arrays. Endomyocardial biopsy–based detections may provide more information about the specific histological- or viral-associated cytokines.

Taken together, sST2 showed superior diagnostic performance to that of cTnI or NT-proBNP in patients with FM. sST2 may be a promising inflammation-associated biomarker for FM in the acute phase as a more reliable indicator for the dynamic onset and resolution of the inflammatory storm triggered by FM.

ARTICLE INFORMATION

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Author contributions: J.W. and C.C. designed the study, conducted the experiments, analyzed and interpreted the data, and drafted the article. M.H., H.L., Y.C., X.N., Y.C., R.X., L.L., P.C., Y.S., C.L., T.Y., H.Z., G.C., K.M., C.Z., and J.J. contributed to data acquisition. B.H., O.B., and A.M. revised the article. D.W.W. designed the study and drafted the article.

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Disclosures

None.

Supplemental Material

Tables S1–S12
Figures S1–S4

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SUPPLEMENTAL MATERIAL

Table S1. Baseline clinical characteristics of controls and patients in the screening cohort (Figure 2).

	control	FM	
		at admission	at discharge
Age (years)	35.0±3.4	33.8±18.4	
Male/Female (n/n)	2/2	2/2	
LVED (cm)	4.2±0.6	4.6±0.6	4.6±0.4
LVEF (%)	68.8±5.6	33.5±10.7*#	53.3±4.9
NT-proBNP (pg/mL)	<70.0	9661.0 (3897.8-26190.5)*#	1473.0 (322.8-2970.5)
cTnI (pg/mL)	<1.9	44364.7 (24052.5-50000.0)*#	265.4 (80.3-6967.5)

LVED, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; cTnI, cardiac troponin I. Data are presented as mean ± standard deviation (SD) if normally distributed, or medians and first to third quartile (Q1-Q3) if not normally distributed, *$p<0.05$ vs control (Mann-Whitney test were used to calculate the significance), #$p<0.05$ vs FM at discharge (Wilcoxon signed-rank test were used to calculate the significance).

Table S2. Baseline clinical characteristics of controls and patients with fulminant myocarditis in the validation cohort (Figure 3A).

	control	FM
Age (years)	38.0 (36.0-39.0)	33.0 (23.3-49.0)
Male/Female (n/n)	4/4	37/39
LVED (cm)	4.6±0.5	4.7±0.6
LVEF (%)	67.5 （61.5-70.7）	30.0 (20.0-41.0)*
NT-proBNP (pg/mL)	<70.0	8528.0 (3484.8-19116.0)*
cTnI (pg/mL)	<1.9	38817.8 (19256.3-50000.0)*

LVED, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; cTnI, cardiac troponin I. Data are presented as mean ± standard deviation (SD) if normally distributed, or medians and first to third quartile (Q1-Q3) if not normally distributed, *p<0.05 vs. control (Student t-test and Mann-Whitney test were used to calculate the significance).

Table S3. Baseline clinical characteristics of FM patients in the in-hospital cohort (Figure 5A-D).

	FM at admission	FM at discharge
Age (years)	28.2 ± 16.1	
Male/Female (n/n)	5/5	
LVED (cm)	4.6±0.3	4.6±0.3
LVEF (%)	28.0 (19.5-41.0)	55.5 (45.5-57.3)*
NT-proBNP (pg/mL)	11148.0 (5502.3-20133.0)	1237.5 (772.8-2523.8)*
cTnI (pg/mL)	50000.0 (23897.8-50000.0)	308.0 (165.6-2793.4)*

LVED, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; cTnI, cardiac troponin I. Data are presented as mean ± standard deviation (SD) if normally distributed, or medians and first to third quartile (Q1-Q3) if not normally distributed, *$p<0.05$ vs. FM at admission (paired Student t-test and Wilcoxon signed-rank test were used to calculate the significance).

Table S4. Clinical characteristics of FM patients in the follow-up cohort (Figure S3).

Patients	Sex	Age (years)	Admission				6-month				1-year				2-year			
			LVED (cm)	LVEF (%)	NT-proBNP (pg/mL)	cTnI (pg/mL)	LVED (cm)	LVEF (%)	NT-proBNP (pg/mL)	cTnI (pg/mL)	LVED (cm)	LVEF (%)	NT-proBNP (pg/mL)	cTnI (pg/mL)	LVED (cm)	LVEF (%)	NT-proBNP (pg/mL)	cTnI (pg/mL)
FM-1	F	29	5.3	38	20842	43351	4.7	67	<5	<1.9	5	59	<5	<1.9			<5	<1.9
FM-2	F	27	4.6	16	40665	24446	4.4	57	<5	<1.9	4.2	67	<5	<1.9			<5	<1.9
FM-3	F	20	4	20	6033	19160	4	69	<5	<1.9	4.6	68	<5	<1.9	4.5	67	<5	<1.9
FM-4	M	19	4.3	41	10617	39843	5.3	51	9	<1.9	5.4	53	<5	<1.9	5.5	48	<5	<1.9
FM-5	M	39	4.5	60	3788	49030	4.8	69	<5	<1.9	4.9	68	20	<1.9	4.5	59	<5	<1.9
FM-6	F	49	4.4	25	10490	18449	4.4	58	<5	<1.9	4.8	59	<5	<1.9			<5	<1.9
FM-7	M	60	4.5	60	516	28122	4.4	67	<5	<1.9	4.4	66	<5	<1.9	4.7	69	<5	<1.9
FM-8	M	15	4.6	26	28282	50000	4.4	61	<5	<1.9	4.4	67	58.6	189	4.6	65	<5	<1.9
FM-9	M	15	4.6	26	28282	50000	4.7	60	9	3.3	4.8	63	58.6	189	4.9	56	<5	<1.9
FM-10	F	26	4.2	47	1850	50000	5.4	46			5.1	32						
FM-11	F	33	4.4	14	5546	50000	4.6	60			4.1	65						
FM-12	M	42	5.5	30	10676	2041	5.1	56			5.1	56						
FM-13	M	32	4.9	20	2910	39457					4.4	57						
FM-14	F	27	4.5	30	9166	26415					4.7	58						
FM-15	F	44	4.6	25	19329	50000	4.7	62			4.9	70						
FM-16	M	37	4.9	12	23301	6032					4.3	58						

LVED, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; cTnI, cardiac troponin I.

Table S5. Clinical characteristics of patients in the validation cohort (Figure 6).

Diagnose	FM	Non-FM control	*P* value
No. of cases	7	19	
Age (years)	37.9 ± 15.2	57.9 ± 14.2	0.004*
Male/Female (n/n)	3/4	12/7	0.461
LVED (cm)	4.8 ± 0.7	5.6 ± 1.1	0.1107
LVEF (%)	36.3 ± 10.5	39.9 ± 14.6	0.5505
NT-proBNP (pg/mL)	5313.0 (2057.0-15353.0)	4944.0 (1608.3-6642.8)	0.7108
cTnI (pg/mL)	21153.4 (2457.4-50000)	208.4 (32.1-6373.0)	0.0147*
sST2 (ng/mL)	109.5 (69.4-200)	25.0 (12.6-44.0)	<0.0001*

LVED, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; cTnI, cardiac troponin I.

Table S6. Plasma concentrations of 8 significantly altered cytokines in patients with FM compared with the controls at admission and discharge (Figure 2D).

Cytokines (pg/mL)	Control (n=4)	FM-admission (n=4)	folds	FM-discharge (n=4)
sST2	12670.0±3028.5	91990.4±29699.7*#	7.3	46665.7±21492.7
PAI-1	2040.7±138.2	14503.2±3696.4*#	7.1	3696.4±4186.1
Siglec-5	4168.2±8046.3	27126.4±3717.4*#	6.5	3717.4±3141.6
CD163	11650.5±1711.8	70218.2±11274.3*#	6.0	11274.3±25084.2
IL-17B	592.1±165.8	2023±588.3*#	3.4	588.3±498.7
IL-4	8.6±1.4	25.4±9.8*#	3.0	9.78±3.6
VEGF-C	29.3±11.3	83.7±45.4*#	2.9	45.4±13.5
CTLA4	1772.8±572.1	559.4±270.6*#	0.3	270.6±268.7

Data are presented as mean ± standard deviation (SD), *p-adjusted<0.05 vs. control (LIMMA test was used to calculate the significance), #p<0.05 vs FM at discharge (paired Student's t test was used to calculate the significance).

Table S7. Baseline clinical characteristics of controls and patients with acute heart failure in the retrospective discovery cohort (Figure 3A).

	control	AHF				FM
		AMI	DCM	VHD	others	
Case number	8	21	15	10	11	76
Age (years)	38.0 (36.0-39.0)	59.0 (50.0-66.0)*#	66.0 (59.0-69.0)*#	58.5 (53.0-67.8)*#	60.0 (49.0-67.0)#	33.0 (23.3-49.0)
Male/Female (n/n)	4/4	13/8	12/3#	5/5	5/6	37/39
LVED (cm)	4.6±0.5	4.9±0.6	6.3±0.8*#	6.1±0.8*#	5.4±0.9*#	4.7±0.6
LVEF (%)	67.5 (61.5-70.7)	40.0 (36.0-48.0)*#	28.0 (25.0-35.0)*	38.0 (32.3-47.5)*	36.0 (28.0-51.0)*	30.0 (20.0-41.0)*
NT-proBNP (pg/mL)	<70.0	1780.0 (1025.5-6918.0)*#	8188.0 (4382.0-10299.0)*	3844.0 (1334.0-17056.5)*	7995.0 (1582.0-20193.0)*	8528.0 (3484.8-19116.0)*
cTnI (pg/mL)	<1.9	50000.0 (25710.1-50000.0)*	36.1 (13.6-88.0)#	56.4 (23.2-253.3)#	26.4 (11.9-134.5)#	38817.8 (19256.3-50000.0)*

AHF, acute heart failure; AMI, acute myocardial infraction; DCM, dilated cardiomyopathy; VHD, valvular heart disease; other, other etiologies including arrhythmias and congenital heart disease; LVED, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; cTnI, cardiac troponin I. Data are presented as mean ± standard deviation (SD) if normally distributed, or medians and first to third quartile (Q1-Q3) if not normally distributed, *$p<0.05$ vs. control (One-way ANOVA and Kruskal-Wallis test were used to calculate the significance), #$p<0.05$ vs FM (One-way ANOVA and Kruskal-Wallis test were used to calculate the significance).

Table S8. The treatment strategy for each FM patient (Figure 3).

Patients	Life support therapy			Immunomodulation therapy			Anti-virus		Survival
	IABP	ECMO	CRRT	IVIG (10g qd)	Dexamethasone (10mg qd)	Methylprednisolone (200mg qd)	Tamiflu (75mg bid)	Penciclovir (500mg qd)	
1	+	-	+	+	-	+	+	+	Yes
2	+	-	+	+	+	+	+	-	Yes
3	+	-	+	+	-	+	+	+	Yes
4	+	-	-	+	-	+	+	-	Yes
5	-	+	+	+	-	+	+	+	Yes
6	+	-	+	+	+	+	+	+	Yes
7	+	-	+	+	-	+	+	-	Yes
8	+	-	+	+	+	+	+	+	Yes
9	+	-	+	+	-	+	-	+	Yes
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43	+	-	-	+	-	+	+	+	Yes

44	+	-	-	+	+	+	+	-	Yes
45	+	-	-	+	+	+	+	+	Yes
46	+	-	-	+	-	+	+	+	Yes
47	+	-	+	+	-	+	+	+	Yes
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49	+	-	-	+	-	+	+	+	Yes
50	+	-	-	+	+	+	+	+	Yes
51	+	-	-	+	-	+	+	-	Yes
52	+	-	+	+	-	+	+	+	Yes
53	+	-	+	+	+	+	+	+	Yes
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62	+	-	+	+	-	+	+	+	Yes
63	-	-	+	+	+	+	-	+	Yes
64	+	-	+	+	-	+	+	-	Yes
65	+	-	+	+	-	+	-	-	Yes
66	+	-	+	+	-	+	-	-	Yes
67	+	-	+	+	-	+	-	-	Yes

68	-	-	+	+	+	+	+	-	Yes
69	+	-	-	+	-	+	+	+	Yes
70	+	-	+	+	+	+	-	-	No
71	+	+	+	+	+	+	+	+	Yes
72	+	-	-	+	-	+	+	+	Yes
73	+	-	-	+	-	+	+	+	Yes
74	+	-	-	+	-	+	+	+	Yes
75	-	-	+	+	-	+	+	-	Yes
76	-	-	-	+	-	+	+	+	Yes

IABP, intra-aortic balloon pump; ECMO, extracorporeal membrane oxygenation; CRRT, continuous renal replacement therapy; IVIG, intravenous immunoglobulin.

Table S9. Diagnostic performance of sST2 to distinguish FM.

Comparation	AUC_{sST2}	95% CI	cut-off value (ng/mL)	specificity (%)	sensitivity (%)	Accuracy (%)	PLR	NLR	PPV (%)	NPV (%)
FM vs NFM	0.94	0.90-0.98	58.39	87.5	88.2	87.9	7.056	0.135	90.5	84.5
FM vs AHF+NFM+control	0.96	0.93-0.98	58.39	90.1	88.2	89.3	8.909	0.131	84.8	92.4

Table S10. sST2 compared with cTnI were used to evaluate the diagnostic performance of FM patients with other patients in the validation cohort.

	Threshold	Sensitivity (%)	Specificity (%)	Accuracy (%)	PLR	NLR	PPV (%)	NPV (%)
Patients (n = 26, with FM n = 7)								
sST2, ng/mL	58.388*	85.7	94.7	92.3	16.2	0.15	85.7	94.7
cTnI, pg/mL	1194.75*	100	63.2	73.1	2.72	0	50	100

PLR positive likelihood ratio; NLR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value; sST2, soluble ST2.

*Optimal threshold value obtained from the data (Table S9), which was the threshold leading to the maximum summation of sensitivity and specificity by the Youden index.

Table S11. Hazard Ratios for primary and secondary outcomes in $sST2_{low}$ and $sST2_{high}$ FM patients.

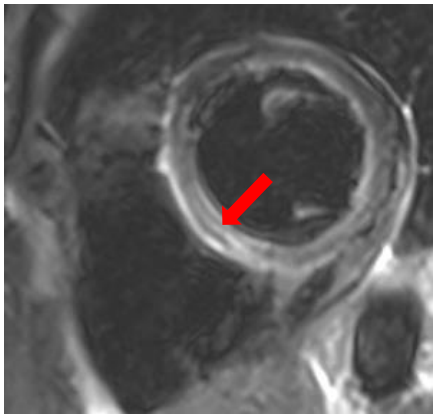
Outcome	$sST2_{low}$ patients (N=37)	$sST2_{high}$ patients (N=37)	HR (95%CI)	*P* value
Primary outcome				
Death from cardiovascular causes	2	5	2.476 (0.480-12,762)	0.254
Secondary outcome				
First rehospitalization for cardiovascular causes	1	2	1.913 (0.173-21.165)	0.597

Table S12. Clinical characteristics of FM patients in the follow-up cohort.

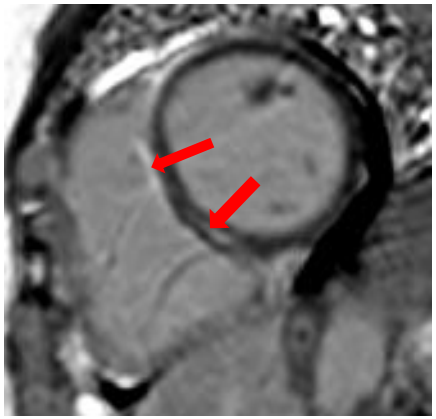
	$sST2_{low}$ group (N=37)	**$sST2_{high}$ group (N=37)**	*P* value
Age (years)	34 (24.5-47.5)	33 (24.5-50.5)	0.888
Male/Female (n/n)	21/16	16/21	0.353
LVED (cm)	4.7 (4.35-5.2)	4.7 (4.35-5.05)	0.803
LVEF (%)	40 (28.5-51)	26 (18.5-36)	<0.0001
NT-proBNP (pg/mL)	4985.0 (2270.0-15390.0)	12289.0 (4696.5.0-25617.0)	0.008
cTnI (pg/mL)	27077.5 (6791.4-50000.0)	39843.5 (22268.3-50000)	0.105

Figure S1. The representative images of cardiovascular magnetic resonance from FM patients.

A

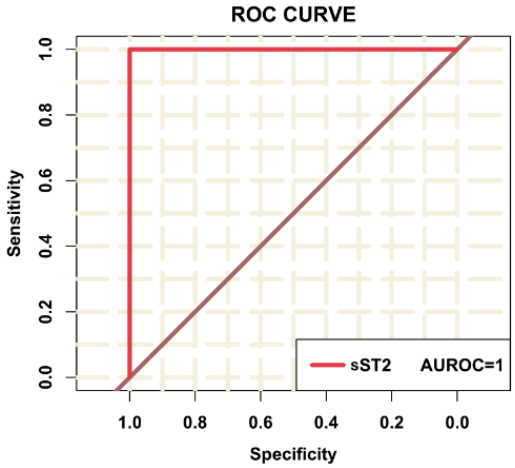


B



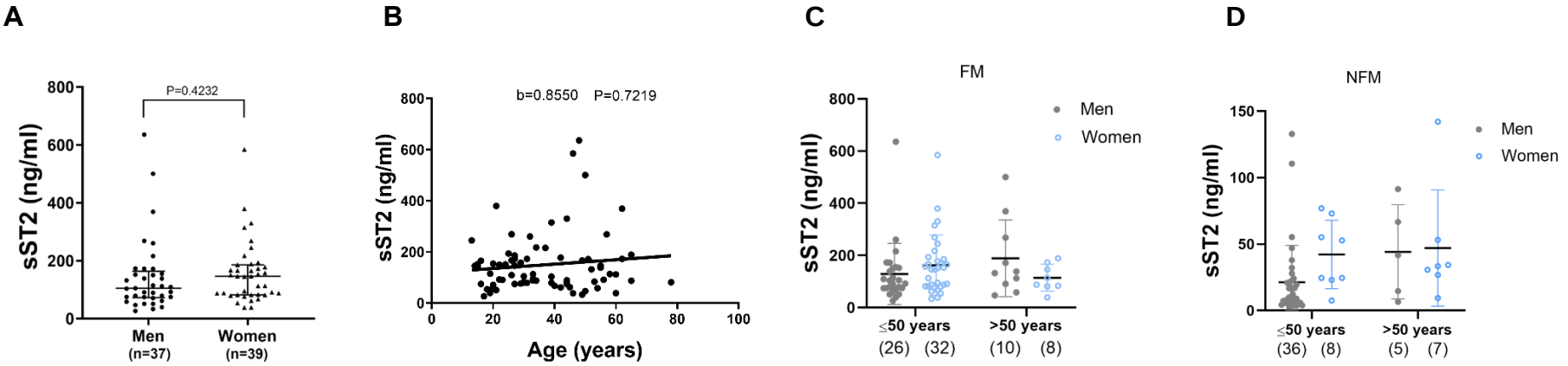
(A) Regional enhancement of the septal wall (arrow). (B) Lateral and midwall enhancement of the septal wall (arrows).

Figure S2. Diagnostic performance of plasma sST2 for the detection of FM.



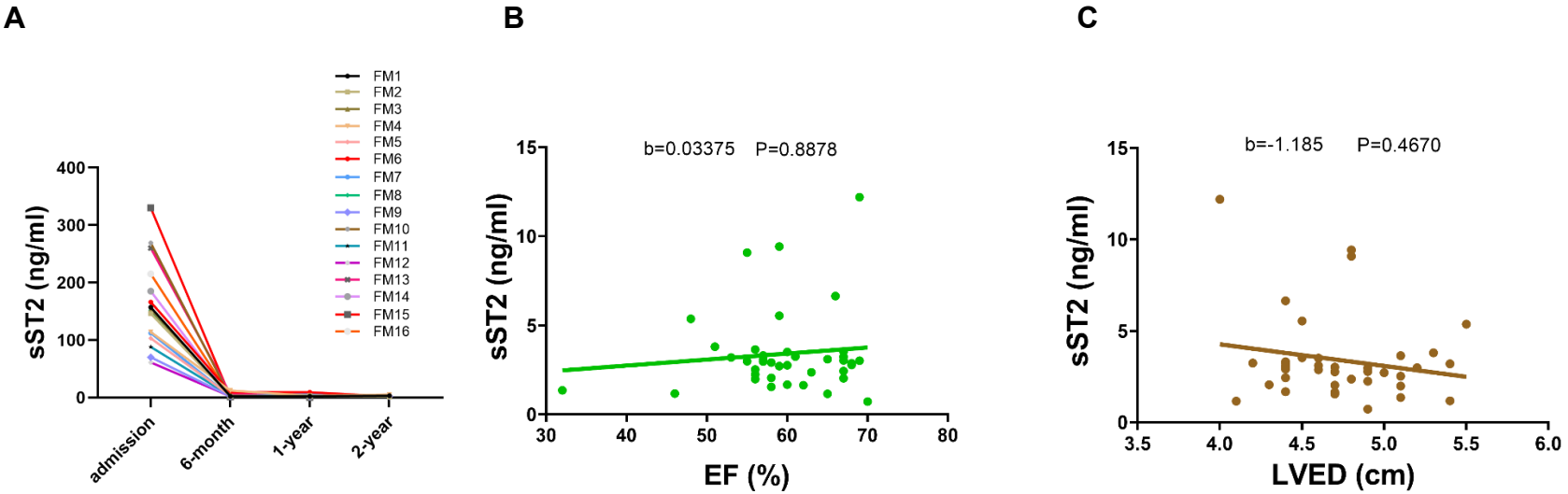
ROC curves of plasma sST2 in 76 patients with FM and 8 control individuals.

Figure S3. Correlations between plasma sST2 level, sex and age.



(A) Circulating concentrations of sST2 in 76 FM patients with different sexes (data are presented as medians and first to third quartile [Q1-Q3], Mann-Whitney test was used to calculate significance). (B) Correlation analysis of plasma sST2 with age in 76 FM patients (Spearman correlation and linear regression analysis were used to calculate significance). (C) Circulating concentrations of sST2 in FM patients under or over 50-year-old (data are presented as medians and first to third quartile [Q1-Q3], Mann-Whitney test was used to calculate significance). (D) Circulating concentrations of sST2 in NFM patients under or over 50-year-old (data are presented as medians and first to third quartile [Q1-Q3], Mann-Whitney test was used to calculate significance).

Figure S4. Plasma levels of sST2 in FM patients during follow-up.



(A) Circulating concentrations of sST2 in 16 patients with FM during 2 years follow-up. (B and C) Correlation analysis of plasma sST2 with EF values and left ventricular end diastolic (LVED) during 2 years follow-up (Spearman correlation and linear regression analysis were used to calculate significance).