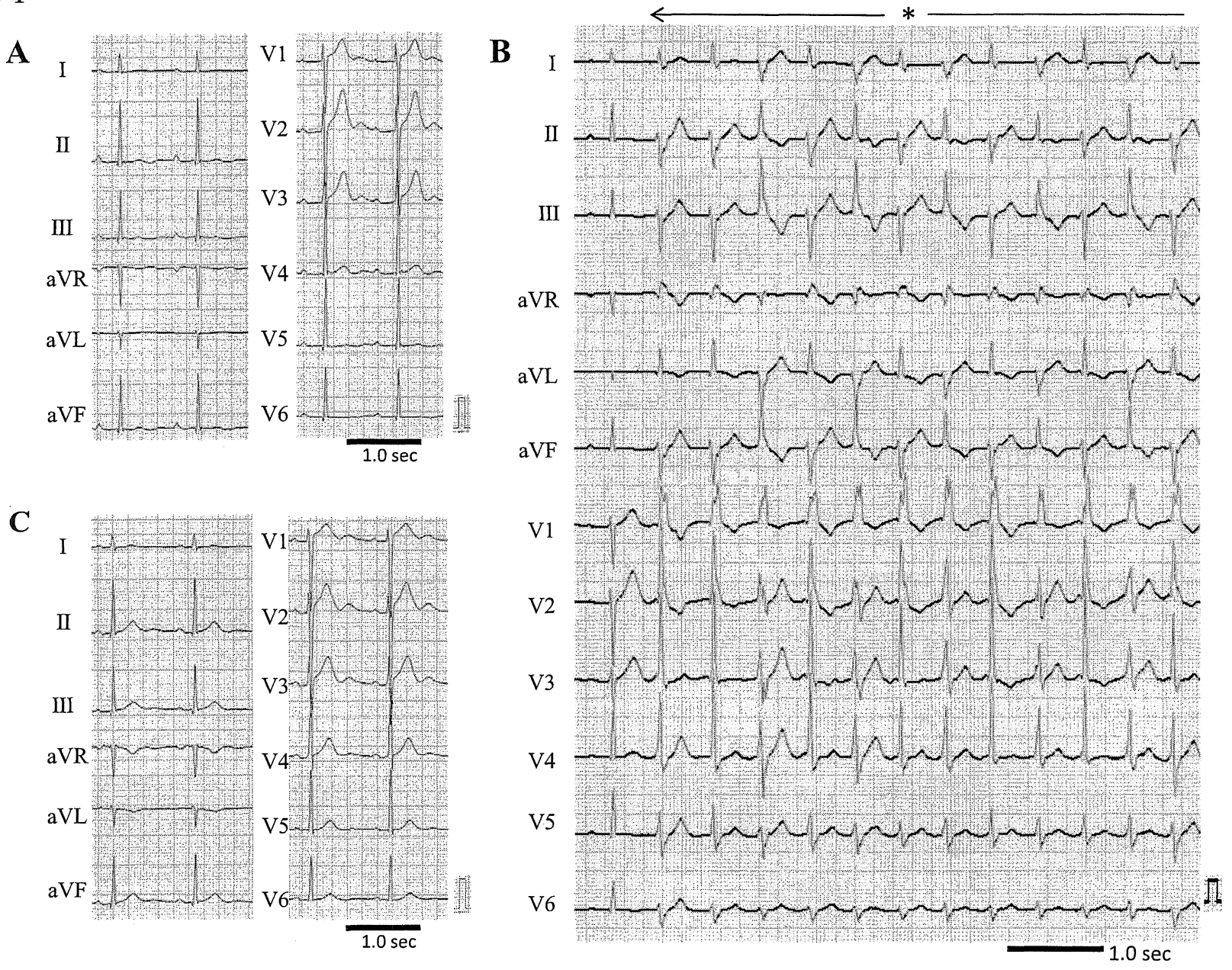
Figure 1



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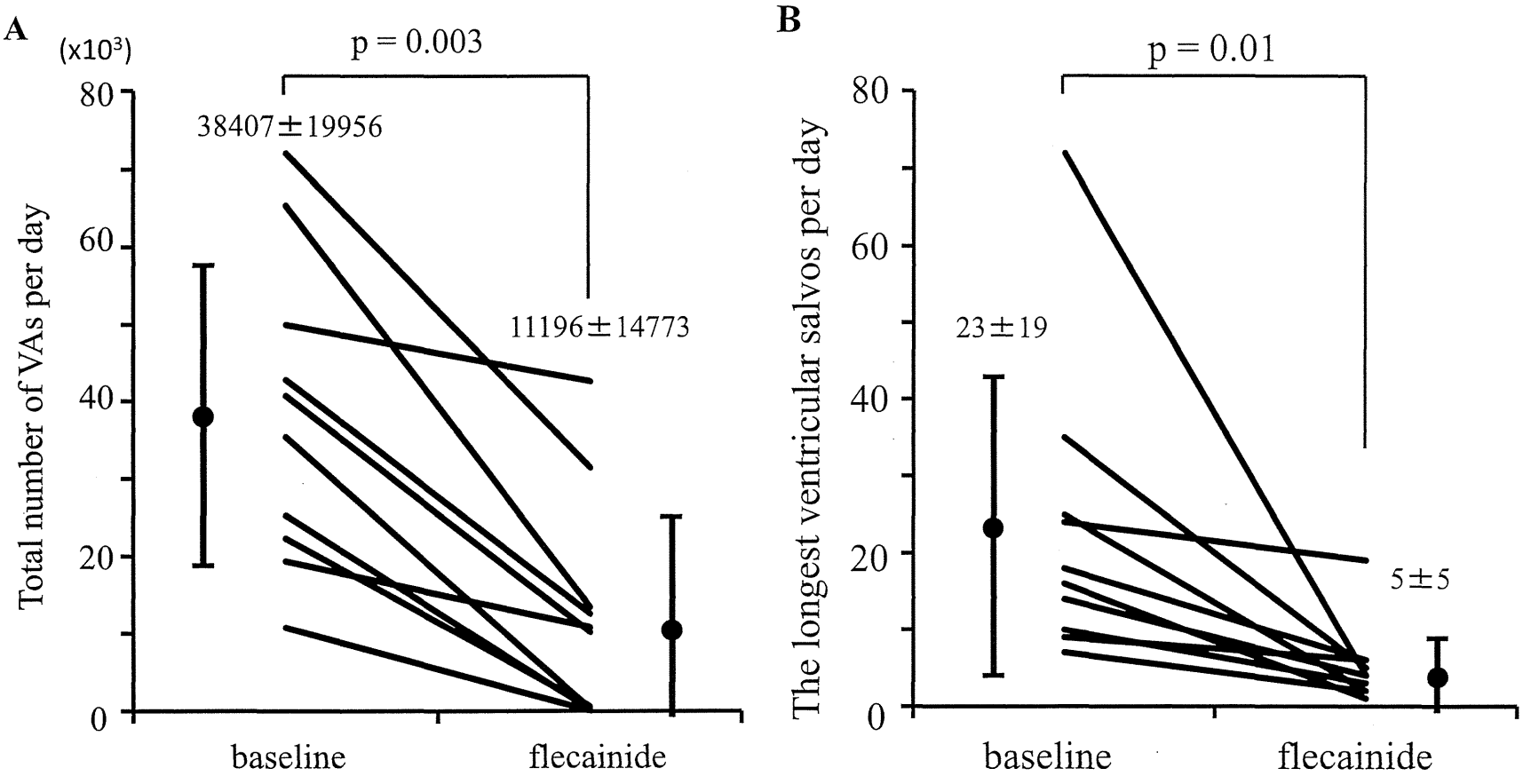

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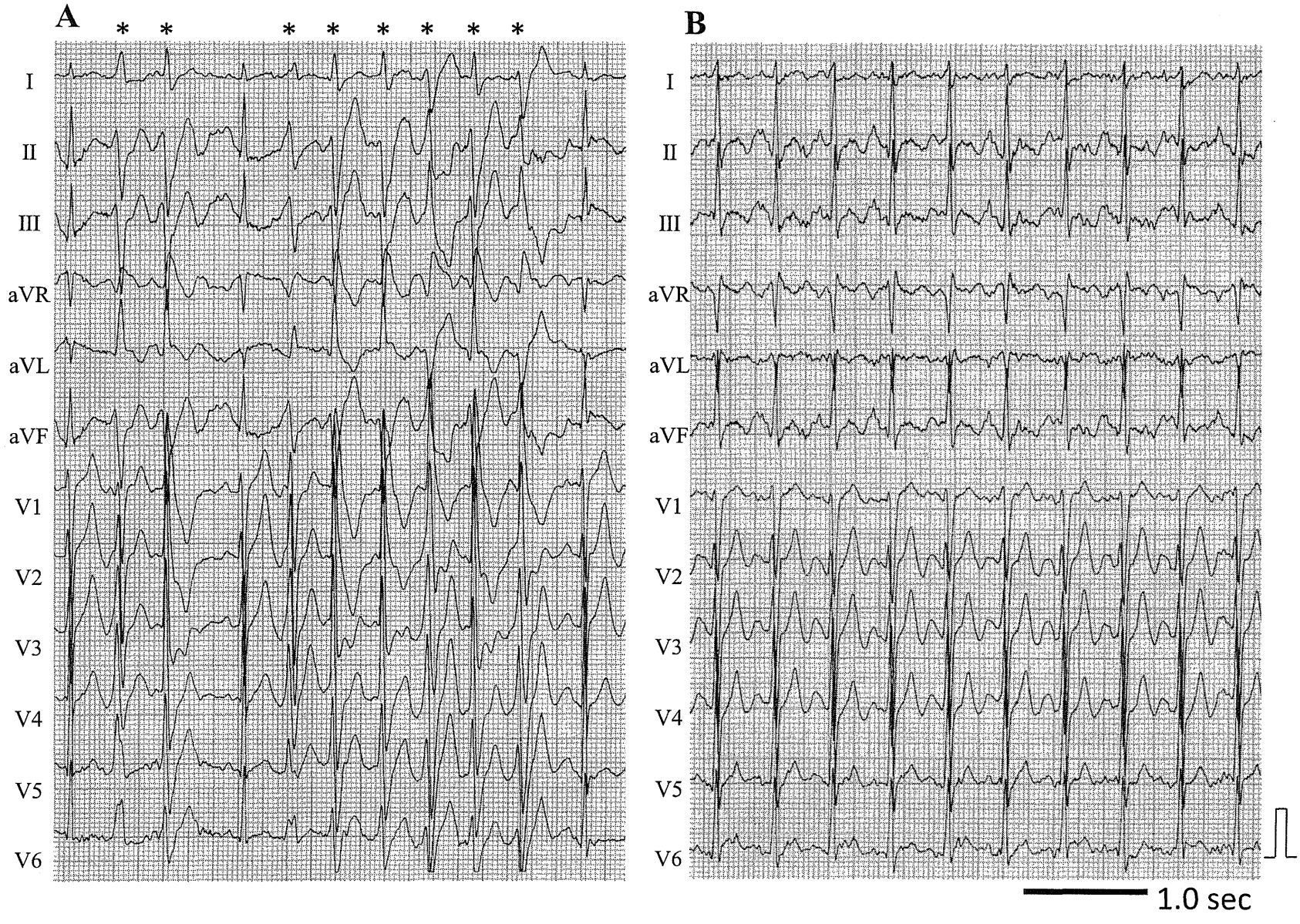

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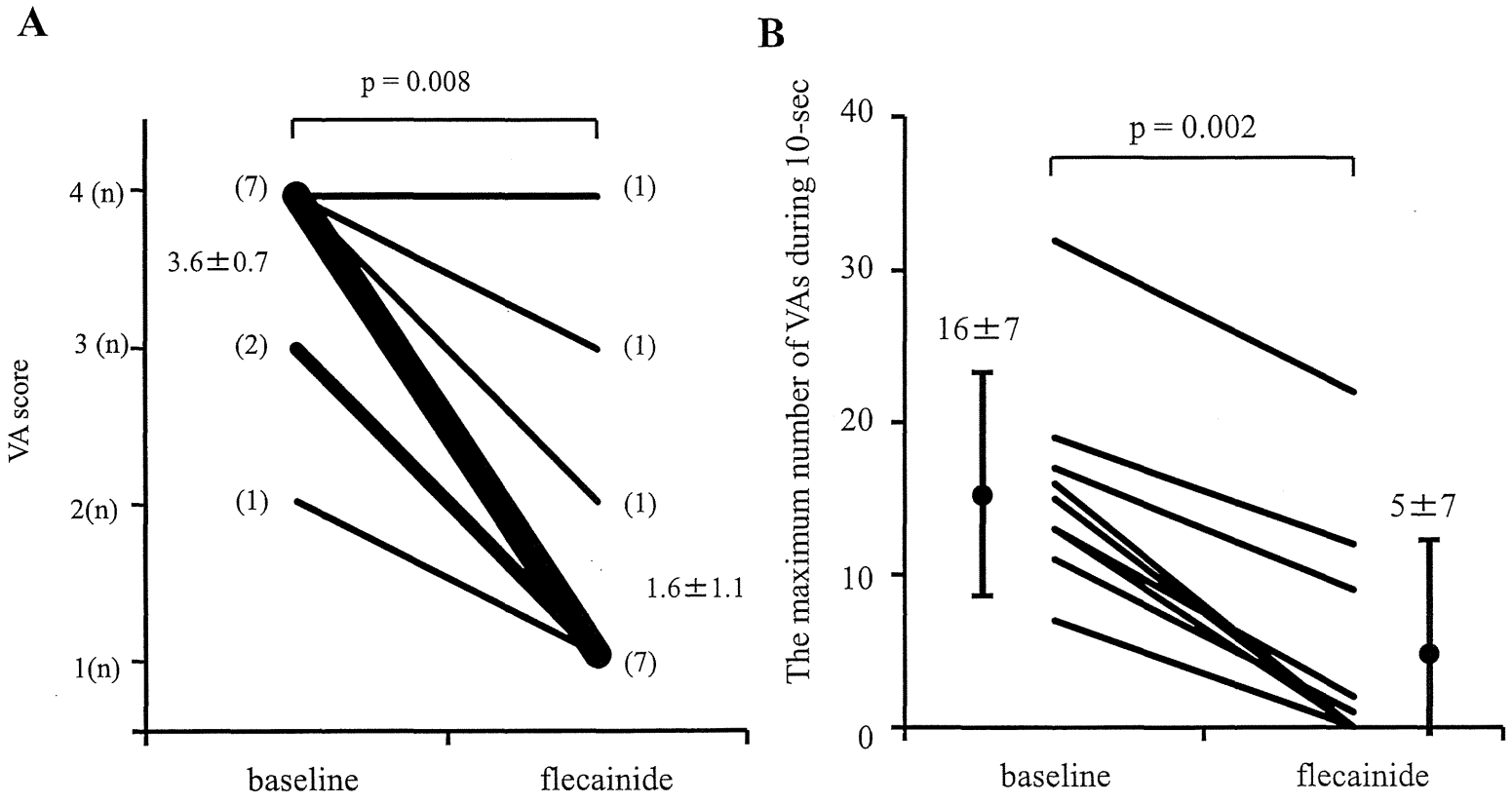

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Risk Stratification in Patients With Brugada Syndrome Without Previous Cardiac Arrest

– Prognostic Value of Combined Risk Factors –

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Background: Risk stratification in patients with Brugada syndrome for primary prevention of sudden cardiac death is still an unsettled issue. A recent consensus statement suggested the indication of implantable cardioverter defibrillator (ICD) depending on the clinical risk factors present (spontaneous type 1 Brugada electrocardiogram (ECG) [Sp1], history of syncope [syncope], and ventricular fibrillation during programmed electrical stimulation [PES+]). The indication of ICD for the majority of patients, however, remains unclear.

Methods and Results: A total of 218 consecutive patients (211 male; aged 46±13 years) with a type 1 Brugada ECG without a history of cardiac arrest who underwent evaluation for ICD including electrophysiological testing were examined retrospectively. During a mean follow-up period of 78 months, 26 patients (12%) developed arrhythmic events. On Kaplan-Meier analysis patients with each of Sp1, syncope, or PES+ suffered arrhythmic events more frequently (P=0.018, P<0.001, and P=0.003, respectively). On multivariate analysis Sp1 and syncope were independent predictors of arrhythmic events. When dividing patients according to the number of these 3 risk factors present, patients with 2 or 3 risk factors experienced arrhythmic events more frequently than those with 0 or 1 risk factor (23/93 vs. 3/125; P<0.001).

Conclusions: Syncope, Sp1, and PES+ are important risk factors and the combination of these risks well stratify the risk of later arrhythmic events.

Key Words: Brugada syndrome; Electrophysiological study; Primary prevention; Risk stratification; Syncope

Brugada syndrome (BrS), characterized by ST-segment elevation in the right precordial leads (type 1 Brugada electrocardiogram [ECG]) and a high incidence of ventricular fibrillation (VF) leading to sudden cardiac death (SCD) in young and otherwise healthy adults, has been reported since the initial description of the disease.[1] It is estimated to be responsible for at least 4% of all sudden deaths and at least 20% of sudden deaths in patients with structurally normal hearts.[2] Clearly, a method for the risk stratification of patients with BrS is required. A number of studies have noted a high recurrence rate of VF in patients with BrS who had previously experienced cardiac arrest.[3–7] Further discussion regarding the indications for implantable cardioverter defibrillator (ICD) in these patients (secondary prevention) is unnecessary. The indications for ICD for primary prevention of SCD in patients with BrS without a history of VF or cardiac arrest, however, have not been well evaluated. A recent consensus statement suggested the indication of ICD for these patients based on the following risk factors: spontaneous diagnostic type 1 Brugada ECG (Sp1), history of syncope likely caused by ventricular

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Table 1. Patient Characteristics vs. Presence of SCD or VF

	SCD or VF (+) (n=26)	SCD or VF (−) (n=192)	P-value	Overall (n=218)
Age (years)	50±11	46±13	0.19	46±13
Male	25 (96)	186 (97)	0.84	211 (96)
Follow-up period (months)	51±54	81±49	0.0023	78±49
ICD implantation	24 (92)	98 (51)	<0.001	122 (56)
ICD period of implantation (months)	44±34	87±40	<0.001	78±43
Sp1	24 (92)	135 (70)	0.018	159 (73)
Syncope likely caused by arrhythmia	21 (81)	66 (34)	<0.001	87 (40)
Inducibility of VF (PES+)	13 (50)	48 (25)	0.0077	61 (28)
Family history of SCD	7 (27)	57 (30)	0.77	64 (29)

Data given as n (%) or mean±Standard Deviation. ICD, implantable cardioverter defibrillator; PES, programmed electrical stimulation; SCD, sudden cardiac death; Sp1, spontaneous type 1 Brugada electrocardiogram; VF, ventricular fibrillation.

arrhythmia (syncope), and development of VF during programmed electrical stimulation (PES+, ie, inducible patients).[8] This statement recommends ICD implantation for primary prevention of SCD as a class IIa indication for patients with Sp1 and syncope and as a class IIb indication for patients with PES+. The indication of ICD for the patients who do not meet these conditions, however, remains unclear. Here we hypothesized that these 3 proposed risk factors may be used to stratify the risk of SCD in patients with BrS and without previous cardiac arrest.

Methods

Patients and Baseline Data

Among 612 patients who had been given the diagnosis of BrS at 2 Japanese institutions, Okayama University Hospital or the National Cerebral and Cardiovascular Center between 1996 and 2012, we retrospectively enrolled 218 consecutive patients with type 1 Brugada ECG, without a history of VF or cardiac arrest, who were hospitalized and underwent electrophysiological testing to evaluate the suitability of ICD therapy. All patients were followed for at least 12 months from the start of the investigation. The primary endpoint was the occurrence of VF or SCD. Patients with structural cardiac abnormalities on transthoracic echocardiography were excluded. Written informed consent regarding the data acquisition was obtained from all individuals. The study conforms to the 1975 Declaration of Helsinki as reflected by approval by the Institutional Review Board in both institutions.

In accordance with the consensus report in 2005, type 1 Brugada ECG was defined as coved-type ST-segment elevation ≥0.2 mV followed by a negative T wave in more than 1 right precordial lead (V1–V3) in the presence or absence of a sodium channel-blocking agent.[5] Although ECG could change during follow-up, Sp1 was defined as spontaneous type 1 Brugada ECG in the absence of a sodium channel-blocking agent at the beginning of the study. ECG was recorded at least 4 different times in each patient. Placement of the right precordial leads in a superior position up to the second intercostal space was performed to increase sensitivity. A sodium channel-blocking agent (i.v. pilsicainide up to 1 mg/kg body weight) was used under careful monitoring to unmask ECG abnormalities when Sp1 was not observed. All patients underwent electrophysiological evaluation to assess the inducibility of VF by PES. The protocol involved up to 3 extrastimuli applied to the right ventricular apex and right ventricular outflow tract at a minimum coupling interval of 180 ms. PES+, however, was defined as VF or polymorphic ventricular tachycardia lasting >30 s or requiring direct current shock induced at a coupling interval ≥200 ms according to the consensus document.[5] Syncope was considered present when a patient had an episode of syncope judged as likely caused by ventricular arrhythmia. Syncope likely due to vasovagal events such as those occurring during abrupt postural changes, exposure to heat and dehydration, or emotional reactions were excluded. Data on family history of SCD prior to age 45 (FH) were also collected. Combinations of the 3 risk factors proposed in the consensus statement, that is, Sp1, syncope, and PES+, were examined for further risk stratification of arrhythmic events.

Follow-up and Arrhythmic Events

Patient treatment, including ICD implantation, was based on physician clinical judgment. All patients were followed up for at least 12 months after the start of the investigation. Patients with ICD were followed every 3 months while those without ICD were followed at least once a year. Arrhythmic events were defined as SCD or appropriate shock delivery by ICD against ventricular tachyarrhythmia.

Statistical Analysis

Data were analyzed using JMP (version 11.0.0, SAS, Cary, NC, USA). For continuous variables, comparisons among groups were made using Student's t-test. Pearson's chi-squared test was used for categorical variables. Event analysis over time was performed using the Cox proportional hazard regression model. Risk was quantified as a hazard ratio (HR) with 95% confidence interval. Survival curves were constructed using the Kaplan-Meier method and compared with the log-rank test. $P<0.05$ was considered statistically significant.

Results

Patient Characteristics and Overall Event Rate

A total of 218 consecutive patients were examined. As shown in **Table 1**, the mean patient age at diagnosis was 46±13 years and most patients were men (n=211, 96%). ICD were implanted in more than half of the patients (n=122, 56%). Sp1 was observed in 159 patients (73%), and 87 patients (40%) had experienced syncope. PES+ was observed in 61 patients (28%), and 64 patients (29%) had FH. During the mean follow-up period of 78±49 months (median, 76 months), 26 patients (12%) experienced at least 1 arrhythmic event. The pe-

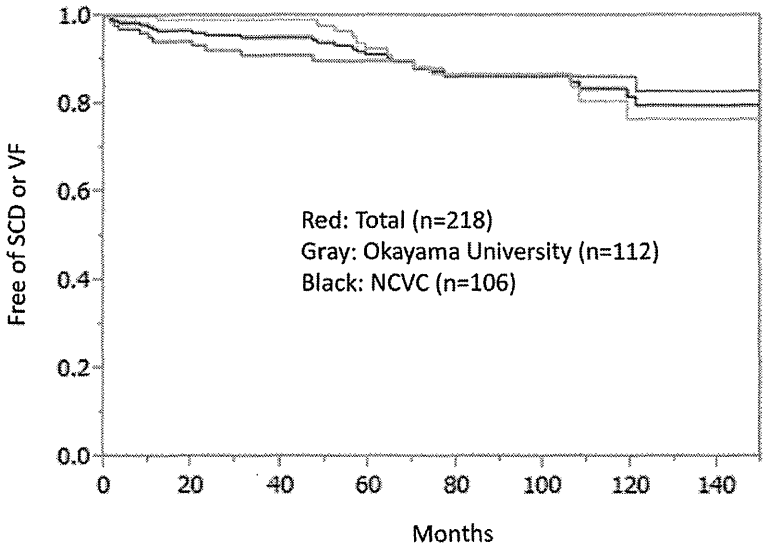

Figure 1. Kaplan-Meier analysis of lethal arrhythmic events during follow-up in 218 patients with Brugada type 1 electrocardiogram but without a history of cardiac arrest, both overall (red line) as well as separately at Okayama University (gray) and the National Cerebral and Cardiovascular Center (NCVC; black). SCD, sudden cardiac death; VF, ventricular fibrillation.

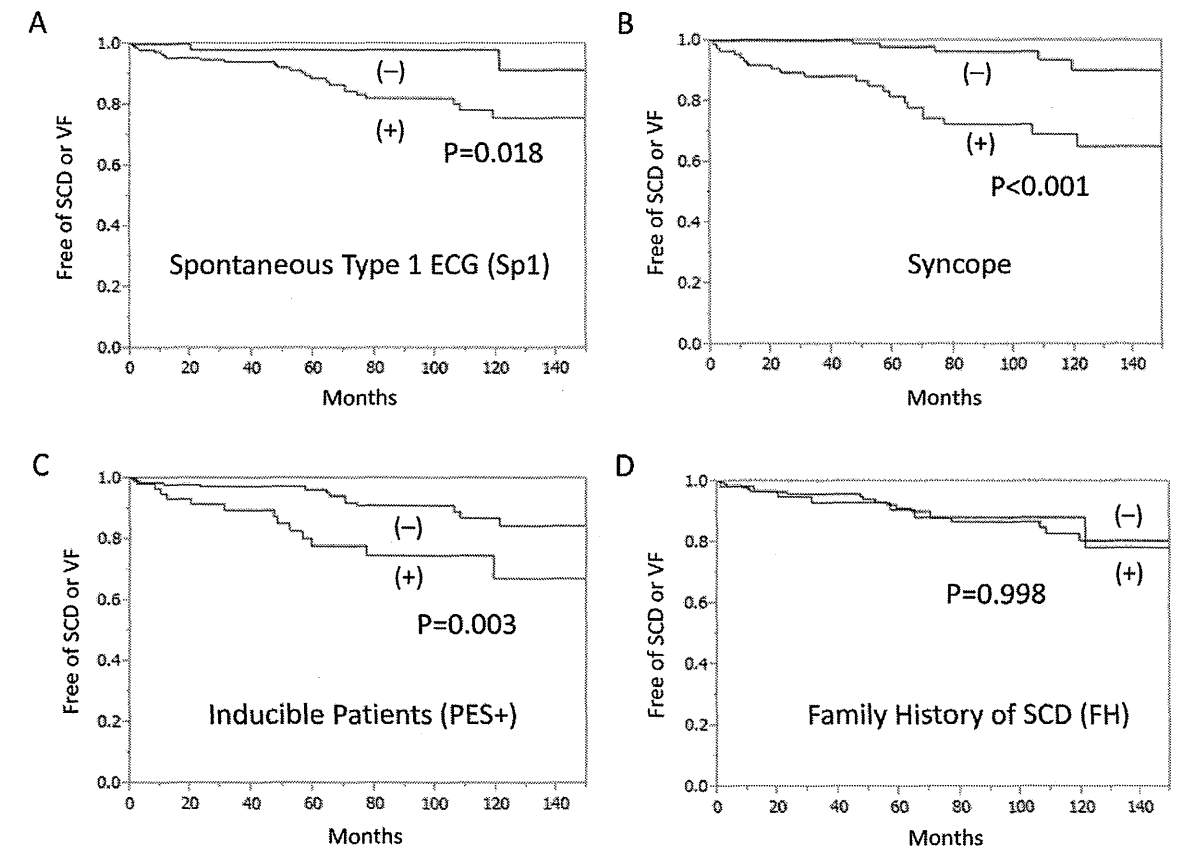

Figure 2. Kaplan-Meier analysis of lethal arrhythmic events during follow-up, categorized by risk factors present: (**A**) spontaneous type 1 Brugada ECG (Sp1− vs. Sp1+); (**B**) history of syncope judged to be likely caused by ventricular arrhythmias (syncope− vs. syncope+); (**C**) inducibility of ventricular fibrillation by programmed electrical stimulation (PES− vs. PES+); and (**D**) family history of SCD (FH− vs. FH+). ECG, electrocardiogram; SCD, sudden cardiac death; VF, ventricular fibrillation.

Table 2. Predictive Factors of SCD or VF

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age (per year)	1.02	0.98–1.05	0.38			
Male	0.72	0.15–12.94	0.72			
Sp1	4.81	1.43–29.92	0.0079	4.51	1.30–28.37	0.014
Syncope	6.87	2.80–20.59	<0.001	6.81	2.76–20.46	<0.001
Inducibility of VF (PES+)	3.01	1.38–6.56	0.0062	2.03	0.92–4.49	0.078
Family history of SCD	0.94	0.37–2.16	0.90			

CI, confidence interval; HR, hazard ratio. Other abbreviations as in Table 1.

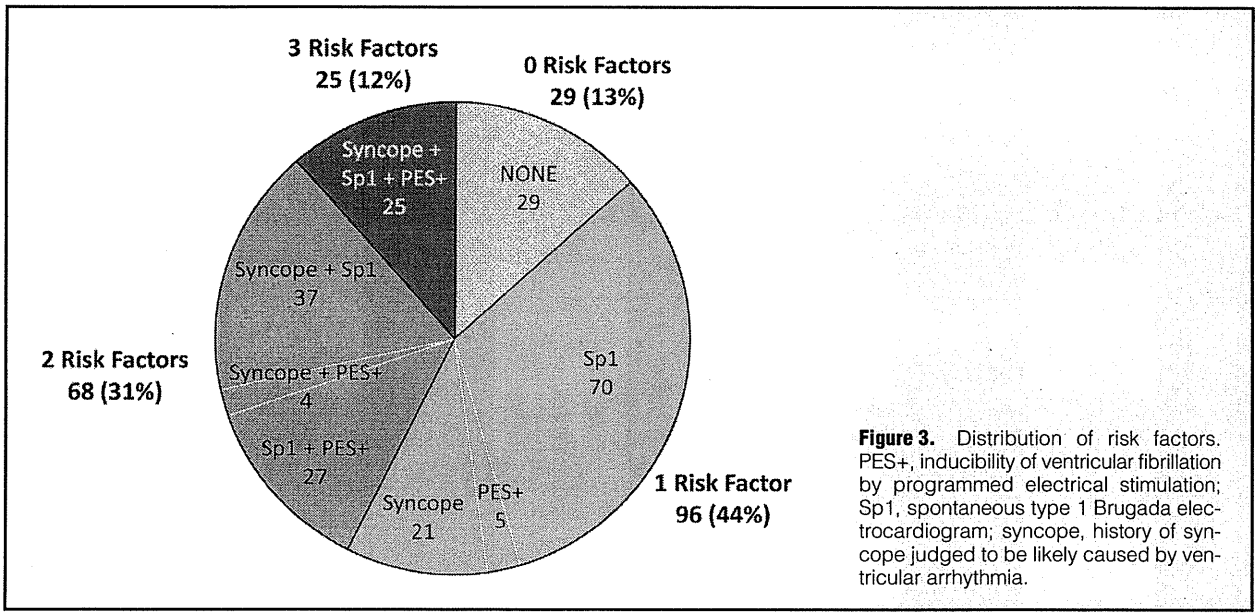

Figure 3. Distribution of risk factors. PES+, inducibility of ventricular fibrillation by programmed electrical stimulation; Sp1, spontaneous type 1 Brugada electrocardiogram; syncope, history of syncope judged to be likely caused by ventricular arrhythmia.

riod from the start of the investigation to the first arrhythmic event was 51±37 months (median, 54 months). Twenty-four patients received appropriate ICD shock delivery against VF with successful termination, while 2 patients who had refused ICD therapy died suddenly. **Figure 1** shows the Kaplan-Meier event-free survival curve for the overall group as well as for each institution. **Table 1** also compares the patient characteristics between 2 groups defined according to the presence (n=26) or absence (n=192) of an arrhythmic event during follow-up. The prevalence of ICD was higher and the follow-up period was shorter in patients with an arrhythmic event (92% vs. 51%, P<0.001; 51 months vs. 81 months, P=0.0023, respectively). The period of ICD implantation was also longer in patients without an arrhythmic event (44 months vs. 87 months, P<0.001). The prevalence of SP1, syncope, and PES+ were higher in patients with an arrhythmic event compared to those without (92% vs. 70%, P=0.018; 81% vs. 34%, P<0.001; and 50% vs. 25%, P<0.0077, respectively). There was no difference in FH between the 2 groups (27% vs. 30%, P=0.77).

Arrhythmic Events

Figure 2 shows the influence of each of the proposed risk factors (Sp1, syncope, and PES+) and FH on the incidence of arrhythmic events during the follow-up period. The prevalence of arrhythmic events was higher in patients with any of the proposed risk factors compared to those without it (Sp1, P=0.018, **Figure 2A**; syncope, P<0.001, **Figure 2B**; PES+, P=0.003, **Figure 2C**). FH, however, had no association with event rate (P=0.998, **Figure 2D**).

Next, univariate and multivariate analysis were used to investigate the possible clinical variables associated with SCD and VF. On multivariate analysis syncope and Sp1 were independent predictors of arrhythmic events during follow-up (HR, 6.81 and 4.51, respectively) as shown in **Table 2**. PES+, however, was not an independent predictor (HR, 2.03, P=0.078).

Risk Factor Combinations

Figure 3 shows the number of risk factors of Sp1, syncope and PES+ in each patient, from 0 to 3. The majority of patients had 1 (44%) or 2 (31%) risk factors. Using event-free survival curve according to the number of risk factors, the event-free rate decreased as the number of risk factors increased (**Figure 4**). The prevalence of arrhythmic events was higher in patients with 2 risk factors compared with patients with 1 risk factor (P<0.001). There was no statistically significant difference in the number of arrhythmic events between patients with 2 and 3 risk factors (P=0.089), and patients without any risk factors had no SCD or VF.

Among patients with either 1 or 2 risk factors, there were no statistical differences in event-free survival rates between

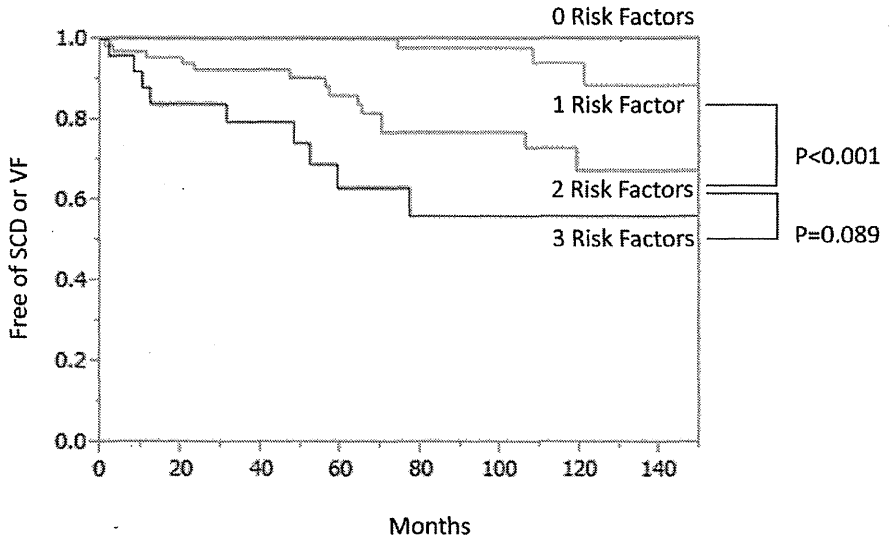

Figure 4. Kaplan-Meier analysis of lethal arrhythmic events during follow-up depending of the number of risks factors present, including syncope, inducibility of ventricular fibrillation, and spontaneous type 1 Brugada electrocardiogram. SCD, sudden cardiac death; VF, ventricular fibrillation.

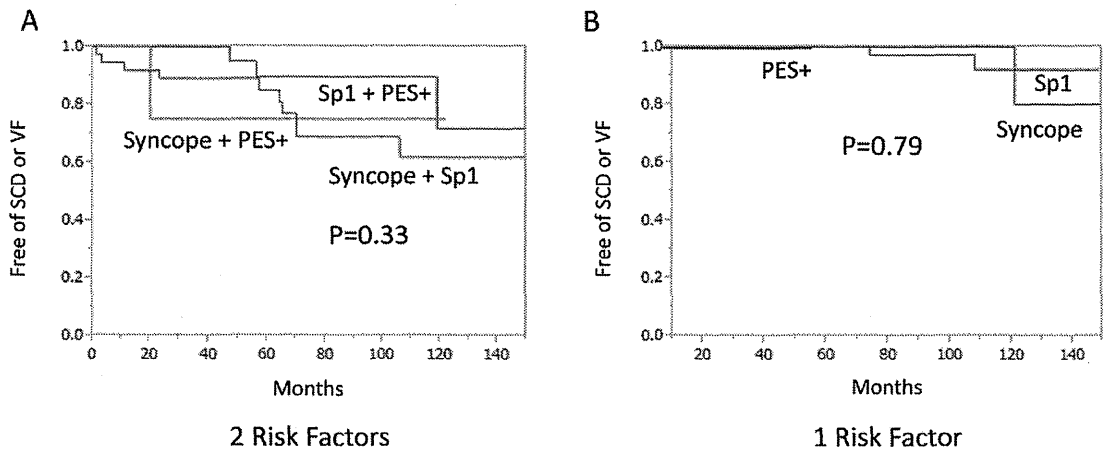

Figure 5. Kaplan-Meier analysis of arrhythmic events during follow-up depending on the presence of (**A**) 2 risks (syncope+Sp1 vs. Sp1+PES+ vs. syncope+PES+) or (**B**) 1 risk (Sp1 vs. syncope vs. PES+). PES+, inducibility of ventricular fibrillation by programmed electrical stimulation; Sp1, spontaneous type 1 Brugada electrocardiogram; syncope, history of syncope judged to be likely caused by ventricular arrhythmia.

subgroups (P=0.33 for 2 risk factors, syncope+Sp1/Sp1+PES+/syncope+PES+; and P=0.79 for 1 risk factor, Sp1/syncope/PES+; **Figure 5**).

Furthermore, there were significant differences in event-free survival rates between all subgroups with 2 risk factors and 1 risk factor (P<0.001 for syncope+Sp1 vs. 1 risk, P=0.03 for Sp1+PES+ vs. 1 risk and P=0.0036 for syncope+PES+ vs. 1 risk; **Figure S1**).

Discussion

Major Finding

This is one of the largest studies on this topic to date, with a subject group of 218 patients with BrS without cardiac arrest who were considered for ICD therapy for primary prevention of SCD. The major finding was that the proposed 3 risk factors, Sp1, syncope, and PES+, successfully stratified the risk of later arrhythmic events in Japanese patients with BrS with-

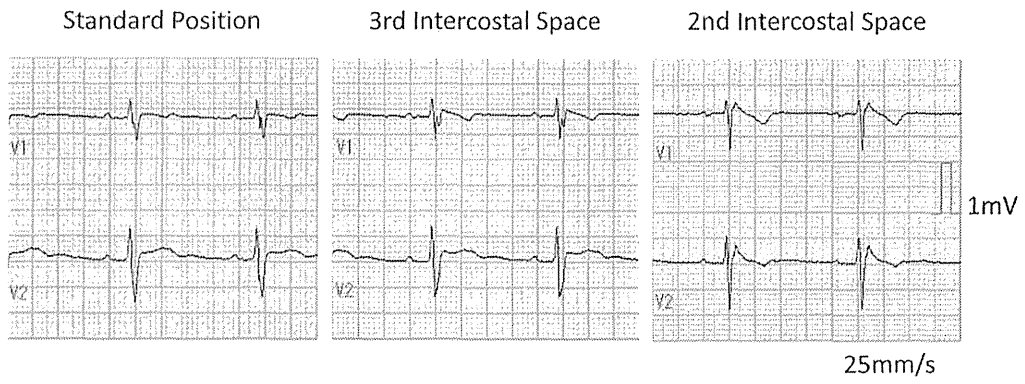

Figure 6. Electrocardiogram (V1 and V2) of a patient with a history of syncope who developed ventricular fibrillation during the follow-up. Spontaneous coved-type ST elevation was seen in the superior position (**Middle**: 3rd intercostal space; **Right**: 2nd intercostal space) not in the (**Left**) standard position.

out prior cardiac arrest. In particular, syncope and Spl were both independent risk factors. To our knowledge, this is the first study to show that the magnitude of the risk of SCD or VF was stratified by combining these 3 risk factors. When a patient had ≥2 risk factors, the risk of fatal arrhythmic events dramatically increased.

Combining Risk Factors for VF

The idea of combining risk factors arose from the guidelines for non-pharmacotherapy of cardiac arrhythmias published by the Japanese Circulation Society in 2011.[9] These guidelines recommended ICD for primary prevention of SCD in patients with BrS according to the number of the following 3 risks factors present: syncope, PES+, and FH. ICD was categorized as a class IIa therapy for patients with ≥2 risk factors and ICD was categorized as class IIb for patients with a single risk factor. Spl was not included as a risk factor in these guidelines. Delise et al reported the risk stratification in a similar patient type as in the present study, with a focus on risk combinations.[10] Although FH itself failed to predict arrhythmic events, the authors concluded that a multi-parametric approach that included syncope, FH, and PES+ helped to identify patients at high risk. They also reported that the patients at highest risk were those with at least 2 risk factors in addition to Spl. In this study, we could stratify the risk of arrhythmic events clearly by combining 3 proposed risk factors according to the 2013 consensus report: Spl, syncope, and PES+.

Japanese Guideline 2011

As noted, the 2011 Japanese guidelines recommended ICD for primary prevention of SCD in patients with BrS according to 3 risk factors: syncope, PES+, and FH. ICD was categorized as a class IIa treatment for patients with ≥2 risk factors and ICD was categorized as class IIb for patients with a single risk factor. Event-free survival curve according to this ICD indication class seemed to well stratify the risk of SCD, although there was a modest but not significant difference between class IIa and class IIb indication (P=0.07; **Figure S2A**). Among patients with class IIa or class IIb indications, however, there were statistically significant differences in event-free survival rate between subgroups (P=0.049 for class IIa indications, syncope+FH+PES+/syncope+FH/FH+PES+/syncope+PES+; and P=0.035 for class IIb indications, FH/syncope/PES+; **Figure S2B,C**). These differences seemed to arise from the lack of predictive value of FH and further discussion on this guideline is required.

Spontaneous Coved-Type ECG

The present results demonstrated the importance of Spl as a predictor of arrhythmic events, confirming similar findings in other studies.[3,10–14] To increase the sensitivity for detecting Spl, ECG were recorded in the superior position up to the second intercostal space as well as in the normal position in all patients, which may have resulted in the higher incidence of Spl (73%) in this study than in other studies (around 55%).[6,10,12,15]

Figure 6 presents a typical ECG of a patient with a history of syncope who developed VF during the follow-up, and in whom ICD was implanted because Spl was recorded in the superior position, not in the normal position. It has been reported that Spl can evolve spontaneously within minutes or can be unmasked by fever, although fever-induced Brugada ECG was not observed in this study.[16–18] Also, seasonal and circadian distributions of arrhythmic events in patients with BrS have been reported.[19] In the present study, ECG were recorded during the day, and circadian variation of ECG pattern was not evaluated. ECG recording immediately after the meal or at various times may further increase the sensitivity for detecting Spl. Interestingly, ECG recording on deep inspiration has been reported to be useful to identify Brugada ECG.[20] Accordingly, repeated recordings of 12-lead ECG should be performed to increase the detection of Spl.

PES

The role of electrophysiological evaluation remains controversial.[5] In this study, PES+ did not serve as an independent predictor of SCD or VF, but patients with PES+ had a higher incidence of arrhythmic events than those without it. Although there are several reports on the predictive value of PES+ that suggest that PES+ plays an important role in risk stratification, several studies including the PRELUDE registry reported by Priori et al argued against its usefulness.[3,11,12,21] The PRELUDE registry was a prospective study in which patients were followed for an average of 34 months, a much shorter duration

than that used in the present study. Furthermore, the median period from the start of the study to the arrhythmic event was 54 months in the present study. Short study duration might be one of the reasons for the discrepancy in PES+ importance between the PRELUDE registry and the present findings. The role of PES was also reported in the meta-analysis by Fauchier et al, which suggested the usefulness of PES in asymptomatic patients and in patients with syncope of unknown origin, not in patients with a history of cardiac arrest.[21] The present result supports the finding of that meta-analysis. Also, the importance of the negative inducibility of VF by PES should be discussed. In the present study, some patients with negative inducibility of VF developed arrhythmic events as well. The previously mentioned meta-analysis by Fauchier et al also concluded that negative inducibility does not portend a good prognosis.[21] Delise et al, however, reported that no arrhythmic events occurred in patients with negative inducibility of VF.[10] Makimoto et al reported that the number of extrastimuli in PES using the minimum coupling interval of 180 ms and the inducibility of VF by up to 2 extrastimuli had significant predictive value for future cardiac events.[22] Further discussion of these unresolved issues should be continued.

Family History of SCD

Although we focused on Spl, syncope and PES in the present study according to the current consensus report published in 2013, we also analyzed the impact of FH on future arrhythmic events, because Kamakura et al reported that FH was one of the predictors of SCD in patients with Spl.[7] Although 45 patients in the Kamakura et al report were included in the present study, the Kamakura et al study included 274 patients without previous cardiac arrest (primary prevention) from 26 institutions across Japan. Also, the Kamakura et al study included patients with aborted SCD (secondary prevention), a patient type that differed from the present study. The FINGER registry, the largest cohort of patients with BrS, also concluded that FH was not a predictor of cardiac events.[3] Although there are limited data on FH as a risk factor for lethal arrhythmia, it should not be disregarded. BrS is considered to be an inherited disease to a certain degree and the importance of FH should be discussed on an individual basis. Also, genetic information may provide important information on the relationship between FH and the risk of SCD. Further study is required on this unsolved issue.

Clinical Implications

The HRS/EHRA/APHRS consensus document 2013 provided partial recommendations for ICD therapy for primary prevention of SCD in patients with BrS.[8] Recently, Takagi et al reported that class IIa indication in this statement could identify a group of patients with increased risk compared to class IIb indication.[23] The indication for ICD for the patients who do not meet the conditions stated in the consensus document, however, remains unclear. In contrast, the combination of the 3 risk factors as proposed in the present study, which was based on the consensus document, covers all patients who are examined for ICD treatment. Patients with ≥2 risk factors are at high risk of SCD, which emphasizes the importance of measuring these 3 risk factors cautiously and exactly. First, repeated 12-lead ECG in the superior position in addition to the normal position should be performed to increase the detection of Spl. Second, to increase the accuracy of risk estimation, syncope judged as likely caused by vasovagal reflex should be excluded. Third, the PES result is important especially when a patient has either Spl or syncope. In this situation, electrophysiological evaluation should be recommended to estimate the risk of VF accurately.

Study Limitations

The retrospective nature of this study is an important limitation. Patients were enrolled from only 2 Japanese institutions and the number of patients in this study was still small. Furthermore, all patients in this study underwent electrophysiological testing to assess suitability for ICD therapy, which was designed to evaluate the 3 proposed risk factors, syncope, Spl, and PES+. Undoubtedly a certain bias existed in physician decision regarding whether or not to perform PES in each patient. Further prospective studies are needed to reach definitive conclusions.

Conclusions

Syncope, Spl, and PES+ were important risk factors for SCD and VF in patients with BrS without previous cardiac arrest. The combination of these proposed risk factors according to the consensus report 2013 accurately stratified the risk of arrhythmic events, which could be of assistance when considering ICD therapy in these patients.

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Disclosures

None.

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Appendix

Number of patients included at each center: Okayama University Hospital, 112; National Cerebral and Cardiovascular Center, 106.

Supplementary Files

Supplementary File 1

Figure S1. Kaplan-Meier analysis of arrhythmic events during follow-up according to the presence of 2 risks (syncope+Sp1 vs. Sp1+PES+ vs. syncope+PES+) and 1 risk.

Figure S2. (A) Kaplan-Meier analysis of lethal arrhythmic events during follow-up depending on implantable cardioverter defibrillator (ICD) indication class defined in the Japanese guideline 2011.

Please find supplementary file(s);
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Decreased Myocardial Dendritic Cells is Associated With Impaired Reparative Fibrosis and Development of Cardiac Rupture After Myocardial Infarction in Humans

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ORIGINAL RESEARCH

American Heart Association | American Stroke Association.

Decreased Myocardial Dendritic Cells is Associated With Impaired Reparative Fibrosis and Development of Cardiac Rupture After Myocardial Infarction in Humans

Toshiyuki Nagai, MD, PhD;* Satoshi Honda, MD;* Yasuo Sugano, MD, PhD; Taka-aki Matsuyama, MD, PhD; Keiko Ohta-Ogo, MD, PhD; Yasuhide Asaumi, MD, PhD; Yoshihiko Ikeda, MD, PhD; Kengo Kusano, MD, PhD; Masaharu Ishihara, MD, PhD, FACC; Satoshi Yasuda, MD, PhD; Hisao Ogawa, MD, PhD, FACC; Hatsue Ishibashi-Ueda, MD, PhD; Toshihisa Anzai, MD, PhD, FACC, FAHA

Background—Dendritic cells (DC) play pivotal roles in regulating the immune system and inflammatory response. We previously reported DC infiltration in the infarcted heart and its immunoprotective roles in the post-infarction healing process after experimental myocardial infarction (MI). However, its clinical significance has not been determined.

Methods and Results—The degree of DC infiltration and its correlation with the post-infarction healing process in the human infarcted heart were investigated in 24 autopsy subjects after ST-elevation MI. Patients were divided into two groups according to the presence (n=13) or absence (n=11) of cardiac rupture. The numbers of infiltrated DC and macrophages and the extent of fibrosis in the infarcted area were examined. In the rupture group, $CD68^+$ macrophage infiltration was increased and $CD209^+$ DC, and $CD11c^+$ DC infiltration and the extent of reparative fibrosis were decreased compared with the non-rupture group, under matched baseline characteristics including the time from onset to death and use of revascularization. Furthermore, there was a significant positive correlation between the number of infiltrating $CD209^+$ DC, and $CD11c^+$ DC and the extent of reparative fibrosis.

Conclusions—Decreased number of DC in human-infarcted myocardial tissue was associated with increased macrophage infiltration, impaired reparative fibrosis, and the development of cardiac rupture after MI. These findings suggest a protective role of DC in post-MI inflammation and the subsequent healing process. (***J Am Heart Assoc.*** **2014;3:e000839 doi: 10.1161/JAHA.114.000839)**

Key Words: cardiac rupture • dendritic cell • inflammation • myocardial infarction • reparative fibrosis

Early reperfusion of the infarcted tissue and reparative fibrosis are essential in preserving the structural integrity of the left ventricle (LV) after myocardial infarction (MI). Cardiac accommodation following MI occurs in different phases, which include inflammatory, early healing, and myocardial remodeling phases.[1,2] An inadequate healing process after MI would result in complications such as cardiac rupture, LV aneurysm, and congestive heart failure due to exaggerated LV remodeling. However, the precise mechanisms and the potential therapeutic targets in the post-MI healing process remain to be clarified.

We previously reported that higher concentrations of serum C-reactive protein (CRP)[3] and plasma interleukin (IL)-6[4] and peripheral monocytosis[5] predict a worse clinical outcome after MI, suggesting that an immune-mediated inflammatory response may adversely affect post-infarction healing and LV remodeling. Recently, we elucidated that dendritic cells (DC) infiltrated into myocardial tissue play immunoprotective roles in post-infarction healing and LV remodeling in animal models.[6,7]

DC are professional antigen-presenting cells (APC), which are found in all organ systems, including the myocardium. Several subtypes have been described thus far, with so-called myeloid DC (mDC) and plasmacytoid DC (pDC) being predominant.[8] We have demonstrated infiltration of mature activated mDC in the infarcted rat heart, peaking on day 7.[6] To elucidate the significance of DC, we generated a mouse model with selective depletion of bone marrow-derived DC,

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and demonstrated that the depletion of bone marrow-derived DC exacerbated post-infarction LV remodeling in association with enhanced inflammatory cytokine expression and matrix metalloproteinase (MMP)-9 activation via marked infiltration of proinflammatory monocytes (Ly6C^{high}) and classically activated M1 macrophages into the infarcted myocardium. On the contrary, decreased IL-10, which has anti-inflammatory activity and myocardial infiltration of anti-inflammatory monocytes (Ly6C^{low}) and alternatively activated M2 macrophages were observed in a rodent model.[7] These findings suggest that DC may play a protective role against post-infarction LV remodeling by regulating the homeostasis of monocytes and macrophages during the transition from inflammation to repair. However, the presence and the clinical significance of DC in the human infarcted heart remain to be determined.

In the present study, we used immunostaining techniques to identify and quantify DC infiltration in the infarcted myocardium in human autopsy samples, to clarify the impact of DC infiltration on the post-MI healing process and the development of cardiac rupture.

Methods

Patients

Among patients who were admitted to our institution with ST-elevation MI (STEMI) and underwent autopsy between December 1978 and May 1998 (n=49), 24 cases with enough preserved infarcted tissue for immunohistochemical analyses were examined.

All autopsies were performed within 24 hours after death. Heart tissue samples were taken from the LV infarcted area of patients who died of cardiac rupture including free wall rupture (n=9) and ventricular septal perforation (n=4), pump failure (n=10), or fatal ventricular arrhythmias (n=1). Patients were divided into two groups according to the presence (n=13) or absence (n=11) of cardiac rupture.

Our study was approved by the ethics committee of the National Cerebral and Cardiovascular Center, and conformed to the principles of the Declaration of Helsinki.

Histological and Immunohistochemical Staining

The ventricular tissue was fixed in formalin and embedded in paraffin using standard histological procedures. The tissue was cut to yield 5-μm-thick cross sections. The sections were subsequently stained with hematoxylin and eosin (HE) and Masson's trichrome staining to determine the extent of fibrosis.

Immunohistochemical examinations were performed on 5-μm-thick formalin-fixed and paraffin-embedded tissue sections. All steps were performed on a Leica Bond III automated system (Leica Microsystems) according to the manufacturer's instructions. In brief, specimens were deparaffinized and antigen was retrieved on the instrument. All slides were incubated with primary antibodies against CD68 (diluted 1:1000; Dako), CD209 (1:1000; BD Pharmingen), or CD11c (1:100; GeneTex) for 16 min, followed by incubation with a mouse-rabbit-horseradish peroxidase polymer and 3,3′-diaminobenzidine substrate. The sections were then incubated in primer (anti-rabbit and anti-mouse) for 8 minutes. Antibody binding was visualized using the avidin-biotin complex method according to the manufacturer's instructions (Vectastain ABC; Vector). The primary antibody was omitted from these protocols as a negative control. The sections were subsequently counterstained with HE.

Quantitative Analyses of Myocardial Inflammatory Cell Infiltration and Tissue Fibrosis

Stained inflammatory cells were counted in the infarcted area of each sample at a magnification of ×100 and in each of ten representative sections (0.1 mm^2), which were randomly chosen from infarct tissue without hemorrhagic change, using ImageJ software (version 1.38x; National Institutes of Health). For each sample, median cell numbers were calculated. For LV tissue fibrosis, percent area fraction (%AF) was measured using ImageJ software. These quantitative analyses were performed by two trained technicians without knowledge of patients' backgrounds.

Statistical Analysis

Continuous data were expressed as mean values±SD. The two groups were compared using the Wilcoxon rank sum test for continuous variables. Categorical variables were reported as frequencies with percentages and compared between the two groups using the Fisher's exact test. The correlation among the infiltration of CD68$^+$ macrophages, CD209$^+$ DC, CD11c$^+$ DC, and the extent of fibrosis were investigated by Pearson or Spearman correlation test. All statistical analyses were performed using the SPSS 13.0 for Windows (SPSS Inc). A P value of <0.05 was considered to be significant.

Results

Study Population

All patients were diagnosed with STEMI, and 50% of patients had anterior infarction. Emergent reperfusion therapy was performed in 8% of patients. Mean age was 68±9 years, and 63% of the patients were men. Baseline characteristics of the

Table. Baseline Characteristics of Patients

	Overall (n=24)	Non-Rupture (n=11)	Rupture (n=13)	*P* Value
Age, y	68±9	68±9	69±10	0.66
Male, n (%)	15 (63)	5 (45)	10 (77)	0.21
Smoking, n (%)	13 (56)	5 (45)	8 (62)	0.69
Hypertension, n (%)	20 (83)	10 (90)	10 (77)	0.60
Diabetes, n (%)	8 (33)	6 (55)	2 (15)	0.08
Dyslipidemia, n (%)	5 (21)	2 (18)	3 (23)	1.00
Prior MI, n (%)	5 (21)	4 (36)	1 (8)	0.14
Killip 3 or 4, n (%)	12 (50)	7 (64)	5 (38)	0.41
STEMI, n (%)	24 (100)	11 (100)	13 (100)	
Onset to death, h	167±148	188±164	148±135	0.44
Anterior infarction, %	12 (50)	6 (55)	6 (46)	1.00
Revascularization, %	2 (8)	1 (9)	1 (8)	1.00
White blood cells, $\times 10^3/\mu L$	13.2±5.7	16.5±6.1	10.5±3.6	0.01
Hemoglobin, g/dL	12.1±2.1	12.4±2.8	11.9±1.3	0.60
Peak CPK, $\times 10^3$ IU/L	4.3±4.3	5.7±5.3	2.7±1.5	0.18
Serum creatinine, mg/dL	1.9±2.0	2.6±2.7	1.3±0.6	0.06
Systolic BP, mm Hg	97±34	84±38	108±27	0.07
Diastolic BP, mm Hg	61±23	52±24	67±22	0.19
Heart rate, bpm	78±34	74±45	82±23	0.56

Continuous variables are presented as mean±SD. Categorical variables are presented as number (percentage). BP indicates blood pressure; bpm, beats per minute; CPK, creatine phosphokinase; MI, myocardial infarction; STEMI, ST elevation MI.

study patients including history of prior MI, time from onset to death, rate of reperfusion therapies, and traditional coronary risk factors, but not white blood cell count on admission, were similar in the rupture and non-rupture groups (Table). The time course from onset to death was not significantly different between the two groups (Figure 1).

Extent of Fibrosis and Infiltration of CD68$^+$ Macrophages and CD209$^+$ DC in Infarcted Myocardium

Staining with Masson's trichrome showed decreased %AF of fibrosis in patients with cardiac rupture compared to those without (Figure 2).

Immunohistochemical staining of the infarcted myocardium showed an increase in the number of infiltrating CD68$^+$ macrophages and a decrease in CD209$^+$ DC in patients with cardiac rupture compared with those without (Figure 2).

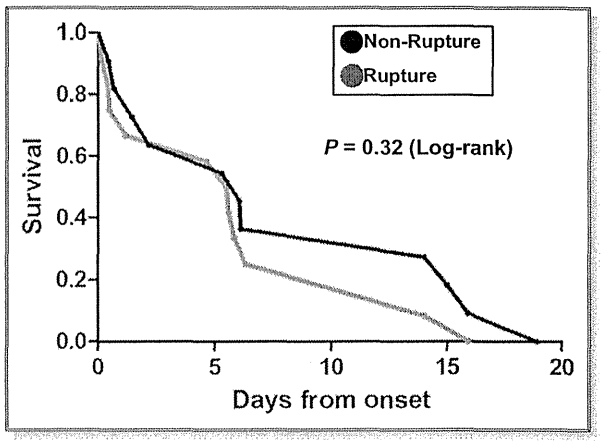

Figure 1. Kaplan-Meier survival analysis in non-rupture and rupture patients after myocardial infarction (MI).

Correlation Among CD68$^+$ macrophages, CD209$^+$ DC and Extent of Reparative Fibrosis

To reveal the possible relationship between inflammatory cell infiltration and the extent of reparative fibrosis, detailed correlation analysis was performed. No significant correlation was found between the number of CD68$^+$ macrophages and% AF of myocardial fibrosis in the infarcted area (R^2=0.01, P=0.39; Figure 3A). However, we found a significant positive correlation between the number of CD209$^+$ DC and %AF of myocardial fibrosis (R^2=0.69, P<0.0001; Figure 3B).

Extent of Fibrosis and Infiltration of CD11c$^+$ DC in Infarcted Myocardium, and Correlation Between CD11c$^+$ DC and Extent of Reparative Fibrosis

To confirm the relationship between DC infiltration and the extent of reparative fibrosis, the same analyses were performed using another DC marker, CD11c.

A decrease in the number of infiltrating CD11c$^+$ DC was observed in patients with cardiac rupture compared to those without (Figure 4A). In addition, we also found a significant positive correlation between the number of CD11c$^+$ DC and % AF of myocardial fibrosis (R^2=0.37, P=0.001; Figure 4B).

Discussion

In the present study, we demonstrated that DC infiltrated human infarcted myocardium after STEMI, and that the degree of infiltration and the extent of reparative fibrosis were significantly lower and the degree of macrophage infiltration was higher in patients with cardiac rupture compared with those without. Interestingly, there was a strong positive correlation between the number of infiltrating DC and the

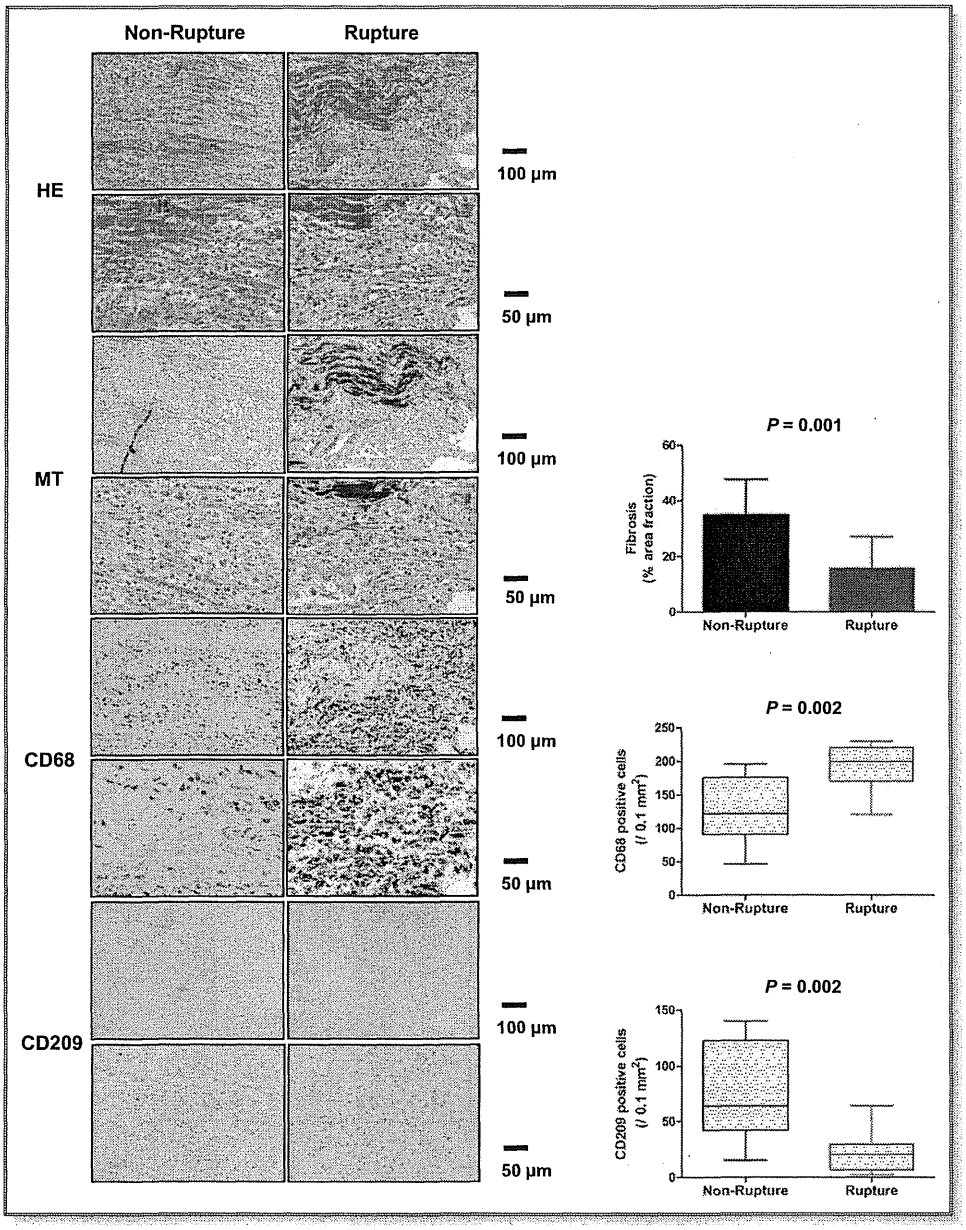

Figure 2. Infiltration of macrophages and DC, and extent of fibrosis in left ventricular infarcted tissue. HE staining, Masson's-Trichrome (MT) staining, immunohistochemical staining for $CD68^+$ macrophages and $CD209^+$ DC in left ventricular infarcted tissue of non-rupture and rupture patients after MI. DC indicates dendritic cells; HE, hematoxylin and eosin; MI, myocardial infarction.

extent of fibrosis in the infarcted myocardium. These findings suggest that DC may play a protective role against cardiac rupture through promotion of reparative fibrosis after MI.

Cardiac rupture is an acute life-threatening complication that occurs in the first several days after MI. Acute myocyte loss and breakdown of extracellular matrix (ECM) promote early ventricular expansion, which is a trigger for subacute cardiac rupture and worsening cardiac function after MI.[9,10] Replacement by collagen is important to provide mechanical stability to the injured tissue, and protects against increased LV dilatation.[11,12] In fact, agents that inhibit collagen synthesis were shown to be associated with an increase in the risk of cardiac rupture in MI patients.[13] These results suggested that appropriate reparative fibrosis is important to prevent cardiac rupture after MI. As well as collagen synthesis, inflammation is also a crucial factor in the

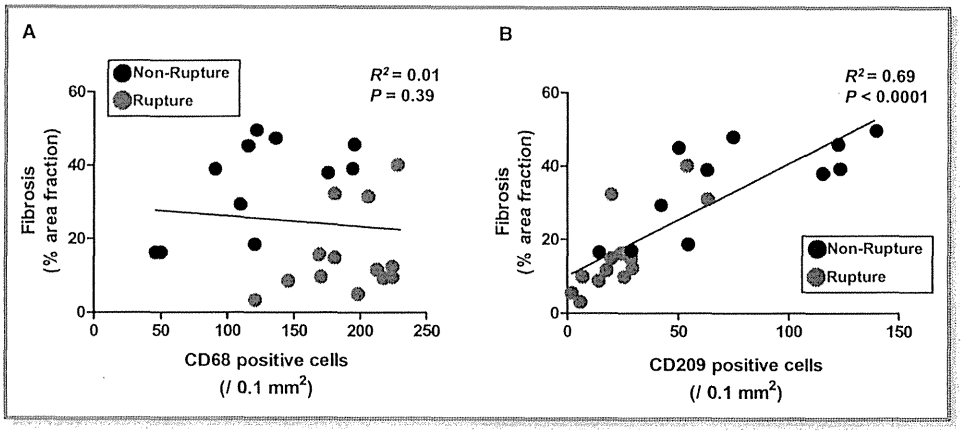

Figure 3. Correlation between extent of fibrosis and infiltration of macrophages and dendritic cells (DC) in left ventricular infarcted tissue. A, Correlation between extent of fibrosis and number of $CD68^+$ macrophages. B, Number of $CD209^+$ DC.

post-MI healing process. An excessive inflammatory response in the infarcted myocardium is related to adverse cardiac events including cardiac rupture;[3,4] however, anti-inflammatory corticosteroid therapy has been reported to increase the incidence of cardiac rupture by delaying collagen accumulation and scar formation, a far from favorable impact on the post-MI healing process.[14,15] These findings indicate that inflammation could be a prerequisite for an adequate post-MI healing process, although excessive inflammation is harmful. Our current study, based on Masson's trichrome staining, showed that the infarcted myocardium in patients with cardiac rupture consisted of disorganized collagen fibers, suggesting the presence of impaired reparative fibrosis. Briefly, impaired reparative fibrosis can be explained by two mechanisms. The first is the degradation of ECM by augmented MMPs secreted from inflammatory cells, predominantly inflammatory monocytes and M1 macrophages, infiltrating the infarcted heart.[7,16,17] Although this process is important for elimination of the necrotic tissue, excessive activation of MMPs could facilitate infarct expansion, resulting in cardiac rupture.[18–21] The second is disordered collagenogenesis by myofibroblasts differentiated from cardiac resident fibroblasts, which are regulated by pro-fibrotic cytokines, such as transforming growth factor beta (TGF-β) secreted mainly from anti-inflammatory monocytes and M2 macrophages during the repair process. M2 macrophages have been reported to promote differentiation of cardiac fibroblasts into myofibroblasts through production of TGF-β.[22] Therefore, adequate regulation of cellular employment is critical for the post-infarction repair process and prevention of cardiac rupture.

DC have come to be appreciated as potent critical controllers that modulate various kinds of inflammatory cells in innate and adoptive immunity.[23–25] Generally, bone-marrow and splenic progenitors and circulating monocytes are reported to differentiate into DC and exert various influences on the immune system at the inflammatory site, such as priming of antigen-specific immune responses, induction of tolerance, and chronic inflammation after tissue injury.[26–28] In the blood, both subtypes of DC are found as circulating DC precursors that lack the expression of costimulatory molecules, so that they are unable to activate other inflammatory cells. In the infarcted myocardium, it was reported that mDC became activated in response to danger signals such as heat shock protein, which is released from necrotic tissue after MI, through the activation of toll-like receptors (TLRs) signaling.[29]

Kretzchmar et al demonstrated a significant decrease in circulating mDC in patients with MI, and also showed significantly higher numbers of markers indicative of mDC and inflammatory cells such as macrophages in the infarcted compared with non-infarcted myocardium. This was accompanied by increased serum levels of an anti-inflammatory cytokine (IL-10) as well as inflammatory cytokines (IL-6, IL-12, and TNFα) in patients with MI.[30] Yilmaz et al also demonstrated that serum high sensitive CRP (hsCRP) and IL-6 levels were decreased, and mDC were partially reconstituted 1 week after the onset in patients with MI. They also showed the number of mDC precursors was negatively correlated with serum hsCRP or IL-6 level, while, in contrast, no significant correlation between pDC precursors and hsCRP or IL-6 was detected.[31] These results suggest that mDC could play important roles in regulating excessive inflammation in the post-MI healing process. Recently, potential mechanisms of suppression of excessive inflammation in a hepatic ischemia-reperfusion mouse model were reported. DC, which were activated by necrotic hepatocytes through TLR9 signaling, might restrict pro-inflammatory monocyte function via production of IL-10.[32] We previously observed mDC infiltrated

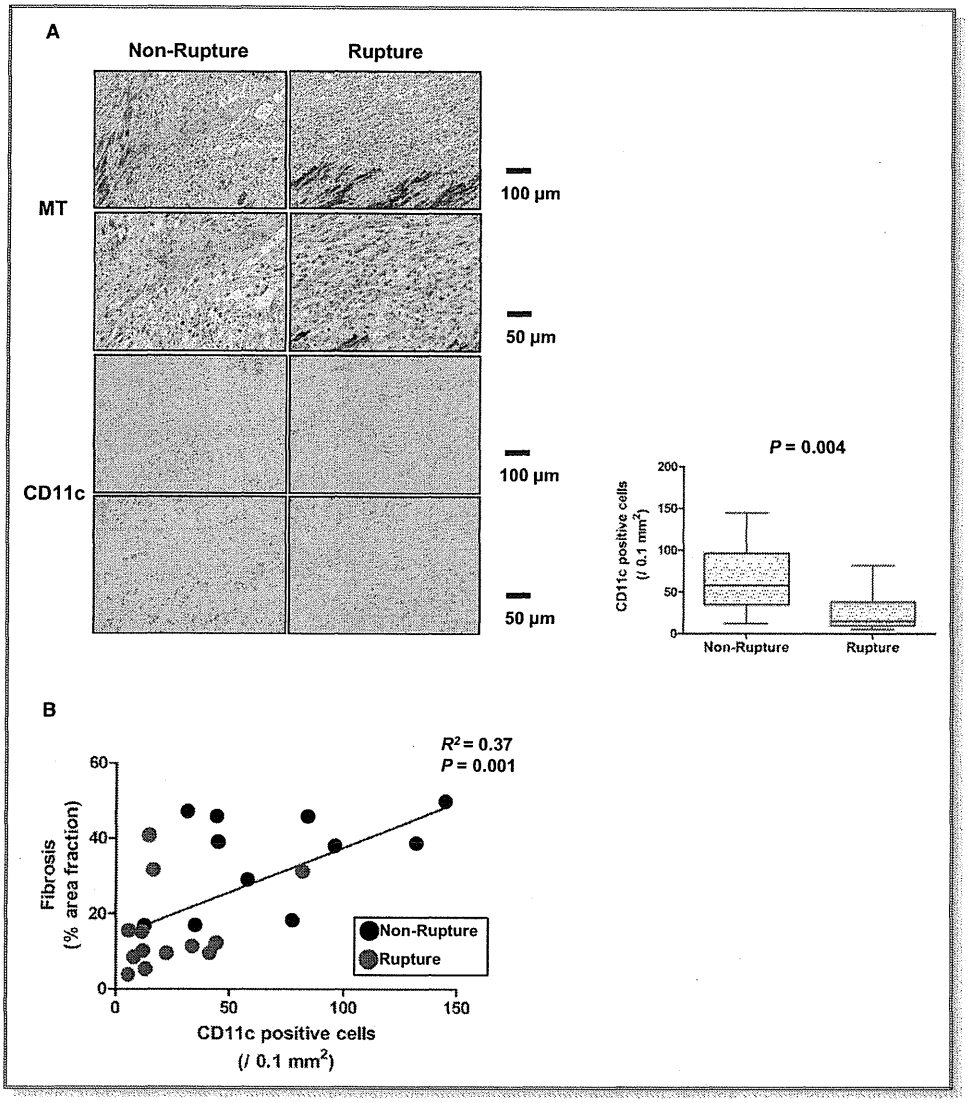

Figure 4. Infiltration of dendritic cells (DC) evaluated by another marker, and extent of fibrosis in left ventricular infarcted tissue. A, Masson's-Trichrome (MT) staining, immunohistochemical staining for $CD11c^+$ DC in left ventricular infarcted tissue. B, Correlation between extent of fibrosis and number of $CD11c^+$ DC.

into the infarcted and border areas, peaking 1 week after MI in a rat model,[6] and also found that depletion of DC resulted in enhanced inflammation and ECM degradation, through activation of pro-inflammatory monocytes and M1 macrophages, and impaired post-infarction healing process, through suppression of infiltration of anti-inflammatory monocytes and M2 macrophages and expression of anti-inflammatory cytokines such as IL-10, in a mouse model.[7] Notably, in the present study, the number of infiltrated CD68-positive macrophages was higher, the number of CD209 and CD11c-positive mDC was lower, and the extent of reparative fibrosis in the infarcted myocardium was less in patients with cardiac rupture compared with those without. Thus, also in human infarcted myocardium, DC may play a protective role against excessive inflammation and cardiac rupture by promoting the post-MI healing process.

Several limitations of this study warrant mention. First, the number of study patients was relatively small. The statistical power might thus not be adequate for any negative results. Second, there were insufficient clinical data regarding inflammatory biomarkers such as monocytes, CRP, and inflammatory cytokines. Third, although we used CD209 (DC-SIGN) in addition to CD11c for identifying DC infiltrated into infarcted myocardium, several potential markers other

than DC-SIGN have been reported for identifying DC. DC-SIGN is a type II transmembrane protein that belongs to a family of calcium-dependent lectins diversely used by human APC, such as tissue-residing mDC, alveolar and lymph node macrophages, and endothelial cells from liver sinusoids,[33–36] and was identified as a novel DC-specific adhesion receptor on human DC that is essential in several key functions throughout the life cycle of DC.[33] Therefore, it was assumed that CD209 was appropriate for identifying mDC infiltrated in human infarcted myocardium. Finally, there is potential for reverse causation and/or confounding factors such as white blood cell count that could also affect inflammatory response including DC recruitment. In addition, patients with cardiac rupture may have had histologic changes due to greater wall tension, such as less collateralization. Since we could not conclude that there was a causal relationship among DC, reparative fibrosis, and cardiac rupture, further study is warranted.

In conclusion, we identified DC infiltration in human infarcted myocardium, and observed a strong association between the number of DC and impaired reparative fibrosis and the development of cardiac rupture, suggesting a protective role of DC during the post-MI healing process.

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Disclosures

None.

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