Circulation

ORIGINAL RESEARCH ARTICLE

Myocardial and Systemic Inflammation in Acute Stress-Induced (Takotsubo) Cardiomyopathy

BACKGROUND: Acute stress-induced (takotsubo) cardiomyopathy can result in a heart failure phenotype with a prognosis comparable with that of myocardial infarction. In this study, we hypothesized that inflammation is central to the pathophysiology and natural history of takotsubo cardiomyopathy.

METHODS: In a multicenter study, we prospectively recruited 55 patients with takotsubo cardiomyopathy and 51 age-, sex-, and comorbidity-matched control subjects. During the index event and at the 5-month follow-up, patients with takotsubo cardiomyopathy underwent multiparametric cardiac magnetic resonance imaging, including ultrasmall superparamagnetic particles of iron oxide (USPIO) enhancement for detection of inflammatory macrophages in the myocardium. Blood monocyte subpopulations and serum cytokines were assessed as measures of systemic inflammation. Matched control subjects underwent investigation at a single time point.

RESULTS: Subjects were predominantly middle-aged (64±14 years) women (90%). Compared with control subjects, patients with takotsubo cardiomyopathy had greater USPIO enhancement (expressed as the difference between pre-USPIO and post-USPIO T2*) in both ballooning (14.3±0.6 milliseconds versus 10.5±0.9 milliseconds; $P<0.001$) and nonballooning (12.9±0.6 milliseconds versus 10.5±0.9 milliseconds; $P=0.02$) left ventricular myocardial segments. Serum interleukin-6 (23.1±4.5 pg/mL versus 6.5±5.8 pg/mL; $P<0.001$) and chemokine (C-X-C motif) ligand 1 (1903±168 pg/mL versus 1272±177 pg/mL; $P=0.01$) concentrations and classic $CD14^{++}CD16^{-}$ monocytes (90±0.5% versus 87±0.9%; $P=0.01$) were also increased whereas intermediate $CD14^{++}CD16^{+}$ (5.4±0.3% versus 6.9±0.6%; $P=0.01$) and nonclassic $CD14^{+}CD16^{++}$ (2.7±0.3% versus 4.2±0.5%; $P=0.006$) monocytes were reduced in patients with takotsubo cardiomyopathy. At 5 months, USPIO enhancement was no longer detectable in the left ventricular myocardium, although persistent elevations in serum interleukin-6 concentrations ($P=0.009$) and reductions in intermediate $CD14^{++}CD16^{+}$ monocytes (5.6±0.4% versus 6.9±0.6%; $P=0.01$) remained.

CONCLUSIONS: We demonstrate for the first time that takotsubo cardiomyopathy is characterized by a myocardial macrophage inflammatory infiltrate, changes in the distribution of monocyte subsets, and an increase in systemic proinflammatory cytokines. Many of these changes persisted for at least 5 months, suggesting a low-grade chronic inflammatory state.

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Clinical Perspective

What Is New?

- Patients with acute takotsubo cardiomyopathy have macrophage-mediated myocardial inflammation.
- Patients with acute takotsubo cardiomyopathy demonstrate modulation of peripheral monocyte subsets and increased systemic proinflammatory cytokines.
- Systemic inflammation persists for at least 5 months.

What Are the Clinical Implications?

- These findings further elucidate the mechanisms and pathogenesis of takotsubo cardiomyopathy.
- Systemic and myocardial inflammation may serve as a therapeutic target for patients with acute takotsubo cardiomyopathy.

Acute stress-induced (takotsubo) cardiomyopathy is a heart failure syndrome that has a presentation and mortality similar to that of acute myocardial infarction.[1–3] Patients with this syndrome, which is often triggered by a major stressful event, have unobstructed coronary arteries and characteristic ballooning of the left ventricle, with subsequent prompt restoration of normal or near-normal ejection fraction. However, we have recently shown that despite previous preconceptions, takotsubo cardiomyopathy results in a long-term heart failure phenotype with persistent symptoms and subclinical cardiac dysfunction.[4] We and others have also shown global severe edema of both the left and right ventricular myocardium that does not completely resolve by 4 months after the acute event despite spontaneous normalization of the ejection fraction.[5–8] Given the persistence of myocardial tissue edema and heart failure symptoms, we hypothesized that the pathophysiology of takotsubo cardiomyopathy may relate to prolonged activation of cellular and humoral inflammatory pathways. Our aim was to investigate whether there is evidence of acute localized macrophage-mediated inflammation within the myocardium (primary end point) with or without evidence of systemic inflammation by assessing monocyte subpopulations and serum cytokine concentrations. Furthermore, we wished to explore the time course and persistence of any of these potential proinflammatory pathways.

METHODS

The data, analytical methods, and study materials will be made available to other researchers for purposes of reproducing or replicating these findings.

Study Design

This was a multicenter, prospective, case–control, mechanistic investigation.

Study Population

Fifty-five patients with acute takotsubo cardiomyopathy were recruited from 5 Scottish cardiac centers (Aberdeen, Dundee, Edinburgh, Glasgow, and Inverness). All patients had invasive coronary angiography and left ventriculography at the time of the diagnosis, and they fulfilled the Mayo Clinic[9] and the European Society of Cardiology–Heart Failure Association diagnostic criteria for takotsubo cardiomyopathy.[10] Specifically, they had typical left ventricular ballooning (apical, midcavity, or basal), normal or near-normal coronary arteries without any evidence of obstructive or culprit coronary plaque, developed QTc prolongation 24 to 48 hours after presentation, and modest cardiac biomarker release. A stressful trigger was identified in the majority (examples shown in Table I in the online-only Data Supplement), and finally, the recovery of left ventricular ejection fraction to normal values seen at follow-up confirmed the initial takotsubo diagnosis. Exclusion criteria were any acute or chronic infectious diseases or other inflammatory conditions such as flu-like illness, upper or lower respiratory tract infection, gastroenteritis, urinary tract infection, any pyrexial illness or septic presentation, asthma, eczema, allergy, rheumatoid arthritis, systemic lupus erythematosus, Crohn disease, ulcerative colitis (list not exhaustive), any concurrent physical illness that in the judgment of investigators was a potential confounder to the hypothesis (eg, concurrent hypertrophic or noncompaction cardiomyopathy, moderate to severe left ventricular hypertrophy of any cause), known allergies or intolerance to intravenous iron compounds, and contraindications to magnetic resonance scanning. In particular, acute pericarditis and acute myocarditis were carefully excluded on both clinical and imaging grounds (presenting history, absence of stressful trigger, ECGs, distribution of wall motion abnormalities with no ballooning, and, when seen, presence of late gadolinium enhancement pattern suggestive of myocarditis). One patient died after hospital discharge, and an additional 6 did not wish to return for follow-up. Age-, sex-, and comorbidity-matched control subjects (n=51) from the University of Aberdeen volunteer database were invited to participate. To match the comorbidities of the participants with takotsubo precisely, control subjects were chosen to be healthy and on no medication, to have isolated hypertension on 1 antihypertensive medication only, or to have diabetes mellitus (diet or metformin controlled).

Study Protocol

The study was approved by the Institutional Review Board and Research Ethics Committee, and all subjects gave written informed consent. Patients underwent prompt assessment (within 14 days) after the onset of takotsubo cardiomyopathy, which was repeated 5 months after the index event. Study assessments included blood sampling, 2-dimensional and Doppler echocardiography, and multiparametric cardiac magnetic resonance (CMR). The last also included cardiac ^{31}P-spectroscopy, late gadolinium enhancement, and repeated scanning exactly 24 hours after intravenous infusion of ultrasmall superparamagnetic particles of iron oxide (USPIO; ferumoxytol, AMAG

Pharmaceuticals, Waltham, MA), as described previously for tracking phagocytic macrophages,[11] including those in the myocardium of patients with acute myocardial infarction.[11–13] At the 5-month follow-up visit, all patients underwent repeat study assessments and an assessment of symptom burden, including New York Heart Association assessment and the Minnesota Living With Heart Failure Questionnaire.

Blood sampling was performed during the acute phase (days 0–13 from acute onset) and at the 5-month follow-up.

Cardiovascular Biomarkers and Inflammatory Cytokines

Blood was clotted, and serum was separated by centrifugation at 50*g* for 10 minutes and stored at –80°C until cytokine analysis. Brain natriuretic peptide concentrations were determined with an immunoassay (Alere Triage MeterPro, Waltham, MA). High-sensitivity troponin I (ARCHITECT$_{STAT}$, Abbott Laboratories, Abbott Park, IL) was obtained at follow-up, in addition to the routine 12-hour clinical troponin from admission. Clinical hematology and biochemistry were performed as part of clinical care. Quantification of serum cytokine concentrations—chemokine (C-X-C motif) ligand (CXCL) 1 or growth-regulated protein, tumor necrosis factor-α, interferon-γ, monocyte chemoattractant protein 1, and the interleukins (IL; IL-1β, IL-6, IL-8 [CXCL8], IL-10, IL-12p40)—was performed with a bespoke commercially available human multiplex cytokine kit (MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel, catalogue No. HCYTOMAG-60K-09, Merck Millipore, Darmstadt, Germany).

Monocyte Phenotyping

Percentages of monocyte subsets were measured from venous blood with a BD LSR Fortessa flow cytometer and analyzed with FlowJo version 10. Anti-human antibodies CD45 V450 (clone HI30), CD14 PE-CF594 (clone MφP9), CD16 PE-Cy7 (clone 3G8), and HLA-DR FITC (clone G46-6) were mixed with 100 μL fresh EDTA-anticoagulated blood. After 20 minutes of incubation in the dark, red blood cells were lysed and fixed with FACSlyse (BD) for 20 minutes, followed by dilution in 2 mL PBS solution. After a final washing step, cells were resuspended in 0.5 mL PBS and subjected to immediate flow cytometric analysis. To identify monocytes, first forward scatter and side scatter were used to identify cells from debris. Then cells were visualized in a side scatter/CD45 plot to gate on a monocyte population. CD45$^+$/HLA-DR–positive cells were gated to exclude any CD16$^+$ natural killer cells and other non–major histocompatibility complex–expressing cells. Unstained, fluorescence minus one, and internal controls were used for setting the boundaries of the gates. Monocyte subpopulations were identified from a CD14 versus CD16 bivariate plot following the criteria defined previously[14] as 3 monocyte subpopulations: CD14^{++}CD16$^-$ (classic, proinflammatory), CD14^{++}CD16$^+$ (intermediate), and CD14$^+$CD16^{++} (nonclassic).

Ex Vivo Monocyte-Macrophage Differentiation

CD14$^+$ peripheral blood monocytes were isolated from whole blood from 5 female patients with acute takotsubo cardiomyopathy (on presentation) and 5 female healthy control subjects, and each was incubated in their autologous serum for 7 days to induce differentiation into macrophages. USPIO (ferumoxytol) was added to the macrophages for 24 hours with final concentrations of 0 (control), 40, and 80 μg/mL. Total iron in cell lysate was quantified colorimetrically with a validated 2,2´-bipyridine assay measuring absorbance at 520 nm. The mean iron of triplicate wells for each USPIO concentration was expressed as nanograms per 1 μg protein.

Transthoracic Echocardiography

Echocardiography was performed with Vivid E9 systems equipped with 2.5 MHz (M5S) transducers (GE Vingmed, Horten, Norway) and analyzed by a single experienced British Society of Echocardiography–accredited sonographer. Three cardiac cycles in each of the standard parasternal long-axis, short-axis, and apical 4-, 3-, and 2-chamber views were obtained at end expiratory breath hold at a frame rate of at least 85 Hz and stored for offline analysis. Any subject with left bundle-branch block on ECG was excluded from the strain and deformation analysis. Image analysis was performed with EchoPAC software (version 1.13, GE Healthcare), as previously described, measuring left ventricular longitudinal, radial, and circumferential strain and deformation indexes.[15]

^{31}P CMR Spectroscopy and CMR Imaging

All participants were scanned on either 3T Philips Achieva TX (Aberdeen) or 3T Siemens Verio (Edinburgh): 48 patients were scanned in Aberdeen and 7 in Edinburgh; all matched control subjects were scanned in Aberdeen. All sequences were validated in Aberdeen and an effort has been made to ensure similar parameters between scanners. ^{31}P CMR spectroscopy (^{31}P-CMRS) was acquired with a 14-cm-diameter transmit-and-receive ^{31}P surface coil as described previously[5] (only patients scanned in Aberdeen underwent ^{31}P-CMRS, n=48). A non–water-suppressed ^{1}H point–resolved spectroscopy acquisition was used to monitor resonance frequency determination and B_0 shimming over the ^{31}P-CMRS volume of interest, which was positioned to cover the entire interventricular septum. The ^{31}P-CMRS acquisition was an ECG-gated image selected in vivo spectroscopy sequence, triggered to mid to late diastole, with a repetition time of at least 10 seconds.

A 6-channel cardiac coil (Philips) or a 32-channel cardiac array coil (Siemens) was used to acquire cine imaging, whole left ventricle precontrast T1 mapping (5 seconds [3 seconds] 3 seconds scheme), whole left ventricle multiecho gradient echo T2* sequence (echo times, 1.15, 2.15, 3.15, 4.15, 5.15, 6.15, 7.15, 8.15, 9.15, and 10.15 milliseconds), early and late gadolinium enhancement (Gadovist, 0.1 mmol/kg) with swap of the phase-encoding direction, and exactly 24-hour post-USPIO acquisition of whole left ventricle multiecho gradient echo T2*. The USPIO (ferumoxytol 4 mg/kg in 50 mL of 0.9% saline) was administered as an intravenous infusion over 30 minutes after baseline CMR. All left ventricular imaging was performed with a slice thickness of 10 mm.

Image Analysis

^{31}P-CMRS data were analyzed in JMRUi3.0 as described previously.[5] The phosphocreatine/γ-adenosine triphosphate

(γATP) ratio (which is the gold standard for in vivo assessment of myocardial energetic status[16]) was determined after the γATP was corrected for blood contamination, and phosphocreatine/γATP ratios were saturation corrected as described previously.[17–19] To ensure that spectra were of good quality, Cramér-Rao SDs of all peaks were calculated, and only those <20% were accepted. T2* and T1 maps were analyzed in 16 segments of the 17-segment model[20] (omitting the true apex) with CMR Tools (Cardiovascular Imaging Solutions, London, UK) and Segment (Medviso, Lund, Sweden), respectively. T2* values were generated for each of the 16 segments from native images before and 24 hours after ferumoxytol. The postferumoxytol values were subtracted from the native values in each segment to derive the change in T2* as a measure of ferumoxytol uptake by tissue-resident macrophages.[11] Left ventricular volumes, mass, and ejection fraction were calculated in CMR Tools. Each segment of the heart was given a wall motion score (1=normal, 2=hypokinesia, 3=akinesia, and 4=dyskinesia); any segment with a score of >1 was assigned as ballooning, and any segment with a score of 1 was assigned as nonballooning. Imaging data are reported grouped by wall motion (ballooning and nonballooning), by left ventricular region (apex, midcavity or base), and for the whole left ventricle.

Our interobserver variabilities for strain echocardiography, ^{31}P-CMRS, and CMR have been reported previously and ranged between 3 to 6±1% to 2% for all strain echocardiography parameters, 1.5 to 2.7±0.5% to 1.5% for CMR inclusive of T1 mapping, and 5±2% for phosphocreatine/γATP ratio.[5,6,15] Interobserver variability for T2* measurements was 5.4±3%.

Statistical Analysis

The main study outcome was myocardial inflammation assessed by the change in T2* from native to post-USPIO images, and the secondary outcome was the presence of systemic inflammation assessed from changes in monocyte subpopulations and serum cytokine concentrations. Data were analyzed by a mixed model with random effects for patient and fixed effects for subject group, with age and sex as covariates, followed by post hoc comparisons of subject groups or time intervals. *P* values for comparisons were calculated with *t* tests with degrees of freedom estimated by the Satterthwaite method. Tabulated data are shown as mean±SEM or median (range). Statistical significance was set at $P<0.05$.

RESULTS

Fifty-five patients presenting with acute takotsubo cardiomyopathy were recruited and assessed at baseline, and 48 were restudied at a mean of 148±7 days after their index event. They were predominantly middle-aged or elderly (median age, 64 years; range, 28–83 years) women (n=50, 91%). Their characteristics are summarized in Table 1. Fifty-one control subjects were well matched with a comparable age (median, 63 years; range, 38–85 years), sex (46 women, 90%), and comorbidity distribution.

Myocardial Inflammation Assessed With USPIO-Enhanced CMR

There was a higher change in T2* values in both the ballooning and the nonballooning segments of patients with acute takotsubo cardiomyopathy compared with control subjects ($P=0.002$ and $P=0.02$, respectively; Table 2 and Figure 1), indicating an increase in myocardial macrophages. Results were similar when analyzed by left ventricular region, with the apex and midcavity demonstrating changes compared with control subjects ($P<0.01$ for both). After 5 months, the post-USPIO change in T2* was comparable to that seen in control subjects both in the ballooning and in the nonballooning segments. The native and post-USPIO T2* values in patients with takotsubo cardiomyopathy and matched control subjects are shown in Table II and Figure I in the online-only Data Supplement.

Myocardial Edema Assessed With Native T1 Mapping

In the scans performed during the acute phase, native T1 values were higher in patients with takotsubo cardiomyopathy in both ballooning and nonballooning segments ($P\leq0.0001$ for both; Table 2). At the 5-month follow-up, T1 values were no longer different from control subjects in the ballooning ($P=0.07$) or nonballooning ($P=0.06$) segments.

Myocardial Energetics Assessed With ^{31}P-CMRS

Resting cardiac energetic status (phosphocreatine/γATP ratio) was markedly reduced in patients with acute takotsubo cardiomyopathy compared with control subjects ($P\leq0.001$), and this showed only partial recovery at follow-up ($P=0.002$; Table 2).

Systemic Inflammatory Cells and Monocyte Subpopulation Phenotyping

Patients with acute takotsubo cardiomyopathy had a higher total white cell and neutrophil count at presentation compared with control subjects ($P<0.001$; Table 1). Although there was no difference in the total monocyte count during the acute phase, patients with takotsubo cardiomyopathy had a higher percentage of classic $CD14^{++}CD16^{-}$–expressing monocytes ($P=0.01$), a lower percentage of intermediate $CD14^{++}CD16^{+}$–expressing monocytes ($P=0.01$), and a lower percentage of nonclassic $CD14^{+}CD16^{++}$–expressing monocytes ($P=0.006$) compared with control subjects (Table 3). When these acute posttakotsubo monocyte subpopulation responses were grouped by days 0 to 3, 4 to 7, and 8 to 12 after the acute event, it became evident

Table 1. Characteristics of the Study Population

	Patients With Takotsubo Cardiomyopathy (n=55)	Control Subjects (n=51)	*P* Value
Female, n (%)	50 (91)	46 (90)	0.93
Age, median (range), y	64 (28–83)	64 (38–85)	0.94
BMI, kg/m^2	26±0.81	26±0.51	0.71
Medical history, n (%)			
Hypertension	15 (27)	13 (25)	0.79
Diabetes mellitus	7 (12)	5 (10)	0.38
Psychiatric disease	11 (20)	0	
Depression	7 (13)	0	
Anxiety	4 (7)	0	
Paroxysmal atrial fibrillation	5 (9)	0	
Presenting symptom, n (%)			
Chest pain	46 (84)	…	
Breathlessness	3 (5)	…	
Syncope	0	…	
Other	6 (11)	…	
LV ballooning type, n (%)			
Apical	48 (87)	…	
Midcavity	4 (7)	…	
Basal	3 (6)		
Presenting ECG, n (%)			
ST-segment elevation	23 (42)	…	
Non–ST-segment elevation	24 (44)	…	
LBBB	2 (4)	…	
Other	6 (10)		
Stressor, n (%)			
Physical	18 (33)	…	
Emotional	27 (49)	…	
None	10 (18)	…	
Blood levels at presentation (upper limit of reference range)			
Troponin I, ng/L (40)	4393±742	…	
BNP, pg/mL (100)	297±65	32.7±4.6	0.002
WCC, ×10^9/L (10)	10.4±0.33	5.6±0.30	<0.001
Neutrophils, ×10^9/L (7)	7.9±0.35	3.1±0.20	<0.001
Eosinophils, ×10^9/L (0.5)	0.15±0.14	0.19±0.09	0.46
Basophils, ×10^9/ L (0.1)	0.04±0.02	0.04±0.02	0.94
Lymphocytes, ×10^9/ L (4)	1.72±0.89	1.74±0.38	0.93
Monocytes, ×10^9/L (0.8)	0.49±0.21	0.45±0.21	0.38
MLWHFQ score at follow-up, median (range)			
Total score (of 105)	5 (0–60)	…	
Physical domain (of 40)	5 (0–30)	…	
Emotional domain (of 25)	0 (0–17)	…	
BNP at follow-up, pg/mL (upper limit of reference range, 35)	77.9±45	32.7±4.6	0.003

Data shown are as mean±SEM unless otherwise stated. BMI indicates body max index; BNP, brain natriuretic peptide; LBBB, left bundle-branch block; LV, left ventricular; MLWHFQ, Minnesota Living With Heart Failure Questionnaire; and WCC, white cell count.

that these changes were most pronounced on days 0 to 3 after presentation (Table 3 and Figure 2). The greatest percentage increase in classic (CD14^{++}CD16$^-$) and greatest percentage decrease in intermediate (CD14^{++}CD16$^+$) and nonclassic (CD14$^+$CD16^{++}) were found in this (days 0–3) time bracket. The percentages of classic (CD14^{++}CD16$^-$) and nonclassic (CD14$^+$CD16^{++}) subpopulations became comparable to that of control subjects after 5 months, whereas the intermediate subset (CD14^{++}CD16$^+$) remained suppressed (*P*=0.01). Ex vivo culture of monocyte-derived macrophages demonstrated no functional difference in the dose-dependent USPIO uptake in cells sampled from patients with acute takotsubo cardiomyopathy compared with those sampled from healthy control subjects (Figure II in the online-only Data Supplement).

Serum Cytokine Profiles

Patients with acute takotsubo cardiomyopathy had higher serum concentrations of IL-6 and CXCL1 (growth-regulated protein) chemokine compared with control subjects (*P*<0.001 and *P*=0.01 respectively; Table 4). Although the concentrations of IL-6 fell at follow-up compared with the initial presentation, they remained elevated compared with those of control subjects (*P*=0.009). Apparent early increases in serum IL-8 concentrations in patients with takotsubo cardiomyopathy (*P*=0.07) became more pronounced by 5 months of follow-up (*P*=0.009).

Standard CMR Imaging and Echocardiography

Consistent with previously reported findings,[7,15,21] patients with takotsubo cardiomyopathy had alterations in left ventricular mass, ejection fraction, and deformation analyses (Table 2). There were no significant correlations between changes from acute to follow-up in any of the functional, structural, or metabolic CMR parameters and either of the measured serum cytokines.

Symptoms and High-Sensitivity Troponin at the 5-Month Follow-Up

At the 5-month interview, 42% of patients reported ongoing symptoms. Of these, the majority (70%) of patients were in New York Heart Association class I, 23% were in class II, and 7% were in class III. Quality of life assessed with Minnesota Living With Heart Failure Questionnaire showed a median score of 5 (range, 0–60 of a maximum of 105) with a median physical domain score of 5 (range, 0–30 of a maximum of 40) and a median emotional domain score of 0 (range, 0–17 of a maximum of 25). The high sensitivity troponin at follow-up was 6.47±0.6 ng/L.

Table 2. MRI and Echocardiography Findings

	Patients With Takotsubo Cardiomyopathy (Acute) (n=550)	Patients With Takotsubo Cardiomyopathy (5 mo) (n=48)	Matched Control Subjects (n=51)	*P* Value, Acute Versus Control	*P* Value, 5 mo Versus Control	*P* Value, Acute Versus 5 mo
Change in T2* after USPIO, ms						
Ballooning segments	14.3±0.65	11.9±0.84	10.5±0.98	0.002	0.28	0.02
Nonballooning segments	12.9±0.54	11.6±0.65	10.6±0.83	0.02	0.32	0.08
Whole left ventricle	13.3±0.44	11.4±0.54	10.9±0.73	0.006	0.62	0.002
Basal	12.1±0.59	11.3±0.73	10.9±0.94	0.33	0.73	0.39
Midcavity	13.8±0.50	11.3±0.62	11.2±0.83	0.009	0.94	0.001
Apical	14.4±0.71	11.7±0.88	10.4±1.14	0.004	0.38	0.01
Native T1, ms						
Ballooning segments	1417±11.82	1257±18.24	1215±17.44	<0.0001	0.07	<0.0001
Nonballooning segments	1329±8.03	1245±10.72	1213±12.07	<0.0001	0.06	<0.0001
Whole left ventricle	1358±9.88	1245±13.41	1204±14.98	<0.0001	0.03	<0.0001
Basal	1311±8.70	1243±12.26	1237±13.34	<0.0001	0.68	<0.0001
Midcavity	1358±9.71	1246±14.79	1214±16.24	<0.0001	0.14	<0.0001
Apical	1398±14.04	1252±18.79	1194±20.43	<0.0001	0.06	<0.0001
PCr/γATP†	1.25±0.10	1.4±0.12	1.9±0.11	<0.001	0.002	0.43
LVEDV index, mL/m^2	73±1.99	71±2.56	72±2.72	0.72	0.63	0.34
LVESV index, mL/m^2	30±1.43	24±1.88	26±1.94	0.13	0.53	0.001
LV mass index, g/m^2	77±1.73	63±2.35	64±2.30	<0.001	0.68	<0.001
LV EF, %	59±1.23	67±1.72	64±1.56	0.01	0.22	<0.001
Echocardiography						
EF, %	54±1.52	64±2.08	64±2.02	<0.001	0.88	<0.001
Estimated RVSP, mm Hg	29±2.44	31±2.91	27±0.98	0.32	0.09	0.54
Global longitudinal strain, %	−12.4±0.51	−18.8±0.72	−19.7±0.78	<0.001	0.48	<0.001
Apical circumferential strain, %	−13.0±0.84	−19.4±1.12	−23.4±1.04	<0.001	0.01	<0.001
LV twist, °	11.9±1.12	13.4±1.64	23.3±1.49	<0.001	<0.001	0.48
LV twist rate, °/s	82.1±5.14	95.0±6.69	114.7±6.22	<0.001	0.03	0.14
LV untwist rate, °/s	−60±7.89	−91±11.21	−112±10.04	<0.01	0.23	0.02

P values for comparisons were calculated with *t* tests, with degrees of freedom estimated by the Satterhwaite method (37–65 *df* for change in T2*).EF indicates ejection fraction; LV, left ventricle; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; MRI, magnetic resonance imaging; PCr/γATP, phosphocreatine/γ-adenosine triphosphate; RVSP, right ventricular systolic pressure; and USPIO, ultrasmall superparamagnetic particles of iron oxide.

† PCr/γATP performed only in patients scanned in Aberdeen, n=48.

DISCUSSION

This is the first prospective evaluation of myocardial and systemic inflammation in acute and 5-month convalescent takotsubo cardiomyopathy. Using USPIO-enhanced magnetic resonance imaging, we demonstrate a macrophage-mediated cellular inflammatory response in the myocardium, superimposed on myocardial edema. Furthermore, we show systemic peripheral inflammatory responses, some of which appear to persist for at least 5 months. Taken together, our data demonstrate both localized and systemic inflammatory responses and uncover a previously unknown mechanistic pathway of takotsubo pathophysiology. These findings provide a potential explanation for the development of the long-term heart failure phenotype and poorer long-term prognosis, as well as suggesting that the acute inflammatory response could be a promising therapeutic target in this condition for which no effective treatment currently exists.

Our study has a number of important strengths. First, we conducted a multicenter study including patients with a clear and rigorously defined diagnosis of takotsubo cardiomyopathy (excluding any possibility of myocardial infarction or myocarditis), ensuring that our findings are robust and generalizable. Second, we had a relatively large sample size and used a control population that was matched not only for age and sex but also for comorbidities found in the study patients. Third, we undertook highly detailed and objective assessments of both myocardial and systemic inflammation using state-of-the-art cardiac imaging, including 24-hour post–USPIO-en-

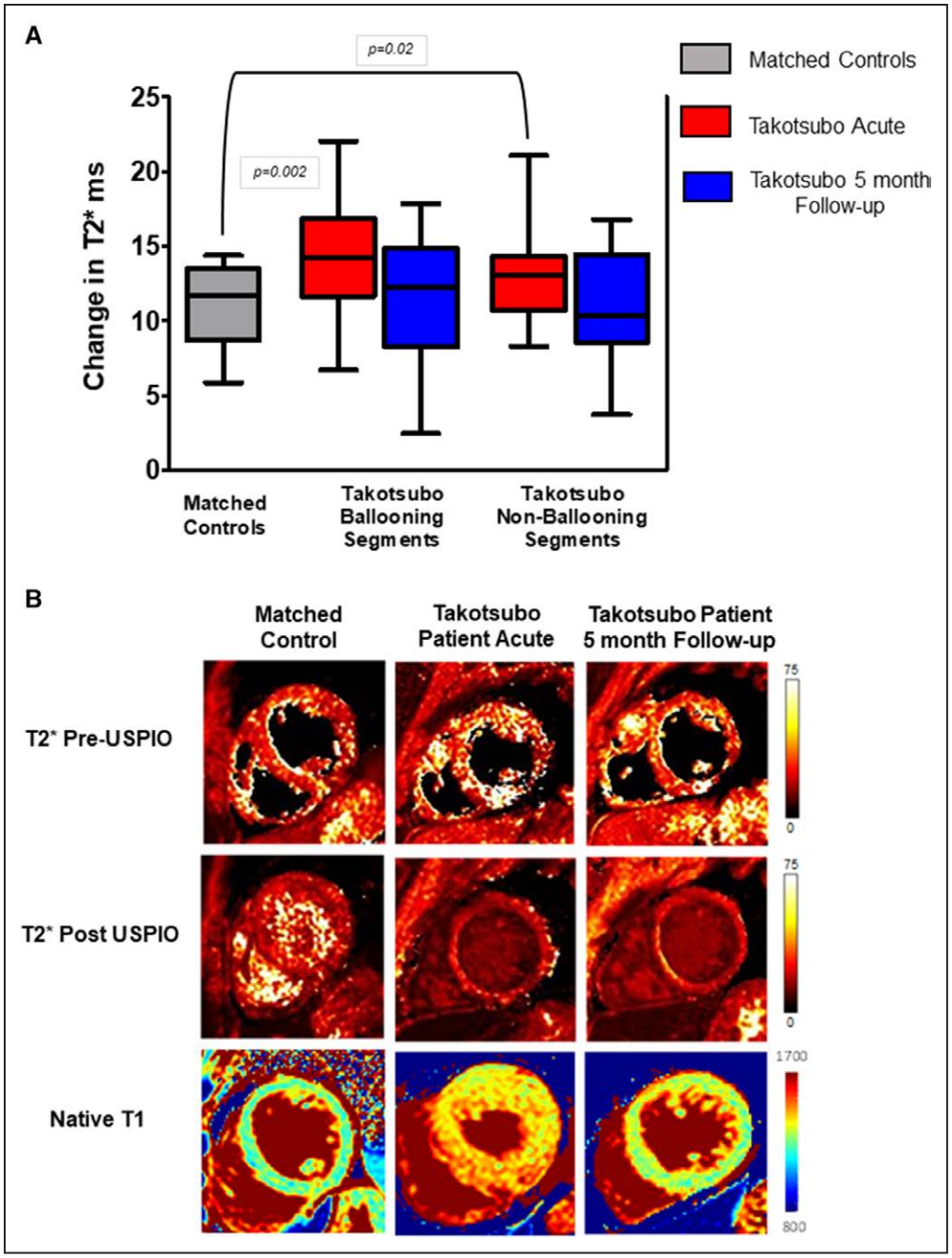

Figure 1. Ultrasmall superparamagnetic iron oxide particles (USPIO) uptake into the myocardium in ballooning and nonballooning segments vs matched control subjects as shown by change in T2*.
A, At acute presentation and at follow-up. Data are shown as median, 25th and 75th percentiles, and maximum and minimum (whiskers). **B**, Example of T2* maps before and after USPIO administration and native T1 maps in a control subject compared with a patient with takotsubo cardiomyopathy at presentation and at follow-up.

hanced magnetic resonance imaging. This enabled us to assess tissue, cellular, and humoral inflammation, including myocardial tissue-resident macrophages.

Study Rationale

The swift recovery of the left ventricular ejection fraction after an acute episode of takotsubo cardiomyopathy has misled clinicians to affirm that takotsubo is a rapidly resolving and self-limiting condition. In contrast to this assumption, 2 large registries reported that patients with takotsubo cardiomyopathy have a long-term prognosis comparable to that of patients with myocardial infarction.[3,22] To provide a mechanistic explanation for these registry data, we have recently shown that patients who with a prior episode of takotsubo cardiomyopathy develop a long-term heart failure phenotype.[4] This, therefore, begs the question: What are the mechanistic processes that account for this evolution toward heart failure?

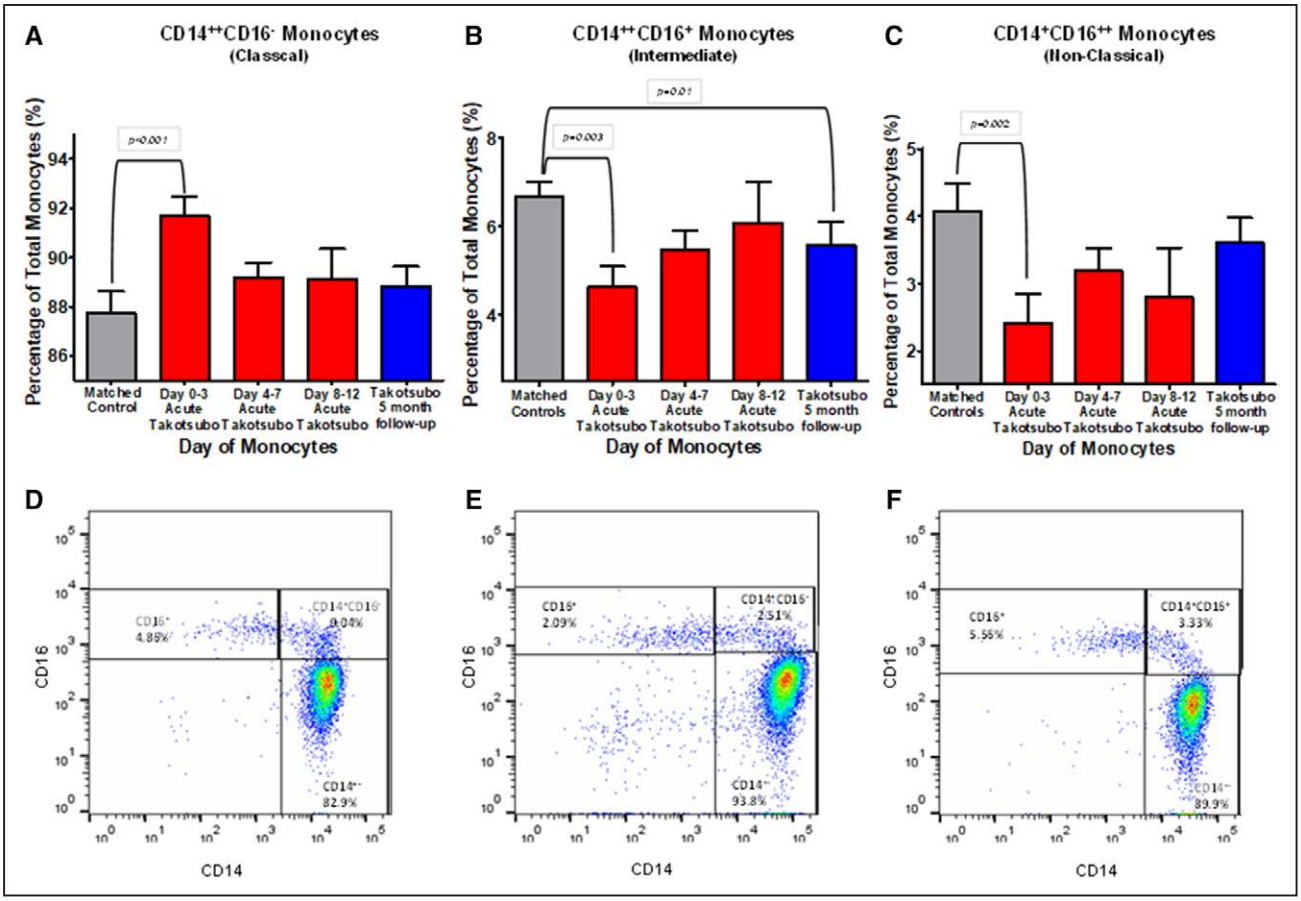

Figure 2. Circulating blood monocytes.
Top, dynamic of each monocyte subpopulation in patients with takotsubo myopathy compared with matched control subjects. The $CD14^{++}CD16^{-}$ (classic, proinflammatory), $CD14^{++}CD16^{+}$ (intermediate), and $CD14^{+}CD16^{++}$ (nonclassic) monocyte subpopulations analyzed at specific time points after acute presentation in patients with takotsubo myopathy compared with matched control subjects (**A** through **C**). Data are shown as mean±SEM. **Bottom**, Representative examples of CD14/CD16 bivariate plots in (**D**) matched control, (**E**) acute-phase takotsubo (sampled on day 2), and (**F**) takotsubo at the 5-month follow-up.

Myocardial Edema, Inflammation, and Energetic Impairment

Previous reports, including our own work, have demonstrated that there is an unprecedented degree of myocardial edema in the myocardium of patients with acute takotsubo cardiomyopathy. Our current larger cohort confirms these findings of pan–left ventricular edema (high native T1 values).[5,23] However, the substrate for this widespread myocardial edema is so far unexplained. In the present study, we have gone on to show, for the first time, that USPIO-enhanced magnetic resonance imaging suggests a macrophage-driven cellular infiltration within the myocardium. As we and others have shown, the main cells capable of phagocytosing USPIO that accumulate in the infarcted myocardium are monocyte-derived macrophages.[12,13,24,25] Indeed, biopsies from patients with acute takotsubo cardiomyopathy have demonstrated the presence of macrophages, albeit from the right ventricular myocardium.[26] We have recently reported postmortem cases of takotsubo cardiomyopathy in which we observed clusters of macrophages ($CD68^{+}$ staining) in the left ventricular myocardium of patients who died within 5 days of acute presentation; these were predominantly M1 macrophages, supporting the proinflammatory findings in the present study.[27] It is therefore most likely that the cellular protagonists responsible for the organ-specific inflammatory response observed herein (USPIO uptake in the myocardium) are macrophages. This is in contrast to other types of acute heart failure presentations such as acute myocarditis, for which our group has previously shown that USPIO-enhanced magnetic resonance imaging was not able to detect a myocardial macrophage infiltrate because the inflammatory cells involved in acute myocarditis are predominantly lymphocytes.[28] Moreover, our ex vivo macrophage culture data suggest that the increase in USPIO uptake in the myocardium of patients with takotsubo cardiomyopathy was not attributable to an increased efficiency of USPIO uptake but to a large increase in tissue-resident myocardial macrophages. This is also in keeping with the most accepted pathophysiological trigger of a catecholamine surge[29]; catecholamines themselves can induce regional myocardial inflammation,[30] possibly enhanced in a susceptible population of women (who have a higher catecholamine sensitivity).[31]

Table 3. Monocyte Profile in Patients With Takotsubo Cardiomyopathy and Matched Control Subjects

Subpopulations of Monocytes	Patients With Takotsubo Cardiomyopathy (Acute) (n=55)	Patients With Takotsubo Cardiomyopathy (5 mo) (n=48)	Matched Control Subjects (n=51)	*P* Value, Acute Versus Control	*P* Value, 5 mo Versus Control	*P* Value, Acute Versus 5 mo
All days, %						
$CD14^{++}CD16^{-}$	90.0±0.54	88.8±0.64	87.1±0.94	0.01	0.20	0.09
$CD14^{++}16^{+}$	5.4±0.34	5.5±0.41	6.9±0.61	0.01	0.01	0.78
$CD14^{+}CD16^{++}$	2.7±0.26	3.6±0.33	4.2±0.48	0.006	0.34	0.03
Days 0–3, %						
$CD14^{++}CD16^{-}$	91.7±0.93	88.8±0.64	87.1±0.94	0.001	0.20	0.008
$CD14^{++}16^{+}$	4.7±0.58	5.5±0.41	6.9±0.61	0.003	0.01	0.21
$CD14^{+}CD16^{++}$	2.4±0.47	3.6±0.33	4.2±0.48	0.002	0.34	0.01
Days 4–7						
$CD14^{++}CD16^{-}$	88.7±0.89	88.8±0.64	87.1±0.94	0.14	0.20	0.72
$CD14^{++}16^{+}$	5.6±0.56	5.5±0.41	6.9±0.61	0.12	0.01	0.48
$CD14^{+}CD16^{++}$	3.0±0.44	3.6±0.33	4.2±0.48	0.12	0.34	0.58
Days 8–12						
$CD14^{++}CD16^{-}$	89.3±1.19	88.8±0.64	87.1±0.94	0.17	0.20	0.73
$CD14^{++}16^{+}$	6.0±0.74	5.5±0.41	6.9±0.61	0.36	0.01	0.41
$CD14^{+}CD16^{++}$	2.8±0.59	3.6±0.33	4.2±0.48	0.03	0.34	0.22

Data are shown as mean±SEM. *P* values for comparisons were calculated with *t* tests with degrees of freedom estimated by the Satterhwaite method (64–87 *df* for $CD14^{++}CD16^{-}$, 60–87 *df* for $CD14^{++}16^{+}$, and 65–87 *df* for $CD14^{+}CD16^{++}$).

Finally, we recapitulated the energetic impairment previously reported in a smaller cohort and its incomplete recovery during follow-up. Whether the inflammatory output and the energetic impairment are causally linked remains to be established.

Monocyte Subpopulations Behavior in Takotsubo Cardiomyopathy

Here, we describe for the first time that patients with takotsubo cardiomyopathy exhibit a substantial increase in the proinflammatory, classic monocyte subset ($CD14^{++}CD16^{-}$) at the expense of a decrease in the other 2 subpopulations: $CD14^{++}CD16^{+}$ (intermediate) and $CD14^{+}CD16^{++}$ (nonclassic, patrolling, and reparative). It is now recognized that the $CD14^{++}CD16^{-}$ (classic) monocytes mature through a continuum to $CD14^{++}CD16^{+}$ (intermediate) and then $CD14^{+}CD16^{++}$ (nonclassic).[32] We propose that the increased percentage of $CD14^{++}CD16^{-}$ (classic) monocytes is the result of an immediate release of $CD14^{++}CD16^{-}$ (classic) monocytes from the bone marrow (and spleen) into the circulation or the infiltration of $CD14^{++}CD16^{+}$ (intermediate) and $CD14^{+}CD16^{++}$ (nonclassic) monocytes into myocardial tissue. Such sequestration would decrease the overall percentages of the last 2 subsets. It is likely that the phagocytosing macrophage infiltrate detected in the myocardium originates from the migration of these circulating monocytes into the heart, rather than proliferation of resident myocardial macrophages, as has been shown in experimental models after insult.[33] Perhaps the most interesting finding is that the intermediate monocyte subset ($CD14^{++}CD16^{+}$) remains low during follow-up, suggesting a lower degree of turnover. This is in complete contrast to patients who have sustained an acute myocardial infarction and experimental models of myocardial infarction in which a 2-phase progression in monocyte activation has been defined: Immediately after myocardial infarction, the classic, proinflammatory $CD14^{++}CD16^{-}$ subset is recruited, whereas by day 7 after myocardial infarction, the nonclassic $CD14^{+}CD16^{++}$ subset becomes dominant, implying lesser proinflammatory response and tissue repair.[34,35] The decrease in percentage of the intermediate subset in patients with takotsubo cardiomyopathy may relate to the failure of their myocardial inflammation to resolve, resulting in a low-level chronic inflammatory state.

Systemic Inflammatory Response

The increase in systemic inflammatory cytokines/chemokines IL-6, IL-8, and CXCL1 (growth-regulated protein) is in keeping with the increase in myocardial inflammation and increase in percentage of blood $CD14^{++}CD16^{-}$ monocytes, highlighting the inflammatory nature of the condition. The increase in IL-8 and CXCL1 (growth-regulated protein) may relate to monocyte adhesion and macrophage infiltration into the myocardium or release of inflammatory cells from the bone marrow,[36] whereas IL-6 is a robust proinflammatory marker.

Table 4. Serum Cytokine Concentrations in Patients With Takotsubo Cardiomyopathy and Matched Control Subjects

	Patients With Takotsubo Cardiomyopathy (Acute) (n=55)	Patients With Takotsubo Cardiomyopathy (5 mo) (n=48)	Matched Control Subjects (n=51)	*P* Value, Acute Versus Control	*P* Value, 5 mo Versus Control	*P* Value, Acute Versus 5 mo
IL-1β, pg/mL	4.2±2.31	3.9±2.36	7.7±2.64	0.32	0.34	0.81
IL-6, pg/mL	23.1±4.54	18.3±5.17	6.5±5.83	<0.001	0.008	0.01
IL-8 (CXCL8), pg/mL	45.5±8.62	61.9±10.28	21.7±10.86	0.07	0.009	0.24
IL-10, pg/mL	6.3±1.10	5.2±1.34	5.7±1.27	0.83	0.78	0.47
IL-12p40, pg/mL	10.2±6.24	16.9±7.72	8.1±6.01	0.82	0.43	0.51
MCP-1, pg/mL	483±38.37	435±50.43	575±42.07	0.14	0.03	0.41
CXCL1 (GROα), pg/mL	1903±168.43	1650±214.02	1272±176.56	0.01	0.15	0.34
TNFα, pg/mL	12.5±1.47	12.8±1.82	12.4±1.89	0.89	0.90	0.86
IFNγ, pg/mL	53.1±13.89	46.9±16.63	31.8±14.81	0.34	0.48	0.67

All data are shown as mean±SEM. *P* values for comparisons were calculated with *t* tests with degrees of freedom estimated by the Satterhwaite method (11–80 *df* for IL-6). CXCL indicates chemokine (C-X-C motif) ligand; GROα, growth regulated protein; IFNγ, interferon-γ; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; and TNFα, tumor necrosis factor-α.

Finally, almost half of the patients remained symptomatic at the time of follow-up. The high-sensitivity cardiac troponin levels were above the 5-ng/mL threshold that defined populations at increased subsequent risk of cardiac events in patients suspected of acute coronary syndromes.[37,38] However, this finding requires validation in larger cohorts given the different pathophysiologies of these conditions.

Study Limitations

There are a number of limitations to the present study. First, we did not obtain biopsies from ballooning or nonballooning areas of the left ventricle in patients with takotsubo cardiomyopathy to demonstrate macrophage-USPIO colocalization; myocardial biopsies would have implied a second invasive procedure during an acute illness combined with participation in a demanding research protocol in patients who experienced a stress-induced condition. However, our published data from postmortem myocardium of patients with takotsubo cardiomyopathy demonstrate the clusters of macrophage infiltrates within the myocardium.[27] Second, we used T1 mapping to identify edema instead of T2 mapping schemes, and this allowed us to compare the current group with our previously published cohorts.[5,15] Both native and post-USPIO myocardial T2* values could be affected by concurrent myocardial pathology such as edema, hemorrhage, vasodilatation, or different proportions of oxygenated or deoxygenated hemoglobin. We are unable to either confirm or refute the possible contribution of some of these to the directly measured T2* values, which may explain some of the differences seen in either native or post-USPIO T2* values between groups (Table II in the online-only Data Supplement). Therefore, we expressed the myocardial USPIO uptake as the change in T2* from the pre-USPIO (native) to post-USPIO images, which were acquired only 24 hours apart. In this way, any contribution of any significant concurrent myocardial pathology should have been subtracted, logically assuming that the subtraction will eliminate the noncontrast agent effects. At the current time, measurements of effect size between health and disease for both 3-T scanners have not been computed, and the majority of patients in this study were scanned in 1 center. A final limitation is not being able to infer from our study whether inflammation is a cause or a consequence of the acute takotsubo event. Moreover, it is unclear whether this inflammation is maladaptive and implicated in the persistence of the long-term consequences of this condition. This can be addressed only by randomized controlled trials of anti-inflammatory interventions.

Conclusions

We demonstrate for the first time that takotsubo cardiomyopathy is accompanied by myocardial and systemic inflammatory activation, with myocardial macrophage infiltration and acute proinflammatory monocyte and cytokine activation. These changes evolve into a low-grade, chronic inflammatory state that remains detectable at least 5 months after acute presentation.

ARTICLE INFORMATION

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Disclosures

None.

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