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Cardiovascular biomarkers in acute Kawasaki disease☆,☆☆

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ABSTRACT

Background: Endomyocardial biopsies have demonstrated that subclinical myocarditis is a universal feature of acute Kawasaki disease (KD).
Methods: We investigated biochemical evidence of myocardial strain, oxidative stress, and cardiomyocyte injury in 55 acute KD subjects (30 with paired convalescent samples), 54 febrile control (FC), and 50 healthy control (HC) children by measuring concentrations of cardiovascular biomarkers.
Results: Levels of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and soluble ST2 (sST2) were elevated in acute vs. convalescent KD, FC, and HC ($p \leq 0.002$), while γ-glutamyl transferase and alanine amino transferase as measures of oxidative stress were increased in acute vs. FC ($p \leq 0.0002$). Cardiac troponin I (cTnI) levels, using a highly sensitive assay, were elevated in 30% and 40% of paired acute and convalescent KD subjects, respectively, and normalized within two years of disease onset. NT-proBNP and sST2 negatively correlated with deceleration time, but only NT-proBNP correlated with MV E:A ratio and internal diameter of the coronary arteries (RCA/LAD Z_{worst}).
Conclusions: NT-proBNP and sST2 were elevated in acute KD subjects and correlated with impaired myocardial relaxation. These findings, combined with elevated levels of cTnI, suggest that both cardiomyocyte stress and cell death are associated with myocardial inflammation in acute KD.

1. Introduction

Kawasaki disease (KD) is an acute inflammatory condition that involves both the arterial wall and the myocardium [1]. While patients uncommonly present with clinically significant systolic dysfunction in the acute phase [2,3], endomyocardial biopsies have documented a range of pathologic findings consistent with diffuse myocardial inflammation [4–6]. Most patients have normal myocardial systolic function after recovery from their acute illness, but diastolic dysfunction has been observed [7,8].

Protein biomarkers of cardiomyocyte strain, injury, and death are used to stratify risk and to monitor response to therapies in adults with congestive heart failure and ischemic heart disease [9]. Some biomarkers, such as troponin, are released by injured or dying cells, but do not directly participate in the pathologic process. Others, such as soluble ST2 (sST2), directly mediate injury and could be targets for therapeutic intervention [10,11]. We tested a panel of cardiovascular biomarkers in acute and convalescent KD patients and compared the results to febrile and healthy controls as well as clinical and echocardiographic data to better understand the mechanisms of myocardial injury in acute KD.

2. Methods

2.1. Patients

KD samples were from consecutive, unselected KD subjects for whom both plasma and serum samples were available. All KD subjects fulfilled American Heart Association diagnostic criteria for KD [12]. Acute KD samples were obtained prior to treatment with intravenous immunoglobulin (IVIG). N-terminal pro-B-type natriuretic peptide (NT-proBNP), sST2, serum cardiac troponin I (cTnI), γ-glutamyl transpeptisdase (GGT), and alanine amino transferase (ALT) concentrations were determined for the following subjects: 55 acute KD (30 of whom had paired convalescent samples; median 46 days, range 26–73 days after onset of KD), 54 age-similar febrile controls (FC), and 50 age-similar healthy controls (HC). cTnI levels were also determined for 17 KD subjects who had late convalescent serum obtained (median 431 days, range 347–757 days after onset of KD).

FC subjects were previously healthy children recruited from the Emergency Department at Rady Children's Hospital San Diego and had ≥3 days of fever and at least

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Table 1
Diagnoses of febrile controls.

	Diagnosis	n
Bacterial infection (n = 8)	Scarlet fever	3
	Staphylococcal scalded skin syndrome	2
	Streptococcal pharyngitis	3
Viral infection (n = 46)	Measles	1
	Culture-proven adenovirus	11
	Viral syndrome defined as self-limited, minor febrile illness with negative throat and rectal viral cultures	34

one of the clinical signs of KD (rash, conjunctival injection, cervical lymphadenopathy, erythematous oral mucosa, and erythematous or edematous hands or feet). Among the 54 FC subjects, 8 had bacterial infection and 46 had viral infections (Table 1).

HC subjects were children undergoing minor elective surgery for polydactyly. The Human Research Protection Program of the University of California, San Diego approved this research protocol and written informed consent was obtained from the parents of all subjects. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

We recorded age, sex, illness day at patient evaluation (first calendar day of fever = illness day 1), and clinical laboratory data. We normalized the hemoglobin concentration for age to allow valid comparisons across the age spectrum of our subjects. For KD subjects only, we recorded response to intravenous immunoglobulin (IVIG) and echocardiographic data. IVIG-resistance was defined as persistent or recrudescent fever (T ≥ 38 °C) at least 36 h after completion of the IVIG infusion (2 g/kg).

2.2. Echocardiography

Echocardiography was performed in acute KD subjects during their initial hospitalization and at 2 and 5 weeks post-IVIG. Dilatation of the right coronary artery (RCA) and left anterior descending coronary artery (LAD) was defined according to the American Heart Association criteria as a Z score of ≥2.5 (standard deviation units from the mean internal diameter normalized for body surface area) [12]. Aneurysms were defined as a focal region of the coronary artery 1.5 times the diameter of the adjacent segment. "Z_{worst}" was defined as the larger of the Z scores for the RCA and LAD at any time point in the illness. The aortic root was measured by standard convention in the parasternal long axis view during mid-systole and the absolute dimension for the aortic sinus was normalized based on body surface area and considered dilated if the Z score was ≥2.0 [13]. Parameters of ventricular diastolic function included mitral inflow velocities during early diastolic filling (E wave velocity) and atrial contraction (A wave velocity), deceleration time (time, in milliseconds, from the peak of the E wave to the baseline), and Doppler measurement of tissue velocity (DTI) at the lateral mitral annulus (E' velocity), septal mitral annulus, and lateral tricuspid annulus during early diastolic filling. DTI was only available for 19 subjects enrolled during the last year of the study. Data, calculated from the diastolic measurements, included the mitral E wave velocity/A wave velocity ratio and the E velocity/E' velocity ratio. Values were compared to published normal values and categorized as either abnormal or normal [14,15]. Fractional shortening (FS) was measured by standard methods (M mode) and normalized for age.

2.3. Biomarker assays

EDTA plasma NT-proBNP concentration was measured with a biotin-coupled anti-NT-proBNP antibody/streptavidin solid-phase chromatographic immunoassay (*Status*First CHF NT-proBNP test devices, Nanogen, San Diego, CA; 99% for reference value for healthy adults = 125 pg/mL), in combination with the DXpress Reader (Nanogen, San Diego, CA). Sodium citrate plasma sST2 levels were determined using the Presage sST2 assay kit (Critical Diagnostics, New York, NY; 99% for reference value for healthy adults = 50.2 ng/mL). Serum cTnI was measured using the Verisens human cTnI assay (Nanosphere, Northbrook, IL), a multi-step and automated assay using functionalized gold nanoparticles with signal enhancement by silver amplification (99% for reference value for healthy adults = 0.0045 ng/mL). Plasma concentrations of GGT and ALT were measured using the VITROS GGT and ALT slides and the VITROS Chemistry Products Calibrator Kit 3 on VITROS Chemistry Systems.

2.4. Statistical analysis

Data were analyzed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA) software, and presented as medians and interquartile range. Mann–Whitney *U* test was used for non-parametric data. Paired data for acute and convalescent KD were analyzed using a Wilcoxon signed rank test. Correlations between continuous variables were

Table 2
Clinical and laboratory characteristics of acute Kawasaki disease (KD) and febrile control (FC) subjects.

Characteristics	Acute KD (n = 55)	FC (n = 54)	p
Median age, yrs. (range)	2.8 (0.4–14.9)	2.4 (0.2–13.5)	NS
Male, n (%)	35 (64)	31 (57)	NS
Median illness day (range) (first day of fever = day 1)	6 (3–10)	4 (2–20)	0.005
Coronary artery status of subjects: n (%)	Normal: 35 (64) Dilated: 11 (20) Aneurysm: 9 (16)	NA	NA
IVIG resistant, n (%)	17 (31)	NA	NA
CRP (mg/dL)[a]	8.2 (5.2–18.5)	2.2 (1.0–4.3)	<0.0001
ESR (mm/h)	62 (44–78)	20 (15–38)	<0.0001
WBC ($\times 10^9$/L)	13.5 (10.7–18.5)	8.7 (6.4–12.8)	<0.0001
% polymorphonuclear leukocytes	56 (46–66)	45 (31–63)	0.03
% bands	12 (8–21)	8 (4–15)	NS
Absolute neutrophil count	9520 (6519–13,090)	4636 (2695–6790)	<0.0001
Age-adjusted Hgb, S.D. units	−1.25 (−2.33 to −0.5)	−0.43 (−1.3–0.86)	0.0004
Platelet count ($\times 10^9$/L)	405 (321–465)	265 (213–349)	<0.0001
ALT (IU/L)	45 (24–102)	24 (17–36)	0.0002
GGT (IU/L)	45 (19–150)	14 (12–17)	<0.0001

IVIG = intravenous immunoglobulin, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, WBC = white blood cell count, Hgb = hemoglobin concentration, ALT = alanine amino transferase, GGT = γ-glutamyl transferase, NA = not available, NS = not significant.

[a] Laboratory data are presented as median (interquartile range).

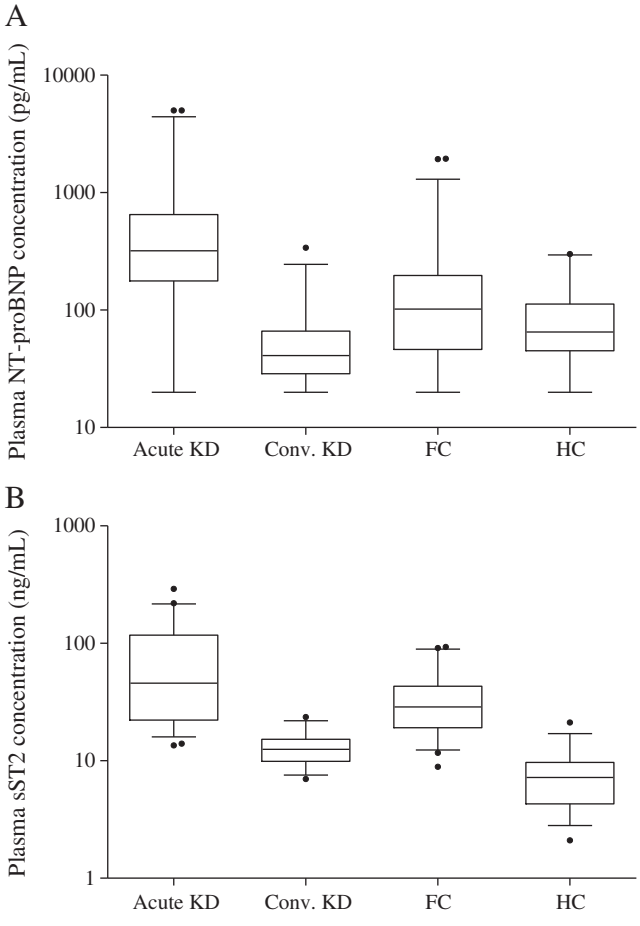

Fig. 1. Plasma concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and soluble ST2 (sST2) in acute Kawasaki disease (KD), convalescent KD, febrile controls (FC), and healthy controls (HC). A) Comparisons of NT-proBNP concentrations ($p < 0.0001$ for acute KD vs. conv. KD, FC, and HC). B) Comparisons of sST2 concentrations ($p \leq 0.002$ for acute KD vs. conv. KD, FC, and HC). Box plot represents median (bar) with interquartile range (box), and T-bars show 5th–95th percentile. Data presented in a logarithmic scale. Outlying values are represented by black dots.