

# Plasma Concentrations of Cytokines and Neurohumoral Factors in a Case of Fulminant Myocarditis Successfully Treated With Intravenous Immunoglobulin and Percutaneous Cardiopulmonary Support

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A 53-year-old Japanese man with fulminant myocarditis was referred. Percutaneous cardiopulmonary support (PCPS) was introduced immediately and intravenous immunoglobulin (IVIG) therapy followed for 2 days. Cardiac function showed signs of recovery on the 4th hospital day and the patient was weaned from PCPS on the 7th hospital day. Creatine kinase-MB peaked at 12 h after admission and was 176 ng/ml. Endomyocardial biopsy showed active myocarditis. A marked increase of the neutralizing antibody titer suggested coxsackievirus B3 infection. Plasma concentrations of cytokines and neurohumoral factors were analyzed. Proinflammatory cytokines, such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF- $\alpha$ ), and anti-inflammatory cytokines, such as IL-1 receptor antagonist, soluble TNF receptor-1 and IL-10, were elevated on admission and all had decreased on the 7th hospital day. Brain natriuretic peptide and noradrenaline were already elevated upon admission (1,940 pg/ml and 4.6 ng/ml, respectively) and decreased thereafter. Although IVIG therapy under PCPS is a common treatment for fulminant myocarditis, the immunological response in vivo remains unclear. This case demonstrated suppression of serum cytokines after IVIG and PCPS treatment. Immunological parameters in those who have been treated with IVIG and PCPS and survived without complications are of great value for evaluation of the therapy. Further analysis with more cases in a multicenter study is necessary. (*Circ J* 2004; 68: 1223–1226)

**Key Words:** Cytokines; Fulminant myocarditis; Intravenous immunoglobulin; Percutaneous cardiopulmonary support

Cardiopulmonary support is regarded as most important for rescue in cases of fulminant myocarditis with circulatory crisis! Despite some case reports suggesting the potential therapeutic efficacy of intravenous immunoglobulin (IVIG) therapy in fulminant myocarditis<sup>2,3</sup> this indication remains controversial and because of the huge cost of that therapy, its biological validity should be examined in humans from an immunological viewpoint<sup>4</sup>. However, the scarcity of fulminant myocarditis itself, vascular trouble associated with percutaneous cardiopulmonary support (PCPS), or multi-organ failure (MOF) that complicates the general condition lessen the opportunity to

examine the pure immunological response to fulminant myocarditis! Therefore, investigating the immunological parameters in those who had been treated with IVIG and survived the cardiopulmonary crisis without complication is of great value for evaluation of IVIG therapy. Herein, we report a rather typical case of fulminant myocarditis in a patient who survived the cardiopulmonary crisis with PCPS and IVIG therapy.

## Case Report

A 53-year-old Japanese man presented with sore throat, epigastric pain and a fever of 40.0°C at Sado General Hospital in August 2002. Ibuprofen, cefixime, and cimetidine were prescribed with a diagnosis of viral infection. One week later, the patient developed nausea, vomiting and general fatigue and visited the hospital again.

The patient was alert, but his pulse was weak and the rate was 95 beats/min. Blood pressure was 80 mmHg by palpation and a diastolic gallop was noted at the apex of the heart. There was a moist rale in the bilateral lower lung field. The extremities were very cold, but there was no pretibial edema. An electrocardiogram (ECG) showed accelerated idioventricular rhythm at the rate of 98 beats/min. The chest

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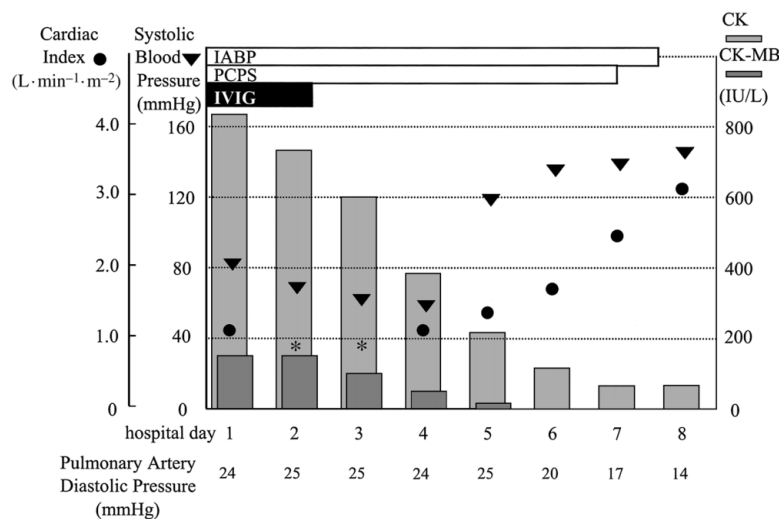


Fig 1. Clinical course. Elevated creatine kinase normalized and the reduced cardiac function improved after intravenous immunoglobulin treatment. \*The cardiac index could not be measured with a Swan-Ganz catheter because of extremely low cardiac output during the second and third hospital days. CK, creatine kinase; IVIG, intravenous immunoglobulin; PCPS, percutaneous cardiopulmonary support; IABP, intra-aortic balloon pump.

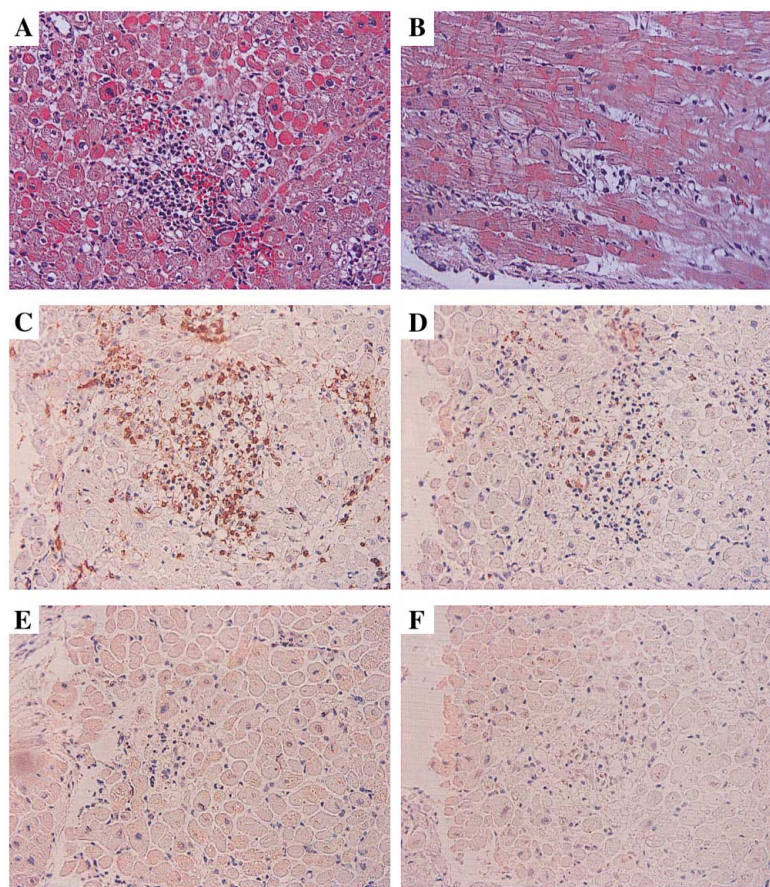


Fig 2. Histopathology and immunohistochemistry of the endomyocardial biopsy on the 10th hospital day. Histopathology (A, B). Myocyte necrosis and degeneration associated with infiltration of the mononuclear cells (H&E; original magnification,  $\times 40$ ). Immunohistochemistry (C, D). Most of the infiltrating cells are positive for CD45RO (T cells) or CD68 (macrophages). (E, F) Only a few cells are positive for CD20 (B cells) or CD1a (dendritic cells) (Original magnification,  $\times 40$ ).

roentgenogram showed cardiomegaly (cardiothoracic ratio 63%) and pulmonary congestion. The leukocyte count was 9,400/ $\mu$ l. Serum concentrations of aspartate aminotransferase (619 U/L), lactic dehydrogenase (1,639 U/L), creatine kinase (825 IU/L), and creatine kinase-MB (149 ng/ml) were also elevated. The echocardiogram showed severe diffuse hypokinesis and wall thickening of the left ventricle with slight pericardial effusion. The thickness of the inter-ventricular septum and posterior wall was 13 mm and 12 mm, respectively. The left ventricular end-diastolic dimension, end-systolic dimension and ejection fraction

(EF) calculated by the Teichholz method were 48 mm, 46 mm, and 10%, respectively. Cardiac valves were normal. In the afternoon of the day of admission, he developed cardiac arrest and intravenous administration of inotropic agents, temporary pacing and intra-aortic balloon pump (IABP) failed to produce hemodynamic improvement. He was transferred to the university hospital in the evening by helicopter with a clinical diagnosis of fulminant myocarditis.

On his arrival, the cardiac index was 1.2 L·min<sup>-1</sup>·m<sup>-2</sup>. We immediately started PCPS and IVIG treatment after ob-

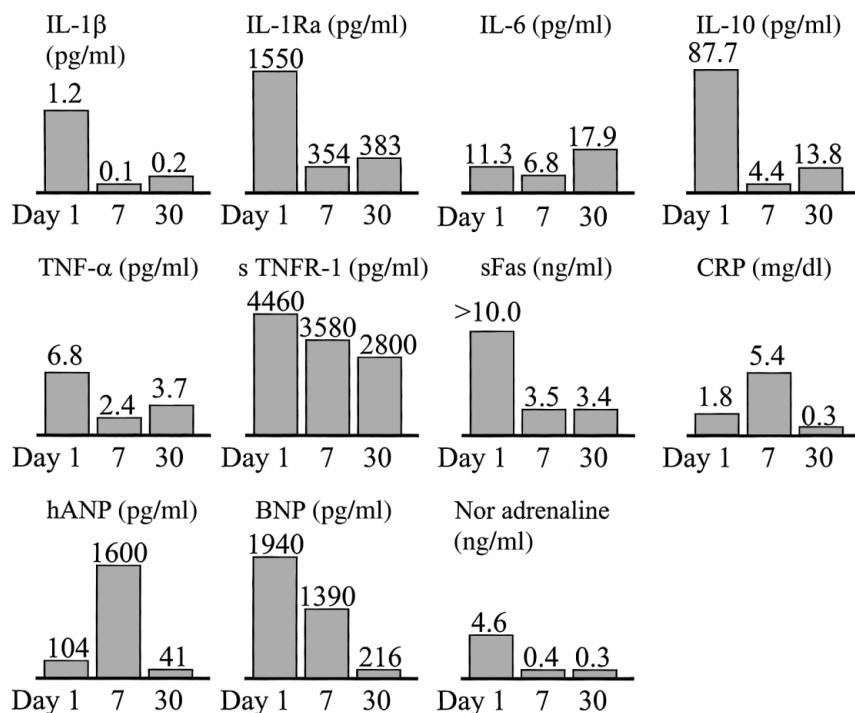


Fig 3. Serum concentrations of cytokines and neurohumoral markers during the clinical course. Day 1, first hospital day. Immunoglobulin was administered on the first and second hospital day. On day 7, the patient was weaned off percutaneous cardiopulmonary support (PCPS) without complications.

taining written informed consent. Gamma-Venin (Aventis Corporation, Japan) of  $0.5\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  was administered for the first 2 days after admission (Fig 1). However, the cardiac index decreased and could not be measured with a Swan-Ganz catheter during the second and third hospital days because of extremely low cardiac output. His ECG showed no spontaneous excitation for the first 3 days. Cardiac arrest continued until the 4th hospital day, when the echocardiogram showed recovery of left ventricular wall motion. On the 7th hospital day, the patient was weaned off the system without complications of PCPS, MOF or serious infection. Endomyocardial biopsy on the 10th hospital day showed infiltrates of mononuclear cells associated with myocyte necrosis (Fig 2). Neither polymorphonuclear cells nor giant cells were observed. Immunohistochemistry using antibodies to CD45RO and CD68 (DAKO Cytomation, Corp) showed that the mononuclear cells were mostly T lymphocytes or macrophages. There were very few CD20 and CD1a positive cells (DAKO Cytomation, Corp), which represent B lymphocytes and dendritic cells, respectively.

One month later, coronary angiography and left ventriculography (LVG) were performed and no significant stenosis in the coronary arteries was observed. The left ventricular ejection fraction (LVEF) improved to 49%. Complete atrio-ventricular block remained, but the patient was discharged with a permanent pacemaker. Neutralizing antibody titers for influenza virus, echovirus, adenovirus and parainfluenza virus did not rise significantly between admission and discharge. However, the neutralizing antibody titer for coxsackievirus B3 showed 256-fold increase on admission and reached a plateau of 512-fold on discharge.

Plasma concentrations of neurohumoral factors and cytokines were examined at 3 time points: on admission (day 1), after weaning from the PCPS (day 7) and on the day of discharge (day 30) (Fig 3). Samples were analyzed at Mitsubishi Kagaku Bio-Clinical Laboratories, Inc in Tokyo, Japan. Samples were collected and stored in accordance with the direction of the laboratories. Proinflammatory

tory cytokines, such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF- $\alpha$ ), were elevated on day 1 and decreased on day 7. Anti-inflammatory cytokines, such as IL-1 receptor antagonist (IL-1Ra), soluble TNF receptor-1 (sTNFR1) and IL-10, demonstrated similar changes to the proinflammatory cytokines. All cytokine concentrations except for sTNFR1 and soluble Fas (sFas) demonstrated a transient fall on day 7, followed by a slight increase that was below that of day 1. C-reactive protein (CRP) also increased in the peripheral blood and peaked not on day 1, but on day 7. Brain natriuretic peptide and noradrenaline were already elevated on admission (1,940 pg/ml and 4.6 ng/ml, respectively) and decreased thereafter. In contrast, human atrial natriuretic peptide (hANP) peaked on day 7. Most data had almost normalized by day 30.

## Discussion

The effect of IVIG has been examined in chronic heart failure<sup>5-7</sup> but a clinical trial of IVIG therapy in fulminant myocarditis does not exist. Intervention in a Myocarditis and Acute Cardiomyopathy (IMAC) trial failed to demonstrate evidence of the therapeutic efficacy of IVIG on recent onset dilated cardiomyopathy (DCM). Forty-five percent of the IMAC population was given  $\beta$ -blockers and improvement in the LVEF was greater than those in a case series by Dec et al<sup>8</sup> and also one by Steimle et al<sup>9</sup>, both of which were conducted before  $\beta$ -blockers were widely used in the management of patients with systolic dysfunction. The effect of IVIG in the IMAC trial proved much slighter than that of  $\beta$ -blockers, angiotensin converting enzyme inhibitors, or angiotensin II receptor antagonists in the management of DCM.<sup>5</sup> However, intervention of the immune system may be more important than that of the neurohumoral system in acute heart failure caused by viral infection, such as fulminant myocarditis.

Lieberman et al first classified myocarditis as either fulminant or acute on the basis of the clinicopathological

criteria, including the severity of illness at presentation.<sup>10</sup> McCarthy et al reported that fulminant myocarditis as an entity has a good long-term outcome and they emphasized the importance of intensive cardiopulmonary support in the event of circulatory crisis.<sup>11</sup> However, the Japanese National Survey demonstrated that MOF, life-threatening arrhythmia and complication of PCPS, such as leg ischemia, often occurred during a circulatory crisis and reduced the survival rate to 58%.<sup>1</sup> A combination of PCPS and IVIG is a promising therapy, but immunological markers that reflect heart inflammation are currently not detectable in the peripheral blood. In addition to the small number of cases of fulminant myocarditis, the low survival rate lessens the opportunity to examine the mechanism of the disease. Therefore, to examine the immunological parameters in those who were treated with IVIG and survived the cardiopulmonary crisis without complication is of great value for verifying IVIG therapy.

Gullestad et al demonstrated an IVIG-induced change in the balance between pro- and anti-inflammatory cytokines in congestive heart failure.<sup>6</sup> Contrary to their observation that improvement of LVEF was associated with an enhanced anti-inflammatory net effect, in the present case both types of cytokines increased at admission and showed a transient fall after IVIG therapy. Because both IL-1 and TNF- $\alpha$  can impair myocardial performance, it is conceivable that inhibition of the effects of these cytokines may be beneficial. Interestingly, both pro- and anti-inflammatory cytokines showed a significant decrease when the patient was successfully weaned of PCPS, despite hemodynamic markers remaining at a high level. That may reflect immunological recovery in vivo proceeding hemodynamic recovery. However, we can not conclude that the immunological effect of IVIG was the main cause of the change in the circulating cytokines concentrations. Recovery of heart failure, dependent or independent of the IVIG effect, might contribute to those alternations. Further observations under various conditions are needed.

Kishimoto et al examined the effects of immunoglobulin on murine myocarditis induced by coxsackievirus B3, encephalomyocarditis virus, and in rat autoimmune myocarditis.<sup>12–14</sup> They reported that immunoglobulin therapy suppressed acute viral myocarditis by an anti-viral effect, an anti-inflammatory effect and improvement in extracellular matrix changes.<sup>12,13</sup> Moreover, immunoglobulin therapy suppressed experimental giant cell myocarditis in rats, associated with the suppression of the expression of dendritic cell via inhibitory Fc receptor.<sup>14</sup> Some immunomodulatory effect of IVIG might have worked favorably in the present case.

We have reported the immunological parameters in those who were treated with IVIG and PCPS and survived without complications. According to our retrospective study conducted before IVIG were routinely used in the management of patients with fulminant myocarditis,<sup>15</sup> the prognosis of the present case was classified as poor because of the high sFas concentrations. Therefore, the addition of

IVIG therapy may be one of the recent improvements in the treatment for fulminant myocarditis. Our observations may show that there is an immunological effect of IVIG; however, it does not assure the clinical efficacy of IVIG therapy in fulminant myocarditis. Accordingly, a large number of patients should be studied and more attention should be paid when interpreting the effect of IVIG on the hemodynamic and immunological variables.

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