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Serum interleukin 6 and 10 levels in Takotsubo cardiomyopathy: Increased admission levels may predict adverse events at follow-up

Francesco Santoro [a, b, 1], Nicola Tarantino [a, 1], Armando Ferraretti [a], Riccardo Ieva [c], Francesco Musaico [a], Francesca Guastafierro [a], Luigi Di Martino [a], Matteo Di Biase [a], Natale Daniele Brunetti [a, *]

[a] University of Foggia, Foggia, Italy
[b] Asklepios Sankt Georg Klinik, Hamburg, Germany
[c] Ospedali Riuniti University Hospital, Foggia, Italy

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ABSTRACT

Background and aims: Systemic inflammation has been hypothesized as a possible mechanism of Takotsubo cardiomyopathy (TTC). Aim of the study was to assess the role of interleukin (IL)-6 and IL-10 in subjects with an episode of TTC.

Methods: Fifty-six consecutive subjects with TTC were prospectively enrolled in the study and followed for a mean of 178 days. Circulating levels of IL-6, IL-10, clinical condition and left ventricular ejection fraction were evaluated at admission. Incidence of death, re-hospitalization and recurrence of TTC during follow-up was also recorded.

Results: 23% of patients experienced in-hospital complications while 20% of patients had adverse events at follow-up.

IL-6 and IL-10 serum levels at admission were higher in subjects with adverse events at follow-up (120 ± 294 *vs.* 22 ± 40 pg/ml, $p<0.05$; 13 ± 35 *vs.* 2 ± 3 pg/ml, $p=0.05$, respectively). Increased serum levels of IL-6 and IL-10 were associated with higher adverse events rates at follow-up (Log-Rank $p<0.001$, <0.05, hazard ratio 8.6, 5.1, respectively) and mortality rates (Log-Rank $p<0.001$, $p<0.05$, hazard ratio 20.8, 7.1, respectively).

Subjects with both increased IL-6 and IL-10 levels were characterized by an increased risk of adverse events when compared to subjects with only IL-6 or IL-10 increased levels or with values below cutoff values (Log-Rank $p<0.01$ for any event, <0.001 for death; hazard ratio 1.20 for any event, 1.31 for death), even after correction for age, LVEF and NTproBNP levels in multivariable Cox analysis.

Conclusions: Serum IL-6 and IL-10 admission levels are associated with higher risk of adverse events during follow-up.

1. Introduction

Takotsubo cardiomyopathy (TTC) is an acute heart failure syndrome, featured by transient and reversible left ventricular dysfunction [1]. Despite the mechanism leading to transient systolic dysfunction characteristic of TTC remains still not completely clear, increased serum levels of catecholamines are considered as one of the drivers [2].

Systemic inflammation has been hypothesized as a possible mechanism of TTC [3]. Inflammation has been extensively studied in the whole spectrum of cardiovascular disease, from acute coronary syndrome [4,5] to arrhythmias [6] and heart failure [7]; higher markers of inflammation are usually found in subjects with a worse outcome.

In subjects with TTC, inflammation has been investigated through its signs, such as myocardial edema at MRI [8] or immune cells infiltrate at biopsy [9]; no studies, however, evaluated the role of inflammation through the evaluation of serum cytokines.

Interleukin (IL)-6 and IL-10 are well studied mediators of inflammation. While IL-6 is usually considered a marker of inflammation, even in cardiovascular disease and atherosclerosis,

* Corresponding author. Viale Pinto 1, 71100, Foggia, Italy.
E-mail address: natale.brunetti@unifg.it (N.D. Brunetti).
[1] Dr. Francesco Santoro and Dr. Nicola Tarantino equally contributed to the paper.

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IL-10 usually features an anti-inflammatory balancing activation [10]. Increased IL-6 levels are found in subjects with acute myocardial infarction [11] and may predict the risk of future ischemic events [12].

IL-10 levels are related to myocardial remodeling after myocardial infarction [13] and increased 30-day mortality [14]. Combined interaction between IL-6 and IL-10 may modulate the prognosis after myocardial infarction [15].

In the present study, we therefore sought to evaluate IL-6 and IL-10 serum levels during a TTC episode and their potential prognostic role.

2. Materials and methods

2.1. Study population

Fifty-six consecutive subjects with TTC were prospectively enrolled at the Department of Cardiology, Ospedali Riuniti University Hospital, Foggia, from January 2012 to December 2014. The diagnosis of TTC was based on Mayo Clinic criteria: a) transient hypokinesis, akinesis, or dyskinesis of the LV mid-segments, with or without apical involvement; b) absence of obstructive coronary disease or angiographic evidence of acute plaque rupture; c) new electrocardiographic abnormalities, either ST-segment elevation and/or T-wave inversion, or modest elevation in cardiac troponin; d) absence of pheochromocytoma and myocarditis [16].

2.2. Exclusion criteria

Patients with chronic inflammatory (systemic *lupus erythematosus*, rheumatoid arthritis, and Crohn's disease) or neoplastic disease, recent infectious disease, fever, immunosuppressive drug therapy (steroids, cyclosporine, or methotrexate), or immunologic disorder were excluded from the study.

2.3. Clinical examination

All patients underwent a clinical examination and age, gender, medical history, kind of stressors and daily ECG were recorded.

2.4. Echocardiograph examination

A two-dimensional Doppler echocardiographic examination, on the day of admission, at the third day, and at discharge was performed. The left ventricular ejection fraction (LVEF) was calculated using the Simpson method from the apical four-chamber and two-chamber view.

2.5. Coronary angiography

All patients underwent coronary angiography at admission.

2.6. Blood sample collection and laboratory

Circulating levels of NT-proBNP, troponin-I, IL-6, IL-10 and C-reactive protein were evaluated by venipuncture at admission, at 3rd day after admission and at discharge.

Blood samples for cytokines analysis were immediately centrifuged and serum separated after coagulum retraction by centrifugation at 2000 rpm for 10 min; it was then frozen at −20 °C until laboratory assay. All cytokines were standardized by inclusion of a titration of the appropriate purified recombinant cytokines of known concentration. Normal values, provided by supplier (Immulite, Siemens, Deerfield, IL, USA) were 1.20–1.95 for IL-6 pg/ml and 0.1–1.8 pg/ml for IL-10 (range values).

Concentration of CRP was determined using a particle-enhanced turbidimetric immunoassay technique. Normal values, provided by furnisher (Immulite, Siemens, Deerfield, IL, USA) were <5.0 mg/dl.

2.7. Follow-up and clinical endpoints

Complete follow-up data were available in all 56 patients. Mean follow-up was 178 ± 165 days. Clinical endpoints were classified according to world health organization classification of disease (ICD-Code 2016) [17]. Clinical end points included were in-hospital complications (Cardiogenic Shock (R57.0), pulmonary edema (J81), Stroke (I64)) and adverse events at follow-up (total mortality (R.99), cardiovascular mortality (sudden and non-sudden cardiovascular death (I46.2) TTC recurrence (I51.81) and re-hospitalization for heart failure (I50) or cardiac arrhythmia (I49.9)). The study was approved by the local ethical committee. All patients signed an informed consent.

2.8. Statistical analysis

Continuous variable were reported as means ± standard deviation and compared with Student's t-test for either paired or unpaired groups as required, dichotomic variables as percentage and compared with χ^2 test of Fisher test as required. Correlations were analyzed with Pearson's test.

Survival rate was reported on Kaplan-Meier plot and analyzed with Log-Rank test and multiple stepwise Cox analysis. Receiver operating characteristic curves were reported and compared with Hanley and McNeil method.

A p value < 0.05 was considered as statistically significant.

2.9. Sample size

We planned a study with a follow-up of 6 months and an enrollment time of 2 years. In a previous study, the median survival time on the control treatment was 10% per person-year [18]. If the true hazard ratio (relative risk) of control subjects relative to experimental subjects was 2, we needed to study 63 experimental subjects and 63 control subjects per year to be able to reject the null hypothesis that the experimental and control survival curves are equal with probability (power) 80%. The Type I error probability associated with the test of this null hypothesis is 0.05.

3. Results

Mean population age was 74 ± 13 years and six out of 56 (11%) patients were men. Clinical characteristics of study population are given in Table 1. Serum levels of IL-6 and IL-10 are given in Fig. 1. No difference was found between subjects with events at follow-up and those without in terms of age, history of hypertension or diabetes, emotional or physical triggers, hospital stay duration, troponin-I at admission and circulating levels of NT-proBNP. Patients with adverse events at follow-up were statistically different in terms of male gender (27% *vs.* 7%, $p<0.05$), LVEF at admission (30% *vs.* 34%, $p=0.05$) and IL levels at admission (IL-6 120 ± 294 *vs.* 22 ± 40 pg/ml, $p<0.05$; IL-10 13 ± 35 *vs.* 2 ± 3 pg/ml, $p=0.05$; Fig. 2).

Of the total cohort, 23% experienced in-hospital complications, while 20% of patients had adverse events at follow-up (14% death, 4% TTC recurrence, 9% CV re-hospitalization for heart failure or cardiac arrhythmias) (Table 2).

3.1. Interleukins and long-term follow-up

ROC curve analysis identified a cut-off value able to predict adverse events at follow-up for IL-6 (>51 pg/ml, 71% positive