The concentration of interleukin-33 in heart failure with reduced ejection fraction

Oliwia Anna Segiet, Ewa Romuk**, Ewa Nowalany-Kozielska*, Celina Wojciechowska*, Adam Piecuch***, Romuald Wojnicz****

Departments of *2nd Cardiology, and **Biochemistry, ***Histology and Cell Pathology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia; Katowice-*Poland*

Abstract

Objective: Despite several improvements in the management of heart failure (HF), it is still an incurable and a progressive disease. Several trials demonstrated that the process of inflammation may be responsible for initiation and progression of HF. The aim of the present study was to investigate the role of interleukin-33 (IL-33) in the pathogenesis of HF and to assess whether disease etiology and course of the disease affect the expression of cytokines.

Methods: The study included 155 (106 male and 49 female) patients with systolic HF with a mean left ventricle ejection fraction of 32.13±12.8% and 60 (36 male and 24 female) healthy individuals. IL-33 concentrations were evaluated using enzyme-linked immunosorbent assay.

Results: The concentration of IL-33 was statistically significantly lower in patients with HF than in healthy subjects, 16.91 (0–81.00) pg/mL and 92.51 (33.61–439.61) pg/mL, respectively. Patients with HF with ischemic etiology had lower concentration of IL-33 (10.75 pg/mL) than subjects with HF with non-ischemic etiology (21.05 pg/mL). Patients with stable HF (10.46 pg/mL) had lower IL-33 levels than those with unstable HF (19.02 pg/mL).

Conclusion: The concentrations of IL-33 were lower in patients with HF than in healthy controls, which may play an important role of above cytokine in HF development and progression. In addition, interleukin concentrations varied depending on the etiology and severity of the course of the disease. *(Anatol J Cardiol 2019; 21: 305-13)*

Keywords: heart failure, interleukin-33, immune system

Introduction

Heart failure (HF) is a cardiovascular disease and a final stage of ischemic and non-ischemic cardiomyopathy. Despite many advantages of present treatment strategies, HF continues to advance. It is the major cause of hospitalization and death among patients >65 years old in Western countries (1, 2). Therefore, it appears to be necessary to investigate novel pathophysiologic mechanisms in view of possible new opportunities in HF treatment, thereby improving survival and slowing the progression of the disease. The role of the immune system in HF development and course of this disease has been established. In HF, there is an imbalance between several proinflammatory and anti-inflammatory cytokines. The concentration of circulating and intracardiac proinflammatory cytokines is upregulated (3-6). These findings resulted in intensive attempts to discover an effective treatment targeting the inflammatory pathways. Unfortunately, many trials were discontinued due to adverse side effects. Infliximab, which is a chimeric monoclonal antibody to tumor necrosis factor (TNF)-α, increased mortality rate (7, 8). Although major improvements have been made in the diagnosis and management of HF, there is still a need to discover new disease markers and therapeutical strategies. Therefore, understanding the underlying molecular mechanisms that predict and contribute to HF is critical.

Interleukin-33 (IL-33), which is a member of the IL-1 superfamily, can be detected in mast cells, lymphoid organs, brain, embryos, macrophages, dendritic cells, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells, and keratinocytes (9). IL-33 acts through interleukin 1 receptor-like 1 (ST2) and IL-1 receptor accessory protein dimeric receptor complex, which activates mitogen-activated protein kinase and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways. There are two isoforms of IL-33 receptor: soluble (sST2) receptor and membrane-bound (ST2L) receptor. ST2L is present in cardiomyocytes and fibroblasts. sST2 is a soluble decoy receptor and a mechanically

Address for correspondence: Oliwia Anna Segiet, MD, 2nd Department of Cardiology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, Marii Curie-Skłodowskiej 10, 41-800 Zabrze, *Poland*
Phone: 0048 32 373 23 72 E-mail: oliwka.anna@gmail.com

Accepted Date: 11.03.2019 **Available Online Date:** 15.05.2019

DOI:10.14744/AnatolJCardiol.2019.64614

Anatol J Cardiol 2019; 21: 305-13
DOI:10.14744/AnatolJCardiol.2019.64614

induced cardiomyocyte protein, which attenuates the antihypertrophic effects of IL-33 (10). This interleukin plays a role in T helper 2 (Th2)-mediated immune response and increases the synthesis of Th2-associated cytokines, mainly through myeloid differentiation primary response protein 88 and IL-1R-associated kinase-1/4. It has been implicated in the pathogenesis of several diseases, such as anaphylaxis, asthma, and atopic dermatitis, and in host defense against parasites. Studies demonstrated that IL-33 is a chemoattractant for human Th2 cells (11). In endothelial cells, IL-33 stimulates the synthesis of IL-6, IL-8, vascular cell adhesion molecule-1, monocyte chemoattractant protein-1 (MCP-1/CCL2), intercellular adhesion molecule-1, and endothelial selectin and, therefore, promotes angiogenesis and enhances vascular permeability (12, 13). Recently, several studies demonstrated a protective effect of IL-33 in atherosclerosis, type 2 diabetes, obesity, and myocardial remodeling. IL-33 inhibited atherosclerotic plaques development through the activation of IL-5 and ox-LDL antibodies (14). IL-33 lowered adiposity, decreased fasting glucose concentration, and improved insulin and glucose tolerance in studies on obesity in animals (15).

Aim of the study

The aim of the present study was to investigate the role of IL-33 in the pathogenesis of HF and to assess whether the etiology and the course of the disease affect this cytokine expression. To this end, plasma and serum levels of IL-33 were measured in patients with both stable and unstable ischemic or non-ischemic HF in comparison with healthy subjects. The correlation of interleukin levels with echocardiographic, clinical, and biochemical parameters, including New York Heart Association (NYHA) functional class, N-terminal prohormone of brain natriuretic peptide (NT-proBNP), C-reactive protein (CRP), and left ventricular ejection fraction (LVEF), was assessed. We wanted also to investigate the influence of accompanying diseases, including chronic kidney disease, diabetes, dyslipidemia, or atrial fibrillation, on the concentration of IL-33.

Methods

Patients' characteristics

Patients with HF with reduced ejection fraction who were admitted to the 2nd Department of Cardiology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia were included in the study. HF was diagnosed based on a history of cardiac diseases, symptoms and signs of cardiac diseases, echocardiographic results, and plasma levels of NT-proBNP according to the guidelines of the Polish Cardiac Society and European Society of Cardiology. Patients with HF were categorized into the NYHA functional classes II–IV based on clinical symptoms. The causes of HF were classified as (1) ischemic HF in patients with a history of myocardial infarction and coronary atherosclerosis with a stenosis >70% in at least one major coronary artery branch or (2) non-ischemic HF in patients with no history of myocardial infarction and angiographically normal coronary arteries. Patients with HF, whose symptoms did not change within 1 month before being included in the study, were classified as stable, whereas patients with exacerbation of HF, whose symptoms worsened during 1 month prior to enrollment, were identified as unstable.

Inclusion criteria were as follows: patients with HF with reduced ejection fraction who were admitted to the 2nd Department of Cardiology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia; written informed consent to participate in the study; and patients aged ≥18 years.

Exclusion criteria were as follows: autoimmune disease, chronic or acute infection, recent use of immunosuppressive treatment, myocardial infarction within 3 months, congenital heart disease, end-stage renal failure, advanced liver cirrhosis, hyperthyroidism and other endocrinology disorders, acute respiratory distress syndrome, cancer, pregnancy, and lack of written informed consent form.

Data collected included medical history, accompanying diseases, demographic information (age, gender, occupation, and diet), physical examination, echocardiographic examination results, electrocardiogram, biochemical blood tests, chest X-ray, and recent pharmacological treatment. A total of 60 healthy subjects without cardiac disease or dyslipidemia with inflammatory markers, including CRP, erythrocyte sedimentation rate, and leukocyte count in the physiological range, were included in the study as the control group. The study was approved by the Bioethical Committee of the Medical University of Silesia. Written informed consent was obtained from all patients and healthy volunteers after oral and written explanation of the study.

Detection of the concentration of IL-33 using ELISA

Blood samples collected from patients on admission in tubes containing EDTA were centrifuged at 3000 g for 15 min at 4°C to isolate the plasma. Blood samples were also collected in tubes with clot activator to form a clot and then centrifuged at 3000 g for 10 min at 4°C to isolate the serum. Plasma and serum samples were stored at –80°C until further use. The concentrations of IL-33 were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's specifications. Levels of IL-33 were measured in duplicate using LEGEND MAX Human IL-33 ELISA Kit with Pre-coated Plates (BioLegend, San Diego, CA, USA).

Blood samples were also obtained for biochemical blood tests, including NT-proBNP, CRP, troponin T, creatine kinase-MB, complete blood count, lipid profile, glucose, electrolyte, creatinine, aspartate transaminase, alanine aminotransferase, bilirubin, and uric acid concentrations.

Echocardiographic measurements

LVEF was evaluated using the biplane Simpson's rule, as recommended by the European Society of Cardiology. In addition, the following measurements were evaluated: left ventricular end-diastolic volume, left ventricular end-systolic volume, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, interventricular septum diameter, diastolic posterior wall diameter, right-ventricular end-diastolic diameter, left atrial

diameter, left atrial area, right atrial diameter, right atrial area, dimensions of the aorta, pulmonary trunk, parameters of left ventricular diastolic function, including peak E-wave velocity, peak A-wave velocity, E/E′ ratio, mitral E/A ratio, mitral A-wave duration, mitral deceleration time, pulsed-wave TDI E′ velocity, left atrial volume index, tricuspid regurgitation, systolic jet velocity, and the presence of valvular abnormalities.

Statistical analysis

All statistical analyses were performed using Statistica 12 Poland software (StatSoft, Inc., Tulsa, OK, USA). Categorical variables were expressed as percentages. Continuous variables were expressed as mean and standard deviation for normally distributed data. Data with non-normal distribution were presented as median with interquartile ranges. Log transformations were performed to normalize non-normally distributed variables. Chi-square or Fisher's exact tests were used for comparison of categorical variables. Student's t-test was used to evaluate the statistical differences for normally distributed data, and post hoc multiple comparison test or Mann–Whitney U test for variables with non-normal distribution. Spearman's correlation was used to analyze the potential correlations between interleukin levels and HF markers of severity. A p-value <0.05 was considered statistically significant.

Results

A total of 155 (106 men and 49 women, mean age 65.39±12.75 years) patients with HF with reduced ejection fraction who were admitted to the 2nd Department of Cardiology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia with a mean left ventricle ejection fraction of 32.13±12.8% and 60 (36 men and 24 women, mean age 62.6±12.4 years) healthy controls were included in the study. HF with ischemic etiology was diagnosed in 107 patients with a history of myocardial infarction and coronary atherosclerosis with a stenosis >70% in at least one major coronary artery branch, whereas HF with non-ischemic etiology was diagnosed in 48 patients. The characteristics of these groups are shown in Table 1. Patients with HF were

Table 1. Characteristics of patients-division due to the etiology of HF

Variable	IHF	NIHF	Statistical significance
Age (years)	67.01±11.27	61.32±14.90	*P*=0.287
Sex (n, % men)	78; 72.90%	29; 60.42%	*P*=0.392
Hypertension (n; %)	81; 75.70%	26; 54.17%	*P*=0.225
Dyslipidemia (n; %)	48; 44.86%	20; 41.67%	*P*=0.099
Diabetes (n; %)	36; 33.64%	9; 18.75%	*P*=0.083
Atrial fibrillation (n; %)	32; 29.91%	24; 49.99%	*P*=0.314
Chronic kidney disease (n; %)	19; 17.76%	7; 14.58%	*P*=0.854
Beta-adrenolytics (n; %)	103; 96.26%	47; 97.92%	*P*=0.886
ACEI/ARB (n; %)	102; 95.33%	45; 93,75%	*P*=0.665
Statins (n; %)	91; 85.05%	25; 52.08%	***P*=0.0001**
Diuretics (n; %)	75; 70.09%	42; 87.50%	*P*=0.446
Aldosterone receptor antagonists (n; %)	67; 62.62%	38; 79.17%	*P*=0.091
Total cholesterol (mg/dL)	173.58±41.09	175.54±54.63	*P*=0.749
HDL cholesterol (mg/dL)	48.54±14.35	46.28±16.83	*P*=0.277
Triglycerides (mg/dL)	149.55±85.27	144.33±75.30	*P*=0.455
NT-proBNP (pg/mL)	1099 (406.9-4765)	1905 (465.9-7127)	*P*=0.536
Creatinine (μmol/L)	96.23±30.39	105.91±75.53	*P*=0.274
eGFR (mL/min/1.73 m^2)	68.67±20.91	68.08±24.02	*P*=0.397
LVEDD (mm)	56.66±10.39	58.74±9.92	*P*=0.565
LVESD (mm)	45.71±12.63	49.6±12.24	*P*=0.291
LVEDV (mL)	140.41±54.43	155.59±82.93	*P*=0.251
LVEF (%)	31.55±12.08	33.43±14.47	*P*=0.527

Categorical variables are expressed as percentages. Continuous variables are expressed as mean and standard deviation or median with interquartile ranges. *P*-values <0.05 were considered statistically significant.
HF - heart failure; IHF - ischemic heart failure; NIHF - non-ischemic heart failure; ACEI - angiotensin-converting-enzyme inhibitor; ARB - angiotensin II receptor blocker; HDL - high-density lipoprotein; NT-proBNP - N-terminal prohormone of brain natriuretic peptide; eGFR - estimated glomerular filtration rate; LVEDD - left ventricular end-diastolic diameter; LVESD - left ventricular end-systolic diameter; LVEDV - left ventricular end-diastolic volume; LVEF - left ventricular ejection fraction

Anatol J Cardiol 2019; 21: 305-13
DOI:10.14744/AnatolJCardiol.2019.64614

categorized into NYHA functional classes II–IV based on clinical symptoms. The study included 76 patients in the NYHA functional class II, 52 patients in the NYHA functional class III, and 27 patients in the NYHA functional class IV. The characteristics of the above-mentioned groups are presented in Table 2. Patients with HF were also divided into groups based on the course of disease on patients with stable and unstable HF. Patients with HF, whose symptoms did not change within 1 month before being included in the study, were classified as stable (n=74), whereas patients with exacerbation of HF, whose symptoms worsened during 1 month prior to enrollment, were identified as unstable (n=81). The characteristics of these groups are presented in Table 3.

The concentration of IL-33 was statistically significantly lower in patients with HF than in healthy subjects, 16.91 (0–81.00) pg/mL and 92.51 (33.61–439.61) pg/mL, respectively (Fig. 1a). Patients with HF with ischemic etiology had lower concentration of IL-33 (10.75 pg/mL) than subjects with HF with non-ischemic etiology (21.05 pg/mL) (Fig. 1b). Cytokine concentration did not differ significantly between patients in different NYHA functional classes (Fig. 1c). Patients with stable HF (10.46 pg/mL) had lower IL-33 levels than those with unstable HF (19.02 pg/mL) (Fig. 1d). Coexisting comorbidities and medications used upon hospital admission did not affect interleukin concentration (Fig. 1e-1h). No statistically significant correlations were observed between IL-33 concentration and echocardiographic, clinical, and biochemical parameters, including NYHA functional class, LVEF, NT-proBNP, and CRP.

Discussion

In our study, the concentration of IL-33 was lower in patients with HF than in healthy volunteers, and patients with stable HF were characterized by lower IL-33 concentration than those with unstable HF. Caselli et al. (16) also demonstrated that patients with HF have decreased IL-33 level. On the contrary, in our study, both IL-33 and ST2 were downregulated in patients with unstable HF submitted to left ventricular assist device support

Table 2. Characteristics of patients-division due to the NYHA functional class

Variable	NYHA II (n=76)	NYHA III (n=52)	NYHA IV (n=27)	Statistical significance
Age (years)	63.57±12.45	68.79±12.3	63.96±13.58	*P*=0.269
Sex (n; % men)	49; 64.47%	36; 69.23%	21; 77.78%	*P*=0.602
Hypertension (n; %)	54; 71.05%	39; 75.00%	14; 51.85%	*P*=0.436
Dyslipidemia (n; %)	42; 55.26%	18; 34.62%	7; 25.93%	*P*=0.155
Diabetes (n; %)	21; 27.63%	17; 32.69%	6; 22.22%	*P*=0.985
Atrial fibrillation (n; %)	22; 28.95%	25; 48.08%	8; 29.63%	*P*=0.463
Chronic kidney disease (n; %)	9; 11.84%	9; 17.31%	7; 25.93%	*P*=0.132
Beta-adrenolytics (n; %)	74; 97.37%	49; 94.23%	27; 100%	*P*=0.506
ACEI/ARB (n; %)	75; 98.68%	48; 92.31%	24; 88.89%	*P*=0.166
Statins (n; %)	63; 82.89%	38; 73.08%	15; 55.56%	*P*=0.676
Diuretics (n; %)	50; 65.79%	40; 76.92%	27; 100%	***P*=0.008**
Aldosterone receptor antagonists (n; %)	47; 61.84%	31; 59.62%	27; 100%	***P*=0.022**
Total cholesterol (mg/dL)	189.63±43.93	165.95±43.27	145.71±33.96	*P*=0.344
HDL cholesterol (mg/dL)	51.25±14.34	46.33±13.77	41.68±17.6	*P*=0.966
Triglycerides (mg/dL)	161.37±91.24	137.65±75.35	126.93±58.61	*P*=0.109
NT pro-BNP (pg/mL)	488.1 (193.9-1193)	1654.5 (881.3-5941)	6309.5 (3414-17508)	***P*=0.001**
Creatinine (μmol/L)	94.57±60.25	100.7±33.51	108.53±28.5	*P*=0.093
eGFR (mL/min/1.73 m^2)	73.61±22.62	64.62±19.39	60.79±20.53	*P*=0.870
LVEDD (mm)	57.05±11.51	56.09±9.24	59.76±8.53	*P*=0.192
LVESD (mm)	45.10±13.75	47.27±12.82	49.71±5.06	*P*=0.549
LVEDV (mL)	133±50.98	140.7±52.82	183.89±95.18	***P*=0.0003**
LVEF (%)	34.59±12.03	33.62±12.17	22.33±11.84	***P*=0.0001**

Categorical variables are expressed as percentages. Continuous variables are expressed as mean and standard deviation or median with interquartile ranges. *P*-values <0.05 were considered statistically significant.
NYHA - New York Heart Association; ACEI - angiotensin-converting-enzyme inhibitor; ARB - angiotensin II receptor blocker; HDL - high-density lipoprotein; NT-proBNP - N-terminal prohormone of brain natriuretic peptide; eGFR - estimated glomerular filtration rate; LVEDD - left ventricular end-diastolic diameter; LVESD - left ventricular end-systolic diameter; LVEDV - left ventricular end-diastolic volume; LVEF - left ventricular ejection fraction

Table 3. Characteristics of patients-division into patients with stable HF and unstable HF.

Variable	Stable HF (n=74)	Unstable HF (n=81)	Statistical significance
Age (years)	63.73±12.53	66.90±12.84	*P*=0.217
Sex (n; % men)	49; 66.22%	57; 70.37%	*P*=0.710
Hypertension (n; %)	53; 71.62%	54; 66.67%	*P*=0.505
Dyslipidemia (n; %)	40; 54.05%	27; 33.33%	*P*=0.488
Diabetes (n; %)	21; 28.38%	23; 28.40%	*P*=0.829
Atrial fibrillation (n; %)	22; 29.73%	33; 40.74%	*P*=0.393
Chronic kidney disease (n; %)	9; 12.16%	16; 19.75%	*P*=0.211
Beta-adrenolytics (n; %)	72; 97.30%	78; 96.30%	*P*=0.823
ACEI/ARB (n; %)	73; 98.65%	74; 91.36%	*P*=0.964
Statins (n; %)	61; 82.43%	57; 67.90%	*P*=0.791
Diuretics (n; %)	49; 66.22%	68; 83.95%	*P*=0.857
Aldosterone receptor antagonists (n; %)	46; 62.16%	59; 72.84%	*P*=0.575
Total cholesterol (mg/dL)	188.44±43.9	161.09±42.44	*P*=0.396
HDL cholesterol (mg/dL)	51.13±14.51	45.05±15.11	*P*=0.052
Triglycerides (mg/dL)	161.33±92.22	134.79±69.52	*P*=0.124
NT pro-BNP (pg/mL)	459.7 (193.9-1193)	3726 (983.7-7803)	***P*=0.001**
Creatinine (μmol/L)	95.58±60.77	102.11±32.36	*P*=0.238
eGFR (mL/min/1.73 m^2)	72.88±22.49	64.3±20.31	*P*=0.122
LVEDD (mm)	57.3±11.58	57.18±9.12	*P*=0.913
LVESD (mm)	45.09±13.75	48.05±10.87	*P*=0.636
LVEDV (mL)	134.21±51.06	154.18±72.87	*P*=0.067
LVEF (%)	34.31±11.81	30.14±13.4	*P*=0.307

Categorical variables are expressed as percentages. Continuous variables are expressed as mean and standard deviation or median with interquartile ranges. *P*-values <0.05 were considered statistically significant.
HF - heart failure; ACEI - angiotensin-converting-enzyme inhibitor; ARB - angiotensin II receptor blocker; HDL - high-density lipoprotein; NT-proBNP - N-terminal prohormone of brain natriuretic peptide; eGFR - estimated glomerular filtration rate; LVEDD - left ventricular end-diastolic diameter; LVESD - left ventricular end-systolic diameter; LVEDV - left ventricular end-diastolic volume; LVEF - left ventricular ejection fraction

(LVAD) compared with those with stable HF. Treatment with LVAD resulted in an increase of IL-33 and ST2 levels. ST2 expression, which is a receptor for IL-33, was proportional to markers of inflammation. These results suggested that IL-33/ST2 pathway might be important in the process of mechanical unloading and attenuating adverse remodeling (16). However, another study showed that IL-33 concentration was enhanced in HF, whereas its bioactivity was decreased (17). IL-33 concentrations were positively correlated with serum levels of oxidative stress markers. The IL-33/sST2 ratio was negatively proportional to malondialdehyde (MDA) level. IL-33 directly inhibited MDA and reactive oxygen species production and activated superoxide dismutase in myocardial cell culture stimulated by angiotensin II (17). IL-33 appears to have protective properties, and it could prevent from myocardial damage and perhaps HF development and progression. Individuals with low concentration of IL-33 more often develop HF. This may be explained why people with HF, including symptomatic patients, had decreased IL-33 level in our study. In our pilot study, a small number of patients were included, and there was no statistically significant correlation between cytokine concentration and NYHA functional classes in our study.

CRP is an acute-phase protein of hepatic origin, whose levels increase in response to inflammation following interleukin-6 secretion by macrophages and T cells. It is considered an unspecific marker of systemic inflammation, and increased levels are found in inflammation, bacterial or viral infections, burns, and pregnancy. Its concentration increases with age. Higher CRP levels are associated with increased risk of diabetes, hypertension, and cardiovascular disease and with higher mortality, especially cardiac mortality. However, a major cause of death among patients with higher CRP levels was not due to cardiovascular disease (18). CRP was not correlated with IL-33 concentration in our study. These findings might suggest that CRP circulating levels could be associated with the grade of inflammation, whereas IL-33 may be more correlated with the HF status and disease etiology, and its concentration may reflect not systemic but rather local myocardial inflammation. Another hypothesis is that HF causes ischemia and intestinal edema, allowing bacte-

Anatol J Cardiol 2019; 21: 305-13
DOI:10.14744/AnatolJCardiol.2019.64614

rial translocation and favoring the formation of endotoxins, contributing to an inflammatory state in individuals with HF. Intestinal blood flow is reduced in patients with HF. This may contribute to juxtamucosal bacterial growth and gastrointestinal symptoms in patients with advanced HF complicated by cachexia (19). This could explain why patients with HF may have increased CRP concentration, regardless of the IL-33 concentration.

Some studies revealed higher IL-33 concentrations in patients with diabetes mellitus than in subjects without diabetes. However, in our study, patients with diabetes were characterized by similar IL-33 levels to patients without diabetes. The explanation could be the fact that diabetes status correlated with myocardial impairment in the course of HF in these patients, and that IL-33 concentration reflects the multiple immunological processes contributing to the acute and chronic aspects of HF (20). Data regarding the association between IL-33 and chronic kidney disease are inconsistent. Several studies implied that IL-33 levels did not differ between patients with chronic kidney

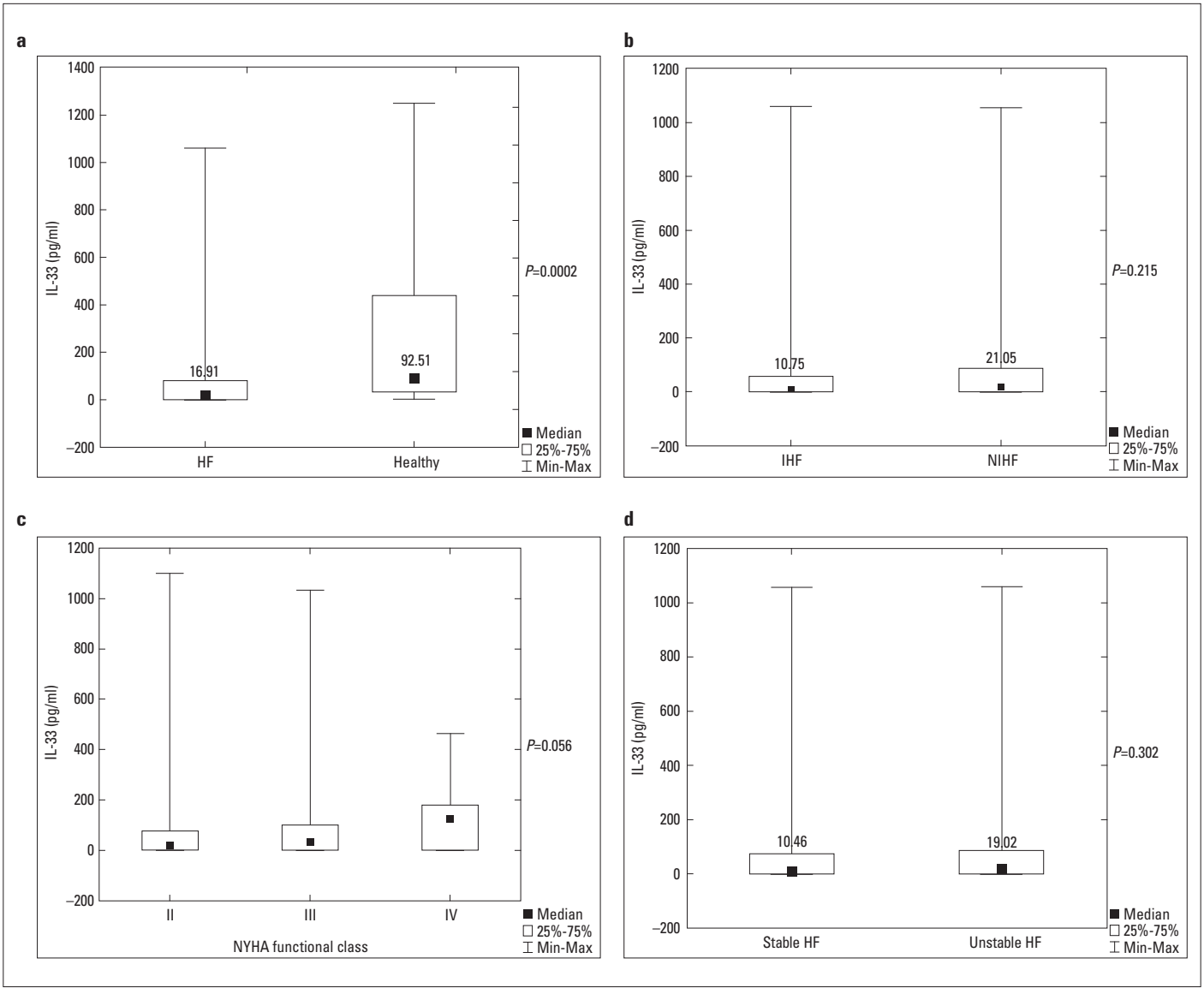

Figure 1. (a) The concentration of IL-33 in patients with HF versus healthy subjects. The concentration of interleukin-33 was statistically significantly lower in patients with HF than in healthy subjects, 16.91 (0–81.00) pg/mL and 92.51 (33.61–439.61) pg/mL, respectively. (b) The concentration of IL-33 in ischemic HF versus non-ischemic HF. Patients with HF with ischemic etiology had lower concentration of interleukin-33 (10.75 pg/mL) than subjects with HF with non-ischemic etiology (21.05 pg/mL). (c) The concentration of IL-33 in patients depending on NYHA functional class. Cytokine concentration did not differ significantly between patients in different NYHA functional classes. (d) The concentration of IL-33 in patients with stable HF versus unstable HF. Patients with stable HF (10.46 pg/mL) had lower IL-33 levels than those with unstable HF (19.02 pg/mL). (e) The concentration of IL-33 in patients with chronic kidney disease versus without chronic kidney disease. (f) The concentration of IL-33 in patients with diabetes versus without diabetes. (g) The concentration of IL-33 in patients with dyslipidemia versus without dyslipidemia. (h) The concentration of IL-33 in patients with atrial fibrillation versus without atrial fibrillation. Data are presented as median with interquartile ranges
HF - heart failure; IHF - ischemic heart failure; NIHF - non-ischemic heart failure

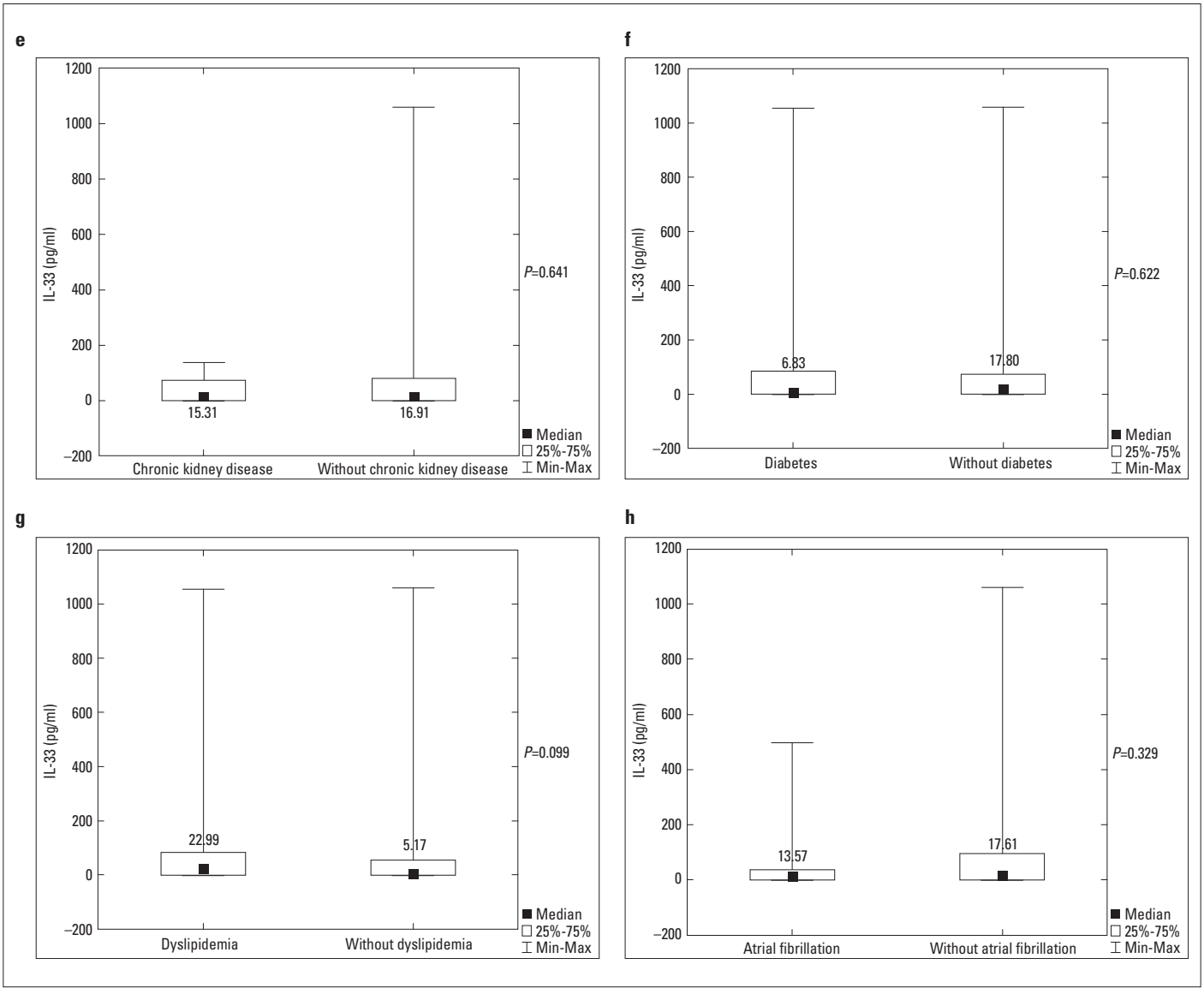

Figure 1. (a) The concentration of IL-33 in patients with HF versus healthy subjects. The concentration of interleukin-33 was statistically significantly lower in patients with HF than in healthy subjects, 16.91 (0–81.00) pg/mL and 92.51 (33.61–439.61) pg/mL, respectively. (b) The concentration of IL-33 in ischemic HF versus non-ischemic HF. Patients with HF with ischemic etiology had lower concentration of interleukin-33 (10.75 pg/mL) than subjects with HF with non-ischemic etiology (21.05 pg/mL). (c) The concentration of IL-33 in patients depending on NYHA functional class. Cytokine concentration did not differ significantly between patients in different NYHA functional classes. (d) The concentration of IL-33 in patients with stable HF versus unstable HF. Patients with stable HF (10.46 pg/mL) had lower IL-33 levels than those with unstable HF (19.02 pg/mL). (e) The concentration of IL-33 in patients with chronic kidney disease versus without chronic kidney disease. (f) The concentration of IL-33 in patients with diabetes versus without diabetes. (g) The concentration of IL-33 in patients with dyslipidemia versus without dyslipidemia. (h) The concentration of IL-33 in patients with atrial fibrillation versus without atrial fibrillation. Data are presented as median with interquartile ranges
HF - heart failure; IHF - ischemic heart failure; NIHF - non-ischemic heart failure

disease and healthy individuals. In addition, IL-33 levels were similar in three stages of kidney injury when they are divided into three groups according to their glomerular filtration rate (21, 22). In another study, IL-33 levels did not differ between multiple myeloma patients with and without kidney failure (23). However, in some trials, IL-33 increased with increasing stage of chronic kidney disease and was associated with vascular dysfunction and also a predictor of fatal and nonfatal cardiovascular events and survival (24).

The important role of IL-33 in the development and progression of HF with both ischemic and non-ischemic etiology was confirmed by several studies on animals. Mechanical stress was found to be the factor stimulating the synthesis of IL-33 in cardiac fibroblasts (25). IL-33 prevented from angiotensin II- and phenylephrine-induced hypertrophy. Moreover, IL-33 activated NF-κB and blocked phosphorylation of inhibitor of NF-κB caused by angiotensin II or phenylephrine (25). IL-33 treatment led to reduced hypertrophy, diminished fibrosis and cardiac remodeling pro-

Anatol J Cardiol 2019; 21: 305-13
DOI:10.14744/AnatolJCardiol.2019.64614

cesses, lower expression of natriuretic peptides RNA, and better survival after pressure overload in mice (25). IL-33 knockout mice, which underwent pressure overload, demonstrated more pronounced deleterious myocardial remodeling and hypertrophic changes, reduced fractional shortening, more severe inflammation, adverse fibrosis, lower survival rate, and increased level of natriuretic peptides RNA, including B-type natriuretic peptide and C-Myc. They had also enhanced Th1 cytokines' mRNA level and infiltration of inflammatory cells in the myocardium (26).

IL-33 has been also implicated in HF caused by ischemic disease. Cardiomyocytes incubated in hypoxia with IL-33 had upregulated level of antiapoptotic proteins, including cIAP1, XIAP, and survivin. Rats, which underwent ischemia and reperfusion, administered with IL-33 demonstrated reduced caspase production and, therefore, decreased apoptosis. This cytokine improved myocardial function, diminished infarct size, and adverse remodeling. IL-33 did not influence the course of ischemia–reperfusion injury in ST2 knockouts. These findings implied that cardioprotective properties of IL-33 are mediated through ST2 signaling (10). The concentration of IL-33 mRNA was upregulated in the myocardium up to 12 weeks after infarction, whereas sST2 expression was increased on week 1 and decreased at 4 weeks after infarction in studies on animals. The concentration of sST2 mRNA was proportional to the expression of inflammatory markers, including TNF-α, IL-6, MCP-1, and transforming growth factor-β, and markers of fibrosis (27).

ST2 knockouts presented more severe systolic dysfunction, adverse remodeling, left ventricular hypertrophy, myocardial fibrosis, and worse survival in both ischemic and non-ischemic HF (25). sST2, which is a soluble isoform of IL-33 receptor, competes with ST2L and inhibits its binding with IL-33. Increased serum ST2 concentration is a well-known predictor of adverse outcome and death in patients with both chronic and acute HF and myocardial infarction (28-30). Moreover, it predicts adverse cardiovascular outcomes in healthy individuals (31). In our study, the concentration of IL-33 was lower in patients with HF than in healthy subjects; however, patients with stable HF were characterized by lower IL-33 concentration than those with unstable HF, which appeared to challenge the hypothesis of a cardioprotective role of IL-33 in HF. In fact, the positive influence of IL-33 in these patients can be blocked by compensatory increased levels of sST2, which is a decoy receptor and inhibits IL-33. This thesis can be supported by several trials showing enhanced concentration of sST2 in HF, and the finding that sST2 is a predictor of adverse outcome in this disease.

IL-33 plays a dual role. Under certain circumstance, it can act both as a proinflammatory and as an anti-inflammatory protein. IL-33 appears to have protective properties, and it could prevent from myocardial damage and perhaps HF development and progression. Individuals with low concentration of IL-33 more often develop HF. This may be explained why patients with HF had decreased IL-33 level. Reduced IL-33 concentrations are responsible for increased sensitivity of the myocardium to ischemia–reperfusion injury. However, the direct effect of this cytokine probably depends on the pathway that it activates, whether it binds to ST2L receptor complex or regulates processes of transcription as an intracellular nuclear factor. IL-33 signaling regulates innate and adaptive immunity and acts as an "alarmin" after cellular damage, including necrosis. Its activation could be cardioprotective, leading to favorable outcomes, or may exacerbate disease, resulting in chronic inflammation, depending on the type of disease, time of its activation, and pathway, which it activates (32). Future investigations to understand the precise mechanisms underlying the role of IL-33 in HF and the clinical significance need to be conducted.

Conclusion

The concentrations of IL-33 were lower in HF patients compared to healthy controls, which may indicate an important role of above cytokine in HF development and progression. In addition, interleukin concentrations varied depending on the etiology and severity of the course of the disease. However, the detailed molecular mechanisms underlying the role of IL-33 as well as possible therapeutic options in heart failure remain to be elucidated.

Ethics Committee Approval: The study was approved by the Local Ethics Committee. All procedures followed were in accordance with the ethical standards of the Responsible Committee on Human Experimentation (Institutional and National) and with the 1975 Declaration of Helsinki, as revised in 2008.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – O.A.S.; Design – O.A.S.; Supervision – O.A.S., E.N.K., R.W.; Fundings – O.A.S., E.N.K., R.W.; Materials – O.A.S., E.R., C.W., A.P.; Data collection &/or processing – O.A.S., E.R., C.W., A.P.; Analysis &/or interpretation – O.A.S., E.R., C.W., A.P.; Literature search – O.A.S., C.W.; Writing – O.A.S., E.R.; Critical review – O.A.S., E.R., E.N.K.

References

1. Braunwald E. Heart failure. JACC Heart Fail 2013; 1: 1-20.
2. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, et al.; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. Circulation 2018; 137: e67-e492.
3. Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). Circulation 2001; 103: 2055-9.
4. Rauchhaus M, Doehner W, Francis DP, Davos C, Kemp M, Liebenthal C, et al. Plasma cytokine parameters and mortality in patients with chronic heart failure. Circulation 2000; 102: 3060-7.

5. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med 1990; 323: 236-41.
6. Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD). J Am Coll Cardiol 1996; 27: 1201-6.
7. Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT; Anti-TNF Therapy Against Congestive Heart Failure Investigators. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation 2003; 107: 3133-40.
8. Mann DL, McMurray JJ, Packer M, Swedberg K, Borer JS, Colucci WS, et al. Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). Circulation 2004; 109: 1594-602.
9. Roussel L, Erard M, Cayrol C, Girard JP. Molecular mimicry between IL-33 and KSHV for attachment to chromatin through the H2A-H2B acidic pocket. EMBO Rep 2008; 9: 1006-12.
10. Seki K, Sanada S, Kudinova AY, Steinhauser ML, Handa V, Gannon J, et al. Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. Circ Heart Fail 2009; 2: 684-91.
11. Rogala B, Glück J. The role of interleukin-33 in rhinitis. Curr Allergy Asthma Rep 2013; 13: 196-202.
12. Aoki S, Hayakawa M, Ozaki H, Takezako N, Obata H, Ibaraki N, et al. ST2 gene expression is proliferation-dependent and its ligand, IL-33, induces inflammatory reaction in endothelial cells. Mol Cell Biochem 2010; 335: 75-81.
13. Choi YS, Choi HJ, Min JK, Pyun BJ, Maeng YS, Park H, et al. Interleukin-33 induces angiogenesis and vascular permeability through ST2/ TRAF6-mediated endothelial nitric oxide production. Blood 2009; 114: 3117-26.
14. Miller AM, Xu D, Asquith DL, Denby L, Li Y, Sattar N, et al. IL-33 reduces the development of atherosclerosis. J Exp Med 2008; 205: 339-46.
15. Miller AM, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, McKenzie AN, et al. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. Circ Res 2010; 107: 650-8.
16. Caselli C, D'Amico A, Ragusa R, Caruso R, Prescimone T, Cabiati M, et al. IL-33/ST2 pathway and classical cytokines in end-stage heart failure patients submitted to left ventricular assist device support: a paradoxic role for inflammatory mediators? Mediators Inflamm 2013; 2013: 498703.
17. Zhang HF, Xie SL, Chen YX, Mai JT, Wang JF, Zhu WL, et al. Altered serum levels of IL-33 in patients with advanced systolic chronic heart failure: correlation with oxidative stress. J Transl Med 2012; 10: 120.
18. Yoshinaga R, Doi Y, Ayukawa K, Ishikawa S. High-sensitivity C reactive protein as a predictor of inhospital mortality in patients with cardiovascular disease at an emergency department: a retrospective cohort study. BMJ Open 2017; 7: e015112.
19. Pasini E, Aquilani R, Testa C, Baiardi P, Angioletti S, Boschi F, et al. Pathogenic Gut Flora in Patients With Chronic Heart Failure. JACC Heart Fail 2016; 4: 220-7.
20. Serrano-Ríos M, Corbatón A. Diabetes mellitus, heart failure and mortality. Med Clin (Barc) 2005; 125: 182-3.
21. Bao YS, Na SP, Zhang P, Jia XB, Liu RC, Yu CY, et al. Characterization of interleukin-33 and soluble ST2 in serum and their association with disease severity in patients with chronic kidney disease. J Clin Immunol 2012; 32: 587-94.
22. Caner S, Usluoğulları CA, Balkan F, Büyükcam F, Kaya C, Saçıkara M, et al. Is IL-33 useful to detect early stage of renal failure? Ren Fail 2014; 36: 78-80.
23. Musolino C, Allegra A, Profita M, Alonci A, Saitta S, Russo S, et al. Reduced IL-33 plasma levels in multiple myeloma patients are associated with more advanced stage of disease. Br J Haematol 2013; 160: 709-10.
24. Gungor O, Unal HU, Guclu A, Gezer M, Eyileten T, Guzel FB, et al. IL-33 and ST2 levels in chronic kidney disease: Associations with inflammation, vascular abnormalities, cardiovascular events, and survival. PLoS One 2017; 12: e0178939.
25. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. J Clin Invest 2007; 117: 1538-49.
26. Veeraveedu PT, Sanada S, Okuda K, Fu HY, Matsuzaki T, Araki R, et al. Ablation of IL-33 gene exacerbate myocardial remodeling in mice with heart failure induced by mechanical stress. Biochem Pharmacol 2017; 138: 73-80.
27. Sánchez-Más J, Lax A, Asensio-López Mdel C, Fernandez-Del Palacio MJ, Caballero L, Santarelli G, et al. Modulation of IL-33/ST2 system in postinfarction heart failure: correlation with cardiac remodelling markers. Eur J Clin Invest 2014; 44: 643-51.
28. Weinberg EO, Shimpo M, Hurwitz S, Tominaga S, Rouleau JL, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. Circulation 2003; 107: 721-6.
29. Rehman SU, Mueller T, Januzzi JL Jr. Characteristics of the novel interleukin family biomarker ST2 in patients with acute heart failure. J Am Coll Cardiol 2008; 52: 1458-65.
30. Boisot S, Beede J, Isakson S, Chiu A, Clopton P, Januzzi J, et al. Serial sampling of ST2 predicts 90-day mortality following destabilized heart failure. J Card Fail 2008; 14: 732-8.
31. Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, et al. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. Circulation 2012; 126: 1596-604.
32. Haraldsen G, Balogh J, Pollheimer J, Sponheim J, Küchler AM. Interleukin-33 - cytokine of dual function or novel alarmin? Trends Immunol 2009; 30: 227-33.