Am J Cardiovasc Dis 2021;11(6):704-709
www.AJCD.us /ISSN:2160-200X/AJCD0139709

Original Article

Circulating interleukin-17A in patients with acute and chronic coronary syndromes

Dinaldo C Oliveira[1], Carolina G C Oliveira[1], Edivaldo B Mendes Jr[1], Maria M Silveira[1], João V Cabral[1], Esmeralci Ferreira[2]

[1]Hospital das Clínicas, Federal University of Pernambuco, Pernambuco, Brazil; [2]Rio de Janeiro State University, Rio de Janeiro, Brazil

Received October 9, 2021; Accepted November 25, 2021; Epub December 15, 2021; Published December 30, 2021

Abstract: Introduction: Interleukin 17 (IL-17) is produced by Th17 and other cells. It is debatable whether IL-17 is atherogenic or atheroprotective. The role of this interleukin in the development and progression of coronary artery disease is unknown. Our aim was to evaluate if there were differences in serum IL-17A levels according to to clinical presentation of coronary artery disease. Methods: This cross-sectional study enrolled 101 patients with acute coronary syndrome (ACS), 100 patients with chronic coronary syndrome (CCS), and 70 healthy volunteers. Blood samples were collected from patients and controls (within 48 h) to analyze IL-17A levels. Clinical characteristics were recorded using questionnaires. This study was approved by the Ethics Committee. Results: Comparisons of the clinical characteristics between patients with ACS and CCS revealed the following: mean age (62 ± 12.4 years vs. 63.3 ± 9.8 years, P = 0.4), male (63.4% vs. 58%, P = 0.4), hypertension (85.1% vs. 79%, P = 0.1), dyslipidemia (48% vs. 31%, P = 0.01), diabetes mellitus (47.5% vs. 41%, P = 0.3), previous myocardial infarction (57.4% vs. 40%, P = 0.01), and smoking (29.7% vs. 38%, P = 1). The peripheral concentrations of IL-17A in ACS, CCS and controls were 5.36 ± 8.83, 6.69 ± 17.92, and 6.26 ± 11.13, respectively, with P = 0.6. In addition, the comparison between ACS and CCS showed: 5.36 ± 8.83 vs. 6.69 ± 17.92%, P = 0.3. Conclusion: The main finding of this study was that circulating IL-17 levels were similar in patients with ACS, CCS, and healthy volunteers. In addition, there was no difference between patients with ACS and those with CCS. Therefore, in patients with ACS and CCS, circulating IL-17A concentrations are low and there were no differences between patients with coronary artery disease and healthy individuals.

Keywords: Interleukin 17A, acute coronary syndrome, chronic coronary syndrome, coronary artery disease, atherosclerosis

Introduction

Acute coronary syndromes are primarily caused by coronary artery disease (CAD) and their high morbidity and mortality rates and high direct and indirect costs make them a global public health issue [1].

The pathology of CAD can be classified as either acute coronary syndrome (ACS) or chronic coronary syndrome (CCS). CAD can limit blood flow in the coronary arteries without lead to clinical symptoms, and deprive a significant amount of muscle of adequate blood supply. However, the actual impact of this finding remains debatable [2, 3].

Cardiovascular diseases (CVDs) are the leading cause of death worldwide, accounting for approximately 18.6 million deaths in 2019. CAD is responsible for the highest proportion of CVD-related deaths at 42.1%. The related direct and indirect costs in the United States of America are estimated to reach over 363 billion [1].

In Brazil, 4.3% of adults aged over 50 years suffer from CAD, and this disease is responsible for 13% of the total deaths here [4]. In 2021, between January 1st and September 27th, 298,538 deaths were reported due to cardiovascular disease in Brazil [5].

In patients with ACS fibrous cap rupture is no longer considered the main event leading to most acute presentations since it is now commonly believed that the erosion of endothelium

is the major triggering factor for acute events [6].

Apart from clinical manifestations, 12-lead electrocardiography and markers of myocardial necrosis are useful for diagnosing coronary syndromes. In the context of cardiac makers for ACS diagnosis, troponin is used as a standard marker, with creatine kinase-MB (CKMB) used when troponin is not available or in exceptional situations.

In addition to the markers known in clinical practice, other substances associated with the inflammatory response, such as cytokines, chemokines, miRNAs, and several others related to the various pathophysiological mechanisms implicated in ACS, have been increasingly investigated [6].

Interleukin-17 (IL-17) is one such marker, and there is an ongoing debate about whether its function is atherogenic or atheroprotective, with no definitive conclusions.

This study analyzed patients with ACS and CCS and healthy controls to comparatively assess the presence and amount of IL-17A in the peripheral blood.

Materials and methods

This was an observational, prospective, and analytical study conducted at a tertiary care hospital and basic science laboratory of a federal university.

The inclusion criteria were age over 18 years, diagnosis of ACS, CCS with refractory symptoms, or confirmed severe ischemia through a non-invasive functional test. The exclusion criteria were previous history of a lower limb, peripheral arterial, or aortic artery disease, active cancer, severe blood dyscrasia, psychiatric disorders, inability to answer questionnaires, life expectancy of less than 1 year, or participation in another study. Patients had to meet one of the inclusion criteria to be included in the study, and the presence of one of the exclusion criteria excluded them from the study.

Seventy healthy volunteers were recruited to establish reference values for normal IL-17A levels.

The clinical variables of interest in this study were collected using previously validated questionnaires. The researchers defined the study sample as 101 ACS patients, 100 CCS patients, and 70 controls.

IL-17 dosage protocol

Enzyme-linked immunosorbent assay (ELISA): IL-17A levels were measured by sandwich ELISA using an Invitrogen kit (Invitrogen, Massachusetts, USA). The plates were tested using a 10x coating buffer solution and capture antibodies from the kit. Each plate contained 5 mL of 10x coating buffer and 20 µL of antibody, with aliquots of 50 µL in each well; the plates were incubated overnight at 4°C. After incubation, the plates were washed thrice using a wash buffer (phosphate-buffered saline [PBS] + Tween 20).

The absorbance standard was prepared using an IL-17A lyophilized standard resuspended in enzyme-linked immunospot (ELISpot).

The standard was added to the plates (50 µL per well) from the lowest to the highest concentration well (3.9-500 pg/mL), which was the detection limit of the kit.

The detection antibody solution was prepared by mixing 20 µL of antibody with 5 mL of ELISpot in each plate, and then adding 50 µL per well, followed by incubation for 1 h at room temperature. The plates were then washed five times with the wash buffer, and 50 µL of a solution containing 20 µL Streptavidin-HRP enzyme diluted in 5 mL of ELISpot per plate was added per well, followed by incubation at room temperature in dark for 30 min.

The reaction was stopped with the STOP solution, and the absorbance of each well was read at 450 nm using an ELISA plate reader. After acquiring the absorbance data, the values were analyzed using an established protocol.

Ethical considerations

This study was conducted in compliance with the laws governing clinical research in Brazil and the ethical guidelines of the 1975 Declaration of Helsinki. This study was approved (number of the approval: 29977220.9.00-0051929) by the Ethics Committee of the com-

Table 1. Clinical characteristics: comparison between ACS and CCS patients

Variables	ACS group	CCS group	*p* value
Age (y)	62 ± 12	63.3 ± 9.8	0.4
Male (%)	64 (63.4)	58 (58)	0.4
Hypertension (%)	86 (85.1)	79 (79)	0.1
Diabetes Mellitus (%)	48 (47.5)	41 (41)	0.3
Dyslipidemia (%)	48 (47.5)	31 (31)	0.01
Stroke (%)	5 (4.9)	6 (6)	0.7

ACS: acute coronary syndrome; CCS: chronic coronary syndrome.

Table 2. Personnel history: comparison between ACS and CCS patients

Variables	ACS group	CCS group	*p* value
CABG (%)	13 (12.9)	63.3 ± 9.8	0.8
PCI (%)	47 (46.5)	58 (58)	< 0.001
MI (%)	58 (57.4)	79 (79)	0.01
Smoking (%)	30 (29.7)	41 (41)	0.01
Drunker (%)	3 (2.9)	13 (13)	0.009
Sedentary (%)	97 (96)	86 (86)	0.01

ACS: acute coronary syndrome; CCS: chronic coronary syndrome; CABG: coronary bypass graft; PCI: percutaneous coronary intervention; MI: myocardial infarction.

plex Hospital HUOC/PROCAPE, Recife, Pernambuco, Brazil.

Statistical analysis

Statistical analysis was performed after exporting the database that was initially built in Excel to SPSS version 21. The numerical variables were tested for normality using the Shapiro-Wilk test, with normal variables presented as mean and standard deviation, and non-normal variables as median and $25^{th}/75^{th}$ percentiles. Categorical variables were presented as absolute values and percentages. Descriptive and comparative analyses of the variables were performed. The chi-square test was used to compare categorical variables, while the Student's t-test, Mann-Whitney U test, or analysis of variance (ANOVA) were used for numerical variables. The *p*-value was considered significant if the *p*-value was < 0.05.

Patient recruitment process

Patient inclusion and exclusion criteria were analyzed in the catheterization laboratory, and those who met the criteria were invited to participate in the study. Those who accepted were informed about the research and informed consent forms were procured. Questionnaires were provided to collect clinical information, blood samples were collected, and patients underwent coronary angiography.

Results

Comparisons of the clinical characteristics between patients with ACS and CCS showed: mean age (62 ± 12.4 y vs. 63.3 ± 9.8 y; P = 0.4), male (63.4% vs. 58%; P = 0.4), hypertension (85.1% vs. 79%; P = 0.1), dyslipidemia (48% vs. 31%; P = 0.01), diabetes mellitus (47.5% vs. 41%; P = 0.3), previous myocardial infarction (57.4% vs. 40%; P = 0.01), and smoking (29.7% vs. 38%; P = 1).

Tables 1 and **2** show all comparisons of the study variables of interest between the ACS and CCS.

There were no reports of death, stroke, emergency revascularization surgery, or procedure-related AMI in hospitalized patients with ACS. One patient had a hematoma smaller than 5 cm (the procedure was performed via femoral access). Radial access route was used in 91% of the patients.

Among the hospitalized CCS patients, there were no reports of death, stroke, emergency revascularization surgery, procedure-related AMI, or complications at the vascular access site. Radial access route was used in 74% of the patients.

Table 3 shows the comparisons of IL-17A levels between the ACS, CCS, and control groups, as well as between the ACS and CCS groups separately. The peripheral concentrations of IL-17A in ACS, CCS and controls were 5.36 ± 8.83, 6.69 ± 17.92, and 6.26 ± 11.13, with P = 0.6. In addition, the comparison between ACS and CCS showed: 5.36 ± 8.83 vs. 6.69 ± 17.92%, P = 0.3.

Discussion

The main findings of this study were the absence of differences in the circulating IL-17A levels between patients with ACS, CCS, and healthy controls, and in the serum IL-17A levels between ACS and CCS patients. ACS patients had higher rates of dyslipidemia, previous coronary bypass graft, previous percutaneous coro-

Table 3. Interleukin-17A: comparison between patients with ACS, CCS, and healthy volunteers

Variables	ACS group	CCS group	Controls	*p* value
IL-17A	5.36 ± 8.83	6.69 ± 17.92	6.26 ± 11.13	0.6
IL-17A	5.36 ± 8.83	6.69 ± 17.92	----------	0.3

ACS: acute coronary syndrome; CCS: chronic coronary syndrome; IL: interleukin.

nary intervention, and sedentary lifestyle. On the other hand, patients with CCS had high rates of smoking and drinking.

The pathophysiology of coronary atherosclerosis is characterized by endothelial dysfunction, low-density lipoprotein (LDL), and immune and inflammatory responses, leading to atheroma formation within the intima [6-8]. In the context of coronary atherosclerosis, a better understanding of IL-17 and Th17 cells is still needed. The IL-17 family is composed of interleukins 17A, B, C, D, E, and F. Such interleukins can be produced by Th17 cells, γδ T cells, neutrophils, monocytes, and NK cells. Studies have shown that both IL-17A and IL-17F are present in atherosclerotic plaques. Some authors believe that IL-17 may be a link between innate and adaptive immunity [9].

Among other functions, IL-17 activates transcription factors (e.g., NK-κB), mitogen-activated kinases, and activator protein 1, stimulates epithelial cells, myeloid cells, and other interleukins, indirectly stimulates the attraction of neutrophils and monocytes, and induces the release of chemokines, production of metalloproteinases, and apoptosis of vascular cells. Therefore, considering the above, the debate on whether this interleukin is atherogenic or atheroprotective remains [8-15].

In this context, some researchers have investigated whether IL-17 could be detected in the peripheral blood of CAD patients and whether this finding would be related to a specific characteristic of CAD or its evolution [12, 15].

Data from patients with ACS and CCS who underwent coronary angiography revealed that circulating IL-17 levels were higher in patients with ACS [12, 16, 17].

On the other hand, Eid et al. investigated 108 patients who underwent coronary angiography and found no differences in serum IL-17 concentrations between CAD patients and controls and between ACS and CCS patients [18]. The study further revealed that there were no differences according to the angiographic severity of the disease.

A study by Patel et al. assessed 100 patients and found no IL-17 expression in patients with ACS [19]. The authors pointed out that the serum IL-17 detection protocol, time taken for blood sample collection (< 48 h vs. ≥ 48 h), the action of statins suppressing IL-17 expression, and ethnic group variations may account for the differences between the cited studies.

Another study used the serum bank of the French ACS registry [20] to investigate 981 patients with a 2 y clinical follow-up. The median IL-17 level of the patients was 6.26 pg/mL. At the end of the second year of follow-up, 176 patients (18%) had died or suffered a myocardial infarction. The authors noted that the prevalence of these events was lower in those with IL-17 levels below the median (21% vs. 15%, P = 0.02). An IL-17 level below the median of 6.26 pg/mL was associated with a greater risk of death or myocardial infarction, even after adjusting for confounding variables (HR = 1.4 [1.03-1.91], P = 0.03). In these patients, the blood samples used for interleukin detection were collected within the first 48 h.

Consistently, our study and others reveal that the levels of IL-17A in patients with CCS are not significant. Most patients in our study had no detectable levels of circulating IL-17 in the peripheral blood. Therefore, significant differences in the circulating IL-17A levels between patients with CCS and controls cannot be evaluated.

As for ACS patients, some studies investigating patients with angiographically documented CAD suggested an increase in circulating IL-17 and linked this finding with the potential proinflammatory and apoptotic effects of this interleukin [12, 16]. However, a study by Patel et al. [19] did not confirm these findings. The most appealing explanation for these differences is related to the time of blood collection (whether or not it was done within the first 48 h), since this is associated with acute inflammation [21].

In our study, blood samples were collected within the first 48 hours, and there was no increase in IL-17 levels in ACS patients.

The study of the French ACS refistry revealed that some patients with blood samples collected within the first 48 h express IL-17 while others do not. Therefore, relevant studies, including ours, are complementary in concluding that there are variations in the expression of IL-17 in ACS patients. It is necessary to have a better understanding of these variations, and the fact that statin intake by patients may account for these findings. In addition, the presence of other inflammatory and autoimmune diseases, the use of medications, and the patient's age group affect the circulating IL-17A levels.

Therefore, our study suggests that circulating IL-17A levels in ACS patients vary, with no uniform pattern that allows generalization. The reasons for such variations need to be better understood, along with the clinical impact of the presence or absence of this interleukin in the peripheral blood of patients with ACS.

Disclosure of conflict of interest

None.

Address correspondence to: Dinaldo C Oliveira, Hospital das Clínicas, Federal University of Pernambuco, Av. Prof. Moraes Rego, No. 1235, Recife, Pernambuco 50670-901, Brazil. E-mail: dinaldo@cardiol.br

References

[1] Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Cheng S, Delling FN, Elkind MSV, Evenson KR, Ferguson JF, Gupta DK, Khan SS, Kissela BM, Knutson KL, Lee CD, Lewis TT, Liu J, Loop MS, Lutsey PL, Ma J, Mackey J, Martin SS, Matchar DB, Mussolino ME, Navaneethan SD, Perak AM, Roth GA, Samad Z, Satou GM, Schroeder EB, Shah SH, Shay CM, Stokes A, VanWagner LB, Wang NY and Tsao CW; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2021 update: a report from the American Heart Association. Circulation 2021; 143: e254-e743.

[2] Collet JP, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, Dendale P, Dorobantu M, Edvardsen T, Folliguet T, Gale C, Gilard M, Jobs A, Jüni P, Lambrinou E, Lewis B, Mehilli J, Meliga E, Merkely B, Mueller C, Roffi M, Rutten F, Sibbing D and Siontis G; ESC Scientific Document Group. 2020 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J 2021; 42: 1289-1367.

[3] Knuuti J, Wijns W, Saraste A, Capodanno D, Barbato E, Funck-Brentano C, Prescott E, Storey RF, Deaton C, Cuisset T, Agewall S, Dickstein K, Edvardsen T, Escaned J, Gersh BJ, Svitil P, Gilard M, Hasdai D, Hatala R, Mahfoud F, Masip J, Muneretto C, Valgimigli M, Achenbach S and Bax JJ; ESC Scientific Document Group. 2019 ESC guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J 2020; 41: 407-477.

[4] Oliveira GMM, Brant LCC, Polanczyk CA, Biolo A, Nascimento BR, Malta DC, Souza MFM, Soares GP, Xavier Junior GF, Machline-Carrion MJ, Bittencourt MS, Pontes Neto OM, Silvestre OM, Teixeira RA, Sampaio RO, Gaziano TA, Roth GA and Ribeiro ALP. Cardiovascular statistics - Brazil 2020. Arq Bras Cardiol 2020; 115: 308-439.

[5] Cardiologia SBD. Cardiômetro 2021 [Available from: cardiometro.com.br].

[6] Libby P. The changing landscape of atherosclerosis. Nature 2021; 592: 524-533.

[7] Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, Tokgözoğlu L and Lewis EF. Atherosclerosis. Nat Rev Dis Primers 2019; 5: 56.

[8] Tousoulis D, Oikonomou E, Economou EK, Crea F and Kaski JC. Inflammatory cytokines in atherosclerosis: current therapeutic approaches. Eur Heart J 2016; 37: 1723-32.

[9] Gong F, Liu Z, Liu J, Zhou P, Liu Y and Lu X. The paradoxical role of IL-17 in atherosclerosis. Cell Immunol 2015; 297: 33-9.

[10] Coto E, Pascual I, Avanzas P, Cuesta-Lavona E, Lorca R, Martín M, Vázquez-Coto D, Díaz-Corte C, Morís C, Rodríguez-Reguero J and Gomes J. IL17RA in early-onset coronary artery disease: total leukocyte transcript analysis and promoter polymorphism (rs4819554) association. Cytokine 2020; 136: 155285.

[11] Garza-Reyes MG, Mora-Ruíz MD, Chávez-Sánchez L, Madrid-Miller A, Cabrera-Quintero AJ, Maravillas-Montero JL, Zentella-Dehesa A, Moreno-Ruíz L, Pastor-Salgado S, Ramírez-Arias E, Pérez-Velázquez N, Chávez-Rueda A, Blanco-Favela F, Vazquez-Gonzalez W and Contreras-Rodríguez A. Effect of interleukin-17 in the activation of monocyte subsets in patients with ST-segment elevation myocardial infarction. J Immunol Res 2020; 2020: 5692829.

[12] Hashmi S and Zeng QT. Role of interleukin-17 and interleukin-17-induced cytokines interleukin-6 and interleukin-8 in unstable coronary artery disease. Coron Artery Dis 2006; 17: 699-706.
[13] Mora-Ruíz MD, Blanco-Favela F, Chávez Rueda AK, Legorreta-Haquet MV and Chávez-Sánchez L. Role of interleukin-17 in acute myocardial infarction. Mol Immunol 2019; 107: 71-78.
[14] Taleb S, Romain M, Ramkhelawon B, Uyttenhove C, Pasterkamp G, Herbin O, Esposito B, Perez N, Yasukawa H, Van Snick J, Yoshimura A, Tedgui A and Mallat Z. Loss of SOCS3 expression in T cells reveals a regulatory role for interleukin-17 in atherosclerosis. J Exp Med 2009; 206: 2067-77.
[15] Tarantino G, Costantini S, Finelli C, Capone F, Guerriero E, La Sala N, Gioia S and Castello G. Is serum interleukin-17 associated with early atherosclerosis in obese patients? J Transl Med 2014; 12: 214.
[16] Cheng X, Yu X, Ding YJ, Fu QQ, Xie JJ, Tang TT, Yao R, Chen Y and Liao YH. The Th17/Treg imbalance in patients with acute coronary syndrome. Clin Immunol 2008; 127: 89-97.
[17] Min X, Lu M, Tu S, Wang X, Zhou C, Wang S, Pang S, Qian J, Ge Y, Guo Y, Xu D and Cao K. Serum cytokine profile in relation to the severity of coronary artery disease. Biomed Res Int 2017; 2017: 4013685.
[18] Min X, Lu M, Tu S, Wang X, Zhou C, Wang S, Pang S, Qian J, Ge Y, Guo Y, Xu D and Cao K. Interleukin-17 and interferon-γ are produced concomitantly by human coronary artery-infiltrating T cells and act synergistically on vascular smooth muscle cells. Circulation 2009; 119: 1424-32.
[19] Patel K, Murphy R, White M, Gasparro D, Kelleher D, Ryan T, McManus R and Ryan A. Interleukin 17: an unlikely marker of acute coronary syndrome? Atherosclerosis 2009; 205: 33-4.
[20] Simon T, Taleb S, Danchin N, Laurans L, Rousseau B, Cattan S, Montely JM, Dubourg O, Tedgui A, Kotti S and Mallat Z. Circulating levels of interleukin-17 and cardiovascular outcomes in patients with acute myocardial infarction. Eur Heart J 2013; 34: 570-7.
[21] Bochaton T, Mewton N, Thiam N, Lavocat F, Baetz D, Dufay N, Prieur C, Bonnefoy-Cudraz E, Miossec P and Ovize M. Early kinetics of serum interleukine-17A and infarct size in patients with reperfused acute ST-elevated myocardial infarction. PLoS One 2017; 12: e0188202.