Association of IL-10 to coronary disease severity in patients with metabolic syndrome

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ABSTRACT

Background: Metabolic syndrome (MetS) is a group of risk factors that increase the risk for heart disease. Little is known about the role of IL-10 in the severity of coronary artery disease (CAD) in patients with MetS. We investigated plasma levels of IL-10 and other pro-inflammatory cytokines in patients with MetS with or without severe CAD.
Methods: Cross-sectional study with healthy and MetS individuals. IL-10 and other pro-inflammatory interleukins were analyzed in 90 subjects divided into 3 groups: group 1 (n = 30), patients with MetS without severe CAD; group 2 (n = 30), patients with MetS and severe CAD (history of myocardial infarction or revascularization performed through surgery or percutaneous transluminal coronary angioplasty with or without stent placement); and group 3 (n = 30), healthy individuals.
Results: Levels of IL-12 (p = .018), TNF-α (p = .007) and IL-6 (p = .010) were significantly higher in group 1 when compared to group 3 (p = .003; p = .002; p = .001, respectively). In addition, group 1 presented significantly higher levels of IL-12 (p = .019), TNF-α (p = .026) and IL-6 (p = .020) when compared to group 2. IL-10 levels were significantly higher in group 1 (p = .003) when compared to group 2 (p = .014) and group 3 (p < .001). Only the level of IL-10 was significant to explain the presence of severe CAD, as a protective factor (OR: 0.896; 95%CI: 0.818–0.981) in the logistic regression model.
Conclusions: Higher IL-10 levels in patients with MetS are associated with lower incidence of severe CAD, suggesting a protective effect through its anti-inflammatory activity even in the presence of higher levels of pro-inflammatory cytokines.

1. Introduction

Metabolic syndrome (MetS) is a group of cardiometabolic risk factors that contribute to the accelerated development of atherosclerosis resulting in coronary artery disease (CAD) [1]. It is characterized by high morbidity and cardiovascular mortality [2]. Currently, the process of atherosclerosis is considered a chronic inflammatory disease including, among others, the expression of adhesion molecules by the inflamed endothelium and leukocyte migration into the intima, the absorption of cholesterol and modified lipoprotein particles and the formation of lipid-laden macrophages [3,4].

During the development of atherosclerotic lesions, T-lymphocytes join macrophages into the intima [5]. This pro-inflammatory cell infiltrate produces cytokines (including tumor necrosis factor (TNF),

interferons (IFNs) and interleukins (ILs)), but can also stimulate a Th2 response, which promotes anti-inflammatory cytokines activities such as IL-10 [6]. ILs are known to influence the cardiovascular system in either a harmful, pro-inflammatory way (IL-1β, IL-6, IL-8, IL-15, IL-17, and IL-18), or in a protective, anti-inflammatory manner (IL-10) [7].

IL-10 is an anti-inflammatory cytokine produced predominantly by macrophages and Th2 lymphocytes. In recent years, there has been evidence that IL-10 plays its anti-inflammatory role in many ways, including the inhibition of the activity of foam cells altering the lipid metabolism of these cells, and the reduction of the expression of metalloproteinase matrix, pro-inflammatory cytokines and cyclooxygenase-2 [8,9]. It also exerts anti-atherogenic effects during plaque development at different stages of atherosclerosis, once atherogenesis is initiated by the recruitment of monocytes into the intima, followed by

an inflammatory activation that differentiates the recruited monocytes to macrophages. The macrophages in the intima layer are incorporated into LDL, such as the particles of ox-LDL, thereby promoting cholesterol transport and the formation of foam cells in the core of the plaque [10]. Recent evidence shows that even in the presence of an inflammatory state, the increase of IL-10 is a protector against cardiovascular diseases [11].

However, little is known about IL-10 activity in CAD and MetS, as well as its relationship with the occurrence of severe ischemic heart disease. Since it inhibits cytokine synthesis, high levels of IL-10 could translate an anti-inflammatory effect, which might correlate with a better prognosis in patients with CAD. Inversely, low IL-10 levels may be associated with a worse clinical outcome in CAD. Thus, the aim of this study was to analyze plasma levels of IL-10, as well as other pro-inflammatory cytokines, such as TNF-α, IL-6, IL-18, IL-12, and IL-1β, in patients with MetS with or without severe CAD. We have also compared the cytokine inflammatory profile to healthy subjects.

2. Materials and methods

2.1. Patients and controls

This is a cross-sectional study in which individuals with or without MetS were included. A total of 90 subjects were recruited and divided in 3 groups: Group 1 (n = 30) presented MetS without severe CAD; Group 2 (n = 30) presented MetS with severe CAD; and Group 3 (n = 30) control healthy subjects. CAD was based on history of myocardial infarction confirmed by electrocardiogram, myocardial scintigraphy or cineangiocoronariography; or revascularization performed through surgery or percutaneous transluminal coronary angioplasty with or without stent placement. All patients with prior myocardial infarction had at least 3 months between the cardiovascular event and the selection.

Exclusion criteria from the study were conditions in which there is direct participation of the immune system, resulting in production of pro-inflammatory cytokines, such as hypothyroidism, kidney failure, liver disease, cancer, gout, sepsis and acquired immunodeficiency syndrome.

Abdominal obesity was diagnosed when the waist circumference (WC) was ≥80 for females and ≥90 for males. MetS was defined as the existence of abdominal obesity and at least 2 of 4 MetS components according to the definition by the International Diabetes Federation (IDF) [12]. All patients included in the study had been receiving statins before the enrollment. Other medication (beta-blockers, ACE inhibitors, ARBs, oral hipoglycemiants) did not differ between groups.

The study protocol (261.855) was approved by the Ethics Research Committee of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), each patient was fully informed about the study and signed the informed consent form prior to inclusion.

2.2. Cytokine analysis

Serum IL-6, IL-8, IL-1β, IL-18, TNF-α, IL-12 and IL-10 levels were measured using the Cytometric Bead Array Kit - CBA (BD Biosciences) and analysis was performed by flow cytometry. The results are expressed as ng/L and the detection limits for these interleukins were, respectively, 2.5, 3.6, 2.0, 3.7, 1.9 and 3.3 ng/L.

2.3. Biochemical analysis

Serum cholesterol, triglyceride and glucose measurements were performed using commercial kits from Labtest Diagnostica S.A. (Lagoa Santa-Minas Gerais- Brazil). The HDL-cholesterol assay method was performed by precipitation of LDL-cholesterol and VLDL-cholesterol. HDL-cholesterol was evaluated by the measurement of cholesterol in the supernatant and then VLDL-cholesterol and LDL-cholesterol were

calculated by the Friedewald formula. Patients with triglycerides above 400 mg/dL were excluded. The final results are expressed in mg/dL.

2.4. Statistical analysis

The Kolmogorov–Smirnov test was used to examine the normality of the variables. All data was presented as mean ± SD and median (interquartile range) for symmetric and asymmetric distributions, respectively. Statistical data treatment was performed by Kruskal-Wallis, one-way ANOVA, Pearson's chi-square test or Fisher's exact test, whenever appropriate and otherwise indicated. Tukey's Post Hoc test was used in multiple comparisons. In addition, binary logistic regression was used to estimate the effects of interleukin plasma levels to identify the highest impact factors to describe severe CAD. A multivariate logistic regression for all interleukins, including sex, age and BMI as covariates was also performed. Values were expressed as odds ratio (OR) with 95% confidence intervals. In all cases significance was set at p < .05.

3. Results

A total of 90 subjects, 30 in each group was included. Baseline anthropometric, biochemical and sociodemographic characteristics showed significant differences between groups (Table 1). Regarding age, groups 1 and 2 were significantly older when compared to group 3 (p < .001). Anthropometry and central obesity evaluation revealed significant differences for BMI (p < .001) and WC (females; p = .001) indicating higher values in groups 1 and 2 when compared to group 3. The WC for males was significantly higher in group 1 when compared to group 3 (p = .003), but did not differ significantly from group 2.

Regarding the laboratory test results, the mean levels of total cholesterol were significantly higher in group 1 when compared to group 2 (p = .018). HDL did not differ between groups in females. However, in males, a lower concentration was found in group 1 compared to groups 2 and 3, as well as in group 2 compared to group 3 (p < .001). The serum levels of TG were significantly higher in group 1 when compared to group 3 (p = .001). As for the LDL, significant higher levels were found in groups 1 and 3 when compared to group 2 (p < .001). Glucose was significantly higher in groups 1 and 2 when compared to group 3 (p = .001).

Table 2 shows the description of drug use in the study population. Regarding statin use, 28 (93.3%) patients in group1 and 30 (100%) patients in group 2 were using the medication, indicating that there was no significant differences between groups (p = .492). Only Antiplatelet agents and beta-blockers were more used in group 2 patients when compared to group 1, justified by previous coronary events. As to other medications (angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, oral hypoglycemic agents and insulin) there were no differences between groups, which demonstrates balance in the treatment of both groups, with and without previous events in patients with MetS.

The results of the ILs measurements are shown in Table 3. Levels of IL-12 (p = .018), TNF-α (p = .007) and IL-6 (p = .010) were significantly higher in group 1 when compared to groups 2 (p = .019; p = .026 and p = .020, respectively) and 3 (p = .003; p = .002 and p = .001, respectively). IL-10 levels were significantly higher in group 1 (p = .003) when compared to groups 2 (p = .014) and 3 (p < .001). In addition, IL-8 was significantly higher (p = .018) in groups 1 and 2 in comparison to group 3. Regarding IL-1β and IL-18 levels, there was no significant differences between groups.

A univariate binary logistic regression model was used to evaluate the influence of ILs (IL-6, IL-1β, IL-8, IL-10, IL-12 and TNF-α) on the presence of CAD. Results have shown that IL-10 was the only significant IL to explain the presence of severe CAD, presenting a protective effect (OR: 0.896; 95%CI: 0.818–0.981) (Table 4). In addition, a multivariate logistic regression including all ILs measured and also including sex, age and BMI as covariates was performed and confirmed that IL-10 was

Table 1
Baseline characteristics of the studied population.

	Patients (n = 90)			p
	Group 1, n = 30	Group 2, n = 30	Group 3, n = 30	
	MetS without severe CAD	MetS with severe CAD	Control	
Age, years	64.3 ± 1.4a	61.5 ± 1.4a	49.5 ± 1.3b	< **0.001**[c]
Gender[a]				
Females	19 (63.3%)	19 (63.3%)	19 (63.3%)	> 0.999[b]
Males	11 (36.7%)	11 (36.7%)	11 (36.7%)	
BMI (kg/m^2)	30.6 ± 0.8a	30.1 ± 0.7a	24.0 ± 0.5b	< **0.001**[c]
WC (cm)				
Females	100.7 ± 3.1a	99.9 ± 3.4a	86.7 ± 0.6b	**0.001**[c]
Males	112.0 ± 2.6a	106.0 ± 2.2ab	101.2 ± 0.5b	**0.003**[c]
Hypertension[a]	30 (100.0%)	30 (100.0%)	- - -	> 0.999[b]
Diabetes Mellitus[a]	18 (60.0%)	25 (83.3%)	- - -	0.079[b]
Cholesterol (mg/dL)	190.9 ± 10.7a	158.5 ± 6.5b	174.9 ± 5.3ab	**0.018**[c]
HDL (mg/dL)				
Females	48.2 ± 3.4	46.3 ± 2.3	52.8 ± 2.3	0.232
Males	33.9 ± 2.5c	41.8 ± 1.3b	50.1 ± 1.9a	< **0.001**[c]
Triglycerides (mg/dL)	283.0 ± 46.1a	204.3 ± 19.3ab	125.0b ± 3.8b	**0.001**[c]
LDL (mg/dL)	128.9 ± 6.6a	101.7 ± 3.7b	113.6 ± 1.7a	< **0.001**[c]
Glucose (mg/dL)	130.0 ± 9.94a	145.5 ± 11.97a	98.15 ± 0.95b	**0.001**[c]

Bold indicates significant difference.
[a] Percentage based on the total number of valid cases for the sample.
[b] Pearson's Chi-square Test.
[c] One Way ANOVA - Post Hoc Tukey. Means followed by the same letters, on the same line, do not differ in a significance level of 5%.

Table 2
Use of drugs in the study population.

Drugs used	Group 1		Group 2		p[a]
	MetS without severe CAD		MetS with severe CAD		
	n	%	n	%	
Beta-blockers	22	73.3	29	96.7	**0.026**
Antiplatelet agents	20	66.7	29	96.7	**0.003**
Statin	28	93.3	30	100.0	0.492
ACEIs/ARBs	27	90.0	26	86.7	1.000
Oral hypoglycemic agents	13	43.3	17	56.7	0.302
Insulin	5	16.7	8	26.7	0.347

ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers. Bold indicates significant difference.
[a] Fisher's exact test.

the only significant (p = .02) factor in the model.

Considering that only IL-10 was significant to indicate the incidence of severe CAD, patients were categorized in quartiles according to its

Table 4
Logistic regression analysis of interleukin levels to identify the severe CAD group.

Variable[A]	OR	95%CI	p
IL-1β	0.997	0.943–1.054	0.916
IL-12	0.910	0.679–1.219	0.526
IL-6	0.963	0.741–1.253	0.781
IL-10	0.896	0.818–0.981	**0.018**
IL-8	1.023	0.982–1.066	0.280
IL-18	0.998	0.992–1.004	0.540
TNF-α	1.014	0.979–1.050	0.435

OR: Odds ratio. Model parameters: ([A]) R^2 de Nalgelkerke = 0.206; Test Hosmer-Lemeshow. p = .491. Bold indicates significant difference.

values (Fig. 1). Data demonstrated that the smaller the IL-10 levels, the greater the risk (OR) to belong to group 2. In the 1st quartile (q1), the risk for severe CAD was almost five times higher when compared to the 4th quartile (q4), which is the group least likely to develop a cardiovascular event or need revascularization.
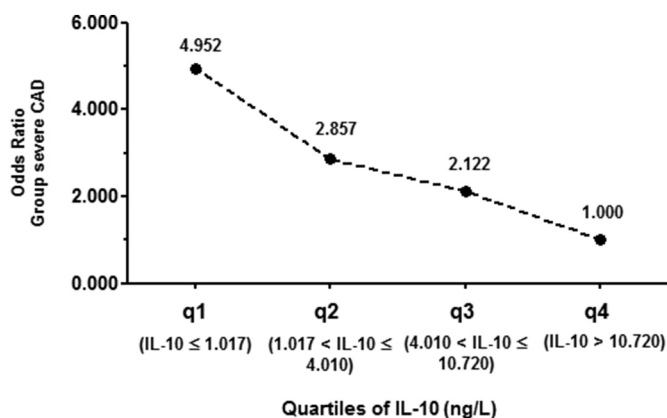
Table 3
Serum levels of interleukins in patients with MetS, MetS with severe CAD and control.

Interleukins	Patients (n = 90)						p[a]
	Group 1, n = 30		Group 2, n = 30		Group 3, n = 30		
	MetS without severe CAD		MetS with severe CAD		Control		
	Mean ± SD	Median (IQ range)	Mean ± SD	Median (IQ range)	Mean ± SD	Median (IQ range)	
IL-8 (ng/L)	23.56 ± 2.6a	18.04 (13.51–31.50)	23.32 ± 3.3a	14.93 (10.29–30.29)	14.61 ± 2.2b	9.80 (7.94–17.43)	**0.018**
IL-12 (ng/L)	7.74 ± 1.7a	4.97 (0.37–12.84)	3.10 ± 0.8b	1.16 (0.04–4.72)	2.10 ± 0.9b	0.04 (0.04–1.05)	**0.018**
TNF-α (ng/L)	78.35 ± 14.0a	56.90 (22.49–108.88)	39.98 ± 8.3b	22.54 (4.07–61.93)	26.80 ± 7.0b	15.94 (4.07–29.83)	**0.007**
IL-6 (ng/L)	6.50 ± 1.3a	3.60 (0.70–9.79)	2.96 ± 0.6b	1.75 (0.02–5.25)	1.80 ± 0.7b	0.02 (0.002–1.84)	**0.010**
IL-1β (ng/L)	17.46 ± 3.3	11.63 (5.34–15.71)	13.45 ± 2.7	11.63 (0.88–21.32)	13.40 ± 2.7	7.34 (1.99–20.97)	0.583
IL-18 (ng/L)	214.58 ± 19.9	186.36 (146.43–264.11)	205.86 ± 11.9	189.50 (160.11–258.51)	175.70 ± 18.6	162.43 (114.71–190.01)	0.302
IL-10 (ng/L)	10.61 ± 1.5a	9.40 (3.41–15.71)	5.86 ± 0.9b	4.56 (1.17–8.37)	4.14 ± 0.9b	2.05 (1.17–5.63)	**0.003**

Bold indicates significant difference.
[a] One Way ANOVA - Post Hoc Tukey. IQ: interquartile. Means followed by the same letters, on the same line, do not differ in a significance level of 5%.

Fig. 1. Patients with lower levels of IL-10 present higher risk for severe CAD.

4. Discussion

MetS is a multifactorial condition that initiates from obesity and results in a systemic oxidative stress linked to a chronic low-grade inflammation. This condition results in endothelial dysfunction, altered lipid metabolism and affects insulin sensitivity [1]. MetS inflammation and abdominal adiposity appear to play a critical role in the development and progression of cardiometabolic risk factors, since the atherosclerotic process arises not only from the accumulation of lipids, but also as result of endothelial dysfunction and activation of the inflammatory system [13]. As inflammation and thrombosis are associated pathologies in the development of atherosclerosis, measurements of inflammatory markers can predict severe CAD risk and prognosis [13]. In this context, this study evaluates pro (IL-8, IL-12, TNF-α, IL-6, IL-1β, IL-18) and anti-inflammatory (IL-10) cytokines as potential plasma biomarkers for CAD risk in patients with MetS. Results have shown that IL-10 was the only significant IL in the prediction of severe CAD, presenting a protective role.

Evidences suggest that elevated plasma levels of IL-8 are associated with an increased risk of CAD in apparently healthy individuals [14,15]. This effect is justified by the fact that IL-8 plays a key role in monocyte migration into the subendothelial space, a crucial process for the initial stages of atherosclerosis [16]. Besides that, several types of cells involved in atherosclerosis produce IL-8, such as endothelial cells, smooth muscle cells and monocytes from peripheral blood [14,15,17,18]. In this study we have found an IL-8 (pro-inflammatory cytokine) increase in patients with MetS when compared to controls, although no differences were found between patients with or without severe CAD.

We have also observed that high levels of IL-12, TNF-α and IL-6 were found in the group of patients with MetS without severe CAD. There is a strong correlation between IL-12 and TNF-α, considering that IL-12 is a cytokine with the ability to induce IFN-γ synthesis in T cells and NK cells [19–22]. IFN-γ is a key mediator in the release of TNF-α by activated macrophages [23]. Studies have shown that in obese patients, when a high fat diet consumption is present, there is activation of pro-inflammatory TH1 cells and macrophages, with subsequent production of IFN-γ, TNF-α and IL-12 [24–26]. TNF-α is a potent cytokine that induces mainly IL-6 production in obese individuals. While many ILs, such as IL-4, IL-5, IL-12, IL-13 and IFN-γ, increase in the presence of MetS, there is evidence highlighting the significant increase of IL-12 in obese and sedentary individuals [27]. Bennet et al. [28] have also demonstrated that elevated levels of TNF-α are associated with increased risk of acute myocardial infarction (AMI) and that obese individuals or individuals that present cytokine polymorphisms have elevated levels of TNF-α and are particularly susceptible.

An interesting finding of present study is that IL-6 serum levels presented lower values in patients affected by severe CAD, similar to the control group. This may be related to clinical stabilization through optimal medical treatment. Studies have shown that in apparently healthy men with high levels of IL-6, there is an association with increased risk of future myocardial ischemia [29,30]. For that, IL-6 has been considered an independent risk factor for coronary artery disease and a great inflammatory marker of atherosclerosis [31]. On the other hand, Anderson et al. [32] point that there is no causal relation of IL-6 and high sensibility c-reactive protein (hs-CRP) with the inflammatory burden in patients with CAD, although they demonstrate increased levels at the time and 24 h after AMI. Bennermo et al. [33] in a study that analyzed IL-6 levels found that patients with cardiac event (recurrent angina, re-hospitalization and myocardial infarction) achieved a significant decrease in the IL-6 levels between discharge and three months of follow-up.

The emergence of chronic sterile inflammation during obesity, in the absence of explicit infection or autoimmune process, is a puzzling phenomenon. The Nod-Like Receptor (NLR) family of innate immune cell sensors, like the NLRP3 inflammasome, is implicated in recognizing certain non-microbial origin danger–signals leading to caspase-1 activation and subsequent IL-1β and IL-18 secretion. In particular, IL-1β is an early and prominent mediator of inflammatory responses in myocardial infarction. However, results suggest that atherogenesis can progress independently of the NLRP3 inflammasome [34]. Our results show that serum levels of IL-1β and IL-18 did not present differences between the studied groups, evidencing that the NLRP3 inflammasome may not be involved in the cardiovascular alterations present in individuals with MetS.

In the past years, the great majority of studies on cardiovascular disease have focused in pro-inflammatory cytokines. However, at present, anti-inflammatory cytokines, especially IL-10 has gained prominence and may have an important role in the pathophysiology of MetS and cardiovascular disease. IL-10 is an anti-inflammatory cytokine with a great variety of anti-atherogenic properties. Some of these properties may occur through several mechanisms, including the inhibition of endothelial infiltration by macrophages/monocytes, the inhibition of NF-κB activation [35], which regulates the expression of many pro-inflammatory molecules-signals, the reduction of matrix metalloproteinases (MMP) production, the inhibition of tissue factor and cyclooxygenase-2 expression and, finally, the inhibition of cell death and apoptosis [10]. In our study, higher IL-10 levels in patients with MetS are associated with lower incidence of severe CAD, suggesting a protective effect through its anti-inflammatory activity even in the presence of higher levels of pro-inflammatory cytokines. On the contrary, lower IL-10 levels are associated with a higher occurrence of severe CAD in this population. Other studies report similar results to ours, showing that low levels of IL-10 were associated with an increased risk of future cardiovascular events and high IL-10 levels were associated with a reduction of these events [36–38].

Regarding statins, a lipid-lowering and anti-inflammatory drug that could interfere with the results, 93.3% of the patients [28] in group 1 and 100% of patients [30] in group 2 were using statin at the highest tolerated dose, associated with fibrates in patients who maintained triglycerides above 200 mg/dL, which demonstrates that both groups with MetS, were oriented to the best available treatment and that there were no significant differences between the treatments of the patients participating in the study. Regarding the hypoglycemic agents, which can also alter the pro/anti-inflammatory balance, our data show that there are no significant differences in the use of these drugs among patients with MetS who participated in the study.

Our study also present limitations, including the limited number of subjects in each group. In addition, even though no differences were found between groups regarding the use of statins, it is possible that this treatment may have influenced the results presented. However, we believe that, in despite of this, our data was able to demonstrate an association of IL-10 with severe CAD in patients with MetS.

Higher IL-10 levels in patients with MetS are associated with lower

incidence of severe CAD, suggesting a protective effect through its anti-inflammatory activity even in the presence of higher levels of pro-inflammatory cytokines.

Conflict of interest

The manuscript is an original work that has not been published and is not under consideration for publication elsewhere in whole or in part in any language. There was no commercial association that might pose a conflict of interest in connection with this manuscript.

Author contributions

Barcelos, A.L.V., Oliveira, E.A., Hauter, G.V., Pedrazza, L. performed laboratory analysis. Barcelos, A.L.V. performed statistical analysis und wrote the manuscript. Bodanese, L.C. investigated study subjects. Costa, B.P. contributed to discussion. Donadio, M.V.F performed statistical analysis and revised the manuscript. Oliveira, J.R. designed the study and wrote the manuscript.

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