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High levels of C-Reactive Protein are Associated with Ischemic Stroke Short-term Outcome in Patients with the T Allele of the *CRP* rs1130864 Variant

Daniela Frizon Alfieri[1]; Tamires Flauzino[1]; Francieli Delongui[1]; Márcio Francisco Lehmann[2]; Maria Caroline Martins De Araújo[3]; Rafaele Maria Tirolla[4]; Nicolas Pivoto[5]; Elaine Regina Delicato de Almeida[1,6]; Andréa Name Colado Simão[1,6]; Mariana Ragassi Urbano[7]; Edna Maria Vissoci Reiche[1,6]*

[1]Laboratory of Research in Applied Immunology, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil.

[2]Department of Clinical Surgery, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil.

[3]Outpatient Clinic for Neurology, University Hospital, State University of Londrina, Londrina, Paraná, Brazil.

[4]Pharmacy School, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil.

[5]Medicine School, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil.

[6]Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil.

[7]Department of Statistics, State University of Londrina, Londrina, Paraná, Brazil.

***Corresponding Author(s): Edna Maria Vissoci Reiche**

Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, Londrina State University, Av. Robert Koch, 60, CEP 86.038-440, Londrina, Paraná, Brazil.

Tel: +55-43-3371-2619, Fax: + 55-43-3371-2619;
Email: reiche@sercomtel.com.br

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Abstract

Objective: C-reactive protein (CRP) has been associated with Ischemic Stroke (IS) risk and short-term outcome. We evaluated the association between the *CRP* rs1130864 variant (+1444 C>T) with susceptibility and short-term outcome of IS and levels of CRP.

Methods: We enrolled 168 IS patients and 166 controls. Baseline characteristics and blood samples were obtained up to 24 hours of hospital admission. The disability was evaluated using the Modified Rankin Scale (mRS) after three months and categorized as mild (mRS<3) and moderate/severe (mRS≥3). The rs1130864 genotyping was determined using polymerase chain reaction and restriction fragment length polymorphism. Serum levels of CRP were determined using high sensitivity turbidimetric assay (hsCRP).

Results: Sex, hypertension, smoking and hsCRP levels were associated with IS. The median of hsCRP was 7.5 mg/L in IS patients and 1.6 mg/L in controls (p<0.001). The rs1130864 genotype distribution did not differ between the groups. However, controls carrying the T allele (CT+TT genotypes) showed higher hsCRP (p=0.005) and more frequency of hsCRP ≥3 mg/L than those carrying the CC genotype (p=0.045). Age and hsCRP predicted moderate/severe disability after three-month only in patients carrying the T allele (p<0.001).

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Conclusion: The rs1130864 *CRP* variant, by itself, may be not a determinant factor for IS susceptibility, as well as for the hsCRP levels in Brazilian IS patients, but in those carrying the T allele, the high levels of hsCRP were associated with poor short-term outcome. The role of CRP as a predictor for IS short-term outcome may differ according to the individual's genotype.

Introduction

Stroke is a major cause of disability and mortality worldwide and has significant clinical and socioeconomic impact. About 80% of all strokes are Ischemic Stroke (IS) due to arterial vascular occlusion caused by any or the combination of unmodifiable risk factors, such as age, sex and genetic variants, as well as by modifiable risk factors, such as hypertension, Type 2 Diabetes Mellitus (T2DM), dyslipidemia, sedentary lifestyle, smoking and obesity [1].

Inflammation plays a key role in the pathogenesis of cerebral ischemic injury through the elevation of the inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 [2]. The IL-6 induces the expression of some acute phase proteins, such as C-Reactive Protein (CRP) that is a sensitive biomarker of inflammation and tissue damage. Increased levels of CRP are independently associated with IS risk in worldwide population [3,4], unfavorable short-term outcome [5,6] and short-term mortality [7]. However, other studies reported that the association of CRP with clinical outcomes disappeared after adjusting for confound variables [8,9].

The combination of sociodemographic and lifestyle factors (smoking, alcohol intake and hormone replacement therapy), obesity and fat patterning and T2DM explained 13-30% the interindividual variability of the baseline CRP levels [10]. On the other hand, heritability estimates suggest that 35-60% of the variance in baseline CRP levels are attributable to genetic variation [10,11]. Genetic variations are very common at *CRP* and each of them seems to affect the CRP serum levels at different ways [12-14]. The rs1130864 variant in the *CRP* is located in the 3'-Untranslated Region (UTR) and consists in a substitution of C to T at +1444 position [15]. The T variant allele was associated with higher serum levels of CRP than the C allele in different inflammatory conditions, such as cardiovascular disease [16], periodontitis [17], T2DM [18] and systemic lupus erythematosus [19]. However, the role of this variant on the CRP serum levels and its association with IS are still unclarified [20-26]. Therefore, the aim of the present study was to evaluate the association between the rs1130864 variant in the *CRP* with the susceptibility for IS and short-term outcome, as well as with the serum levels of hsCRP.

Methods

Study Subjects

The protocol was approved by the Institutional Research Ethic Committee of the State University of Londrina, Paraná State, Brazil (CAAE 0250.0.268.000-11) and a written consent form was obtained from all the individuals. A total of 168 patients with IS were consecutively recruited during 2013-2015 from the Emergency Room of the University Hospital of State University of Londrina, Southern Brazil. The baseline characteristics of the patients with IS were described elsewhere [5,7]. Briefly, the patients were diagnosed with focal neurological signs or symptoms thought to be of vascular origin that persisted for >24 hours using brain Computed Tomography (CT) and clinic examination in baseline conditions. The IS subtypes were defined using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria [27], which was conducted by two training neurologists. As controls, 166 consecutively healthy volunteers with no history of stroke and/or myocardial infarction were enrolled in the same period. Patients and controls with fever, acute infections, hematological, inflammatory or autoimmune diseases, renal or liver failure, cancer, cerebral hemorrhage and using inflammatory and/or antioxidant supplementation were excluded. A standard questionnaire was used at the admission of the individuals in the study to obtain demographic, anthropometric and clinical data of traditional risk factors for IS and the use of any therapeutic drugs (antihypertensive, lipid-lowering and hypoglycemic) before the inclusion in this study. Body weight (kg) and height (cm) were reported by the individuals, when it was possible, or by the patient's family. Body Mass Index (BMI) was calculated as weight (kg) divided by height (cm) squared. The ethnicity was self-reported as Caucasian and non-Caucasian (Asiatic, Black and Afro-Brazilian) [28].

Baseline blood pressure evaluations were also obtained at the admission using digital apparatus properly calibrated and the mean of two measurements was used in the analysis. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg in the chronic stage, or as a previous history of treatment with antihypertensive drugs [6,29]; T2DM was defined as a fasting serum glucose ≥126 mg/dL, a non-fasting serum glucose ≥200 mg/dL and/or use of hypoglycemic medication [30]; dyslipidemia was defined by the presence of one or more than one of the abnormal serum lipid concentration: Total cholesterol ≥200 mg/dL, low-density lipoprotein cholesterol ≥130 mg/dL; high-density lipoprotein cholesterol <40 mg/dL, triglycerides >150 mg/dL [31]. Smoking was defined as current or former cigarette smoking and alcohol intake was defined as habitual consumption of alcohol beverages before onset of stroke [6].

The short-term outcome was the disability evaluated using the Modified Rankin Scale (mRS) after three-month follow-up [32], applied through clinical examination or using telephone interviews with the patients or their relatives [33]. The disability was categorized as mild (mRS<3) and moderate/severe (mRS ≥3) [34].

Inflammatory biomarkers

Peripheral blood samples were obtained under non-fasting state, with and without EDTA as anticoagulant. From the IS patients, the samples were obtained within 24 hours after the hospital admission; from controls, the samples were obtained at the time of inclusion in the study. Plasma, serum and buffy-coat were immediately separated by centrifugation (2,500 rpm for 15 min) and stored in aliquots at - 80°C until analyzes.

Plasma levels of IL-6 were determined using a sandwich enzyme-linked immunosorbent assay (ELISA, eBioscience, San Diego, California, USA). Serum levels of CRP were determined using high-sensitivity turbidimetric assay (hsCRP) with 0.175 mg/L as limit of detection (Architect c8000, Abbott, Abbott Park, IL, USA). The serum levels of hsCRP were further categorized as < 3 mg/L and ≥ 3 mg/L [35].

The rs1130864 variation in *CRP* gene

Genomic DNA was extracted from a buffy-coat of peripheral

blood cells using a resin column procedure (Biopur, Biometrix Diagnóstica, Curitiba, PR, Brazil). A 460 Base Pairs (bp) sequence of the *CRP* was amplified by Polymerase Chain Reaction (PCR) as previously reported [36] with some modifications. Briefly, the primers used were determined according to the GenBank n° M11880.1 [36]. PCR was performed with a final volume of 25 µL, with 0.15mM of each primer, 1.50 mM MgCl2, 0.10 mM dNTP, 1.25 units thermostable DNA polymerase (Invitrogen TM, Life Technologies, Carlsbad, CA, USA) and 100 ng of the genomic DNA sample. PCR conditions were performed in a thermocycler (Applied Biosystems Veriti TM 96-Well Thermal Cycler, Life Technologies, Foster City, CA, USA) and comprised of 5 min denaturation at 95°C for initial denaturation; 37 cycles of 45 sec at 95°C for denaturation, 45 sec at 56°C for the annealing and 45 sec at 72°C for the elongation; and 10 min at 72°C for final elongation. In all PCR analyses, a negative control (without a DNA sample) was included. The PCR products were subjected to Restriction Fragment Length Polymorphism (RFLP) analysis as described previously [19]. The C allele includes a restriction site for *Hpy*CH4III, which resulted in 311bp and 149bp fragments and the T allele does not include the restriction site for *Hpy*CH4III, resulting in a fragment of 460bp. The heterozygous genotype results in three fragments (460, 311 and 149bp).

Statistical analysis

Statistical analyses were performed to compare the variables between the two groups (IS patients and controls). Categorical variables were expressed and number (n) and percentage (%). The continuous variables were expressed as median and Interquartile Range (IQR) of 25% and 75%. The Chi-square test was also used to test Hardy-Weinberg Equilibrium (HWE). In the univariate analysis for the quantitative variables, the Wilcoxon test was used because the assumption for the t test was not attended (normality of the data in both groups). For the qualitative variables, the Chi-square test or Fisher exact test was used. After the univariate analysis, to identify the risk factors for IS, the multivariate logistic regression was assayed. Automatic stepwise regression analysis was employed to assess the most significant demographic/clinical and inflammatory biomarkers that predict mRS after three-month follow-up (data natural logarithm transformed). Odds Ratio (OR) and 95% Confidence Interval (CI) were also determined. The statistical significance level used was 0.05 and when the p-value was <0.05, there was difference between the variables in the groups. The analysis was performed in software R [37].

Results

Characteristics of the subjects

Table 1 shows the baseline characteristics of IS patients and controls. In the univariate analyses, the IS patients were more likely to be older and males with higher frequency of smoking, hypertension, T2DM, use of anti-hypertensive and hypoglycemic drugs compared to the controls. Moreover, the IS patients presented higher IL-6serum levels compared to the controls. The median (IQR) of hsCRP was 7.5 mg/L (2.6-24.8) in IS patients and 1.6 mg/L (0.7-3.7) in controls. While 118 (70.7%) patients presented hsCRP ≥3 mg/L, 44 (27.3%) controls presented hsCRP ≥3 mg/L. After multivariate analysis, some of these variables were estimated as independent predictors of IS, such as sex (male), hypertension, smoking and hsCRP serum levels. Each increase of 1 mg/L of hsCRP, the odds increases 1.23 times to the IS occurrence (95% CI 1.13-1.33). With this multivariate analysis, 77.6% of all IS patients were correctly classified with sensitivity of 79.2% and specificity of 76.1%. Further, the results of the multivariate analyzes were used to adjust the evaluation of the association between the rs1130864 variant and IS susceptibility.

As regard the stroke subtypes, 59 (35.1%) patients had large artery atherosclerosis stroke (LAAS), 51 (30.4%) lacunar infarct (LAC), 26 (15.5%) Cardio-Embolic Infarct (CEI), 6 (3.5%) other determined etiology (ODE) and 26 (15.5%) had Undetermined Etiology (UDE). After three-month follow-up, 90 (67.2%) patients showed moderate/severe disability with median mRS of 4.0 (IQR: 5.0-2.0).

3'UTR polymorphic variation (rs1130864) in *CRP* gene

The genotype distribution of rs1130864 in IS patients and controls were consistent with those expected from the HWE (p>0.05). No differences were observed in genotype distribution when evaluated in an additive as well as in a dominant model, among IS patients and controls before and after adjustment for sex, hypertension and smoking. The dominant model allowed to examine the association between the presence of T allele and hsCRP levels; therefore, this model was used for sequential analysis (CC vs CT+TT) (Table 2). The rs1130864 variant (CC vs CT+TT genotypes) was not associated with stroke subtypes (p>0.05) (data not shown). Moreover, the T allele was not associated with the short-term outcome (p>0.05) (Table 3).

The 3'UTR rs1130864 variant was not associated with clinical and IL-6 as well as hsCRP levels in patients with IS (p>0.05). However, controls carrying the T allele (CT+TT genotypes) showed higher hsCRP levels (p=0.005) and more frequency of hsCRP ≥3 mg/L (OR 2.03, 95% CI: 1.01-4.10, p=0.045) than those carrying the CC genotype (Table 4). In contrast, IL-6 levels did not differ regarding the genotypes of the IS patients.

In order to delineate the predictors to endpoint (mRS as continuous variables) in patients with CC genotype and CT+TT genotypes, we carried out two automatic stepwise univariate regression analyses with mRS short-term outcome values as dependent variables and the demographic and IL-6 and hsCRP levels as explanatory variables. (Table 5) shows that among the IS patients with CT+TT genotypes, 18.2% of the variance in endpoint disability were explained by age and hsCRP (positively related) (p<0.001). On the other hand, among IS patients with CC genotype, 16.5% of the variance in the endpoint disability were explained by hypertension (positively related) (p=0.001).

Table 1: Baseline characteristics of acute ischemic stroke patients and healthy controls from Brazilian population.

	Univariate			Multivariate	
Characteristics	**Healthy Controls (n=166)**	**Stroke Patients (n=168)**	***p* value**	**OR (95%CI)**	***p* value**
Age (year)	63.00 (53.00-73.00)	69.00 (59.00-77.00)	0.010		
Sex					

Female/Male	113 (68.07)/53 (31.93)	71 (42.26)/97 (57.74)	<0.001	11.8 (3.99-34.94)	<0.001
Ethnicity					
Caucasian	125 (75.30)	124 (76.54)	0.793		
Non-Caucasian	41 (24.70)	38 (23.46)			
BMI (kg/m^2)	26.00 (23.73-28.94)	25.39 (22.19-29.05)	0.107		
Hypertension	69 (43.67)	136 (82.42)	<0.001	5.84 (3.03-11.24)	<0.001
T2DM	36 (21.69)	65 (39.39)	<0.001		
Dyslipidemia	64 (40.51)	59 (35.76)	0.380		
Smoking	12 (7.23)	37 (22.56)	<0.001	3.13 (1.24-7.88)	0.015
Antihypertensive	63 (39.90)	124 (78.50)	<0.001		
Hypoglycemic	24 (15.19)	43 (27.74)	0.007		
Lipid-lowering	33 (20.89)	46 (29.68)	0.073		
hsCRP ≥ 3 (mg/L)	1.63 (0.76-3.77)	7.50 (2.60-24.80)	<0.001	1.23 (1.13-1.33)	<0.001
≥3 mg/L	44 (27.3)	118 (70.7)	<0.001		
IL-6 (pg/mL)	3.00 (1.00-5.00)	11.00 (5.00-26.00)	<0.001		

The continuous variables were expressed as median and interquartile range (25%-75%); the categorical variables were expressed as number (n) and percentage (%); OR: Odds Ratio; CI: Confidence Interval; BMI: Body Mass Index; T2DM: Type 2 Diabetes Mellitus; hsCRP: C reactive protein with high sensitivity assay; IL: Interleukin.

Table 2: Frequency of the 3'untranslated region variation (rs1130864) in *CRP* gene of patients with acute ischemic stroke and healthy controls from Brazilian population.

	Overall rs1130864 *CRP* genetic variant				
	Controls (n=166)	**Stroke patients (n=168)**	**p value**	**Adjusted OR (95% CI)[a]**	**Adjusted p value[a]**
Codominant model					
CC	91 (54.82)	95 (56.55)	0.911	Reference	0.750
CT	63 (37.95)	60 (35.71)		0.90 (0.48-1.69)	
TT	12 (7.23)	13 (7.74)		0.57 (0.16-1.94)	0.370
Dominant model					
CC	91 (54.82)	95 (56.55)	0.751	Reference	0.568
CT+TT	75 (45.18)	73 (43.45)		1.19 (0.65-2.17)	

a. Adjusted for sex, hypertension and smoking; categorical data were expressed as absolute number (n) and percentage (%); the distribution of genotypes and allelic frequencies were in Hardy-Weinberg Equilibrium in patients and controls (chi-square test, p>0.05). CI: Confidence Interval; OR: Odds Ratio.

Table 3: Frequency of 3'untranslated region variation (rs1130864) in *CRP* gene according to the modified Rankin Scale at short-term outcome of acute ischemic stroke patients.

	Short-term outcome			
	mRS <3	**mRS ≥3**	**P value**	**OR (95% CI)**
	(n=78)	**(n=59)**		
Codominant model				
CC	56 (60.20)	22 (50.0)	0.314	-
CT	28 (30.10)	19 (43.20)		1.72 (0.80-3.70)
TT	9 (9.00)	3 (6.80)		0.56 (0.11-2.83)

Dominant model				
CC	56 (71.79)	37 (62.71)	0.26	1.51 (0.73-3.11)
CT+TT	22 (28.21)	22 (37.29)		

Categorical data were expressed as absolute number (n) and percentage (%); mRS: modified Rankin Scale; mRS <3: mild functional impairment; mRS ≥3: moderate/severe functional impairment; CI: Confidence Interval; OR: Odds Ratio.

Table 4: Demographic, clinical and inflammatory biomarkers according to the 3'untranslated region variation (rs1130864) in the CRP gene in ischemic stroke patients and controls from Brazilian population.

	Controls			Stroke patients		
	CC (n=91)	**CT+TT (n=75)**	**P value**	**CC (n=95)**	**CT+TT (n=73)**	**P value**
Age (year)	65.00 (51.00- 74.00)	63.00 (55.00-71.00)	0.969	68.50 (59.00-77.00)	70.50 (60.0-77.50)	0.859
Sex						
Male/Female	34 (37.36)/57 (62.64)	19 (25.33)/ 56 (74.67)	0.098	53 (55.79)/ 42 (44.21)	44 (60.27)/ 29 (39.73)	0.56
Ethnicity						
Caucasian	65 (71.43)	15 (20.00)	0.203	66 (73.33)	58 (80.56)	0.281
Non-Caucasian	26 (28.57)	5 (80.00)		24 (26.67)	14 (19.44)	
BMI (kg/m^2)	25.46 (23.66-28.80)	26.94 (24.00-29.00)	0.301	25.40 (22.03-29.00)	24.46 (22.61-29.06)	0.551
Hypertension	34 (38.64)	35 (50.00)	0.153	82 (87.23)	54 (76.06)	0.072
T2DM	20 (21.98)	16 (21.33)	0.92	38 (40.43)	27 (38.03)	0.755
Dyslipidemia	33 (37.50)	31 (44.29)	0.388	35 (37.23)	24 (33.80)	0.649
Smoking	7 (7.69)	5 (6.67)	0.8	20 (21.28)	17 (24.29)	0.648
hsCRP ≥ 3 (mg/L)	1.22 (0.55-2.61)	2.00 (1.20-5.16)	0.005	6.96 (2.90-21.9)	9.50 (2.60-28.90)	0.578
≥3mg/L	19 (21.10)	25 (35.20)	0.045	67 (71.30)	31 (69.9)	0.842
IL-6 (pg/mL)	3.00 (1.00-5.00)	3.00 (1.00-7.00)	0.95	11.00 (6.00-29.00)	11.00 (5.00-22.0)	0.505

The continuous variables were expressed as median and interquartile range (25%-75%); the categorical variables were expressed as number (n) and percentage (%). BMI: Body Mass Index; T2DM: Type 2 Diabetes Mellitus; hsCRP: C reactive protein with high sensitivity assay; IL: Interleukin.

Table 5: Results of automatic stepwise multiple regression analyses with disability at short-term outcome as dependent variable in patients with acute ischemic stroke according to the 3'untranslated region variation (rs1130864) in *CRP* gene.

Genotype	**Dependent variable**	**Explanatory variables**	**t**	**P value**	**F**	***df***	**P value**	**R^2**
CT+TT	Short-term outcome	Age[a]	2.628	0.011	7.457	Feb-58	0.001	0.182
		hsCRP[a]	2.552	0.013				
CC	Short-term outcome	Hypertension	2.053	0.001	8.704	Feb-78	<0.001	0.165
		hsCRP[a]	0.074	1.181				

a. natural logarithm transformed; hsCRP: C reactive protein with high sensitivity assay; R^2: Nagelkerke analysis.

Discussion

The main findings of the present study were that the T allele, in heterozygosity or in homozygosity (CT+TT genotypes), of 3'UTR rs1130864 variant in *CRP* was not associated with IS susceptibility, as well as with hsCRP levels in Brazilian patients; however, after three-month follow-up, only the IS patients carrying the T allele (CT+TT genotypes) showed a positive association between hsCRP and poor short-term outcome. Other important results of the study were the higher levels of hsCRP among the controls carrying the T allele in heterozygosity or homozygosity (CT+TT genotypes) than those with the C allele in homozygosity. Interestingly, the elevated hsCRP levels among those carrying the T allele were not accompanied by higher levels of IL-6, which suggests that the higher hsCRP levels may be associated with the presence of the T allele in these individuals and not as result of the IL-6-induced immune response. Moreover, the study reinforced the variables sex (male), hypertension, smoking and high serum levels of hsCRP as some of predictors of IS. We will now discuss these findings on a point-by-point basis.

To our knowledge, this was the first study carried out to investigate the association between the rs1130864 *CRP* variant with IS susceptibility, short-term outcome and hsCRP levels in Brazilian population. The overall distribution of genotypes and alleles obtained in this cohort is in agreement with previous studies [38,39], but discordant with those carried out in more genetically homogeneous populations [23,26]. This discrepancy could be explained based, at least, on genetic differences of our subjects [40]. However, the lack of association of this variant with IS obtained in the present study is in agreement with previous studies [20-22,25,26,41,42]. Moreover, high baseline of hsCRP levels and the presence of the T allele were associated with high risk of recurrent ischemic events in patients with symptomatic intracranial atherostenoses [43]. Other study carried out in Germany population reported an association between rs1130864 variant and IS and that C allele was associated with microangiopathic but not macroangiopathic or CEI stroke subtypes [24].

Regarding the association between the 3'UTR rs1130864 variant in *CRP* and hsCRP serum levels, the present study observed that controls carrying the T allele (CT+TT genotypes) showed higher hsCRP than those carrying the C allele in homozygosity, as described previously [16,39]. This variant influences the CRP levels, probably because its location in the disproportionately long length 3'UTR of the *CRP*, indicating a regulatory role, which could affect the stability of the mRNA and, therefore, increased the CRP production [16, 39]. Brull et al., [16] showed that this variant was associated with, approximately, 2-fold difference of CRP levels in healthy volunteers (0.55 *vs*. 1.04 mg/L for C allele carriers and TT homozygotes, respectively). A meta-analysis showed that the mean CRP concentration in C allele carriers without CVD was 2.01 mg/L and those homozygous for the T allele had a circulating CRP concentration 0.68 mg/L higher than those carrying the C allele [44]. Further, other studies showed that the T allele was associated with higher CPR levels in healthy subjects [13,14,37,45].

Functional genetic variants of *CRP* seem to influence its protein level independently of other variables, such as BMI and IL-6 levels [12,16,46]. Genetic variation within the 3' UTR of immune genes is a strong determinant of immune response interfering in the mRNA stability/degradation, nuclear export, subcellular localization and translation efficiency [47,48]. Therefore, regulation of mRNA stability is a potentially important step in CRP production, because mRNA for CRP is known to have the short half-life of approximately 2.5 hours [49]. Moreover, sequence variations can disrupt binding sites for microRNAs (miRNAs) and/or RNA-Binding Proteins (RBPs), altering their ability to regulate transcripts [50]. Therefore, it is assumed, that individuals carrying certain *CRP* alleles associated with higher CRP levels are at higher risk for CVD [25].

However, in IS patients evaluated by previous studies, the association between the rs1130864 variant on *CRP* and CRP levels is conflicting. Case-control studies showed that individuals with CT+TT genotypes presented higher hsCRP levels than those with CC genotype [25,51]. On the other hand, one study carried out in a Germany cohort demonstrated that the CT+TT genotypes of patients with myocardial infarct, stroke and Transient Ischemic Attack (TIA) were not associated with elevated hsCRP levels after adjustment for age, sex, BMI, smoking and T2DM [39]. Other studies with IS patients also found no association between the T allele and CRP levels [26,41,42]. These controversial results could be due to differences in genetic profile, race, sample size, methods of genotyping and study design and argue for further studies in different ethnic groups to validate these finding.

One explanation for the increased levels of CRP among the IS patients independently of their rs1130864 genotypes could be due the acute ischemic event pathophysiology, by itself and the association with other environmental stimuli. CRP is induced by inflammatory cytokines and additional genes may regulate CRP levels in IS [52].

The present study reinforces that high hsCRP serum levels as predictor of IS, independently of the others risk biomarkers. This finding is consistent with previous studies carried out in our population [3,5,53] and others nationwide [4,54]. Many inflammatory biomarkers have been reported to be useful in predicting clinical outcome after IS and hsCRP remains one of the most widely used in clinical practice [54,55]. Framingham study shows that high CRP is associated with an increased risk for IS or TIA [56]. Moreover, acutely elevated CRP showed significant and positive association with unfavorable outcome after adjusting for age, sex, baseline functional impairment when evaluated with neurological impairment, stroke subtype and conventional risk factors [6] and independent predictor of mortality [7,57]. Increases in CRP may reflect a systemic inflammatory response following ischemia and the extent of injury, it is considering a prognostic biomarker of IS outcome [58]. It is unclear whether the CRP exert a role in the pathogenesis of IS or is a marker of inflammatory processes that have a causal role in the ischemic event. CRP has the capacity to bind oxidized LDL and to induce adhesion molecule and tissue factor expression in endothelial cells and monocytes, respectively and has been found within atherosclerotic plaques [59]. Moreover, circulating level of CRP correlates inversely with endothelium-dependent vasodilatation in patients with cardiovascular heart disease, which is itself predictive of adverse outcome [60]. Taken together, these results may reflect a pathogenic role of CRP in the IS, mainly in those individuals who are carries of genotypes that are associated with high levels of this acute phase protein.

Few studies analyzed the association between rs1130864 *CRP* variant and stroke outcome [23,41,42]. We did not find association between the frequency rs1130864 *CRP* variant and the functional impairment after three-month follow up. However, hsCRP predicted mRS after three-month follow up only in IS patients carrying the T allele. One explanation for this result could be that after an ischemic event, inflammatory molecules remained upregulated for several weeks [61] and sequential blood study demonstrated significantly elevated CRP, ESR and WBC counts even three-month follow up after the onset of stroke [62]. This inflammatory environment increases RBPs and miRNA [50] that upregulate CRP expression [63] and enhance the functional impairment outcome. A previous study suggested that genetic variant in the *CRP* can disrupt the interaction sites for miRNA binding, which usually leads to stabilization of the mRNA transcript and increased protein levels [64]. Therefore, only the IS patients carrying the T allele could present correlation between hsCRP levels and mRS after three-month follow-up.

Potential limitations of our study merit consideration. First, the number of subjects included in our study is small to exclude an association between rs1130864 CRP variant and IS susceptibility. Second, among the 166 IS patients, 29 (17.46%) were not evaluated during the follow-up study period. However, the largest study (50,816 subjects), using a retrospective case control design, on the effects of CRP variants on CRP levels and

the risk of IS strongly indicated that the relation is not causal [65]. Third, the study evaluated only the baseline hsCRP levels and one outcome point (three months). In further studies, the hsCRP levels should be measured during the follow-up period as well as other outcome points should be included, such as one-year follow-up. This design may provide evidence for the predictive role of CRP levels only in the T allele carries and not in those CC carriers. Finally, although patients with clinical autoimmune and infectious conditions were previously excluded from this study, it is always possible that subclinical diseases could contribute to changes in the CRP levels.

Conclusion

Taken all the results into consideration, it is reasonable to suggest that the rs1130864 *CRP* variant, by itself, is not a determinant factor for IS susceptibility, as well as for the hsCRP levels in Brazilian IS patients, but in the patients carrying the T allele, the high levels of hsCRP were associated with the IS short-term outcome. This result suggests that the role of CRP as a predictor for the IS short-term outcome may differ according to the individual's genotype.

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Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. The protocol was approved by the Institutional Research Ethic Committee of the State University of Londrina, Paraná State, Brazil (CAAE 0250.0.268.000-11).

Author's contribution statement

Conceptualization: DFA, ANCS, EMVR; Methodology: MFL, MCdeAM, NP, DFA, TF, FD, RMT, ERDdeA; Formal analysis and investigation: DFA, MRU; Writing: DFA, ANCS, EMVR; Review and editing: DFA, EMVR; Supervision: EMVR

Consent to participate

Written consent form was obtained from all individual participants included in the study.

Data protection, confidentiality and privacy

The samples were consecutively and anonymously coded to guarantee the confidentiality.

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