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ORIGINAL ARTICLE

Analysis of the acute systemic and tissue inflammatory response following carotid endarterectomy

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ABSTRACT

BACKGROUND: We aimed to investigate the acute systemic and tissue inflammatory response associated with carotid endarterectomy (CEA) and analyze the association between serum and tissue biomarkers and histological features of carotid plaques between symptomatic and asymptomatic patients.

METHODS: We studied 11 patients (6 symptomatic and 5 asymptomatic) with ≥70% internal carotid stenosis treated with CEA. Serum expression of interleukin (IL) 1β, IL-4, IL-6, IL-8, IL-10, metalloproteinase (MMP) 8, MMP-9, tumor necrosis factor-α (TNF-α), high-sensitive C-reactive protein (hs-CRP), and vascular endothelial growth factor (VEGF) were measured in the preoperative period and 1, 6 and 24 hours after CEA. Assessment of tissue biomarkers (IL-1β, IL-4, IL-6, IL-8, IL-10, MMP-8, MMP-9, TNF-α, and VEGF) and histological analyses were performed on carotid plaques.

RESULTS: Peak serum values for MMP-8, MMP-9, IL-6, and IL 10 were observed 6 hours after CEA, whereas for hs-CRP, TNF-α, and VEGF, they were identified 24 hours after the procedure. Symptomatic patients exhibited higher serum concentrations of MMP-8, MMP-9, IL-1β, IL-4, IL-8, hs-CRP and TNF-α and higher tissue concentrations of MMP-8, MMP-9, IL-1β, IL-6 and VEGF than asymptomatic patients. Significant difference was found between symptomatic and asymptomatic patients in tissue IL-6 levels (30.95 pg/mL and 9.33 pg/mL, respectively; P=0.028).

CONCLUSIONS: Systemic and tissue inflammatory response occurs even after CEA, being observed important activity of inflammatory and anti-inflammatory cytokines at 6 and 24 hours after CEA. Symptomatic patients show higher concentrations of serum and tissue biomarkers in comparison to asymptomatic patients.

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Inflammation is currently recognized as a factor that plays an important role in the development of atherosclerosis and its clinical manifestations. Inflammation also contributes to the progression and rupture of atherosclerotic plaques, increasing patients' risk of future adverse cardiovascular events, such as myocardial infarction and stroke.[1, 2]

Recent studies suggest an association between inflammation, carotid artery stenosis and risk of stroke.[3, 4] Many enzymes involved in these processes play their

respective roles as serum and tissue biomarkers, reflecting the activity and the dynamics of reactions that contribute to the vulnerability of carotid atheromatous plaque and accelerate the clinical manifestation of a future transient ischemic attack (TIA) and/or stroke.[2-4]

The inflammatory response in patients with carotid stenosis can be evaluated from the expression of circulating and tissue acute-phase proteins and cytokines, which reflect the atherosclerosis process and may thus serve as diagnostic and prognostic markers of the disease.[2-4] The cells of the atherosclerotic plaque are a source of cytokines acting both locally and sistemically.[5] The aim of our study was to investigate the acute systemic and tissue inflammatory response associated with CEA and analyze the association between serum and tissue biomarkers with histological features of carotid plaques between symptomatic and asymptomatic patients.

Material and methods

Patients

Between May and August 2012, 11 patients with greater than 70% internal carotid artery (ICA) stenosis were admitted to our center for CEA. The selected patients were 8 men and 3 women, with ages ranging from 55 to 81 years (mean 69.09±8.30 years). Six patients (54.50%) had experienced a previous neurological event (four strokes and two TIAs), and 5 (45.50%) patients had no symptoms. These patients were referred from the Division of Vascular and Endovascular Surgery at the Pontifical Catholic University of Campinas (PUC, Campinas). The study was performed according to the Guidelines of the World Medical Association's Declaration of Helsinki and was approved by the Research Ethics Committees at PUC, Campinas and Santa Casa de Sao Paulo School of Medical Sciences. All patients gave their full informed consent prior to participating in the study.

The inclusion criteria were: asymptomatic or symptomatic patients with ≥70% ICA stenosis. The exclusion criteria comprised: patients who had already undergone CEA; occlusion or <70% ICA stenosis; patients admitted for carotid artery stenting (post-CEA restenosis, postirradiation ICA stenosis, high carotid bifurcation); any clinical and/or laboratory suspicion of infection; presence of autoimmune or systemic disease; use of anti-inflammatory drugs, chemotherapy treatment or immunossupressants; recent (<1 month) severe infection or recent (<1 month) stroke.

TABLE I.—*Clinical and laboratorial characteristics of study population.*

Variables		Values
Age (years)		69.09±8.30
Gender	Male	72.70%
	Female	27.30%
Hypertension	Yes	90.90%
	No	9.10%
Diabetes mellitus	Yes	54.50%
	No	45.50%
Smoking	Yes	36.40%
	No	63.60%
Obesity	Yes	9.10%
	No	90.90%
BMI (kg/m^2)		26.64±3.61
Total Cholesterol (mg/dL)		189.45±22.39
HDL (mg/dL)		47.55±11.26
LDL (mg/dL)		110.64±27.23
Triglycerides (mg/dL)		156.09±50.08
Glucose (mg/dL)		129.36±53.82
Carotid cross - clamping (minutes)		44.18±7.22
Contralateral carotid stenosis	<50%	54.50%
	50% a 69%	45.50%
	≥70%	0.00%
Ischemic heart disease	Yes	36.40%
	No	63.60%
Myocardial Revascularization	Yes	36.40%
	No	63.60%

Data shown as mean±standard deviation or percentage. BMI: Body Mass Index; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Preoperative period

Baseline data were obtained from clinical records, physical examinations, routine laboratory measurements, and from a study protocol filled out by the participating patients, as summarized in Table I. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg, or current use of antihypertensive medication at the time of CEA. Diabetes mellitus was diagnosed in patients with fasting blood glucose levels ≥126 mg/dL and/or current use of hypoglycemic agents. Smoker was defined as currently smoking or cessation of smoking less than 1 month prior to entering the study. Hypercholesterolemia was defined as a total cholesterol concentration ≥200 mg/dL or current use of cholesterol-lowering

TABLE II.—*Inflammatory activity of serum biomarkers during the four moments of analysis.*

	Preoperative	1h after CEA	6h after CEA	24h after CEA	P
IL-1β (pg/mL)	0.24±0.15	0.21±0.12	0.23±0.09	0.22±0.10	0.560
IL-4 (pg/mL)	0.09±0.12	0.06±0.06	0.09±0.09	0.11±0.10	0.691
IL-6 (pg/mL)	1.14±0.56	1.70±0.64	4.58±2.56	3.18±3.27	<0.001
IL-8 (pg/mL)	1.07±0.61	1.05±0.66	1.33±0.71	1.09±0.60	0.477
IL-10 (pg/mL)	0.42±0.24	1.68±3.03	3.27±5.09	0.59±0.31	0.018
MMP-8 (pg/mL)	460.58±320.57	408.54±334.77	1329.75±1077.83	794.48±577.92	0.008
MMP-9 (pg/mL)	69069.88±57324.15	42807.69±29689.96	248583.46±242990.51	65938.49±36335.00	0.022
hsCRP (mg/L)	2.03±1.14	1.48±0.86	2.25±1.72	32.16±23.82	<0.001
TNF-α (pg/mL)	2.01±2.20	1.63±1.57	2.19±1.26	2.28±1.70	0.260
VEGF (pg/mL)	1.23±1.13	0.37±0.49	2.74±3.67	2.97±3.97	0.010

IL: interleukin. MMP: metalloproteinase. hsCRP: high sensitive C reactive protein. TNF: tumor necrosis factor. VEGF: vascular endothelial growth factor.

agents. Abdominal obesity was diagnosed as patient's Body Mass Index ≥ 0 Kg/m^2.

The degree of carotid stenosis was determined by duplex ultrasonography investigation. In patients with greater than 70% ICA stenosis, carotid disease was confirmed by cerebral angiography performed one week prior to CEA. All patients were examined by a neurologist for assessment of their preoperative neurological status. As observed in previous publications, we followed the North American Symptomatic Carotid Endarterectomy Trial Criteria for classifying patients as being neurologically symptomatic or asymptomatic.[6]

Carotid endarterectomy

All patients received statin and antiplatelet therapy with acetylsalicylic acid for at least six months before surgery. CEA was performed under general anesthesia. All endarterectomies were performed by an open, non-eversion technique, with careful surgical exposure of the bifurcation into the internal and external carotid arteries. Patients received 5000IU heparin intravenously before cross-clamping.

Measurement of serum biomarkers

Samples of approximately 15 mL of venous blood were obtained via puncture of peripheral veins with needles at four moments: 24 hours prior to surgery and at 1 hour, 6 hours and 24 hours after carotid cross-clamping. Serum levels of MMP-8, MMP-9, IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α, VEGF and hs-CRP were determined at each point in time of follow-up.

Collected blood was distributed in three vacuum tubes: two purple tubes containing ethylenediamine tetra-acetic acid (EDTA) and one red, dry tube containing gel in its interior. The samples containing EDTA were firstly centrifuged at 1000 rpm for 15 minutes and then at 10,000 rpm for 10 minutes. The samples in the presence of gel were centrifuged at 4500 rpm for 20 minutes. Plasma was divided into aliquots after sample centrifugation and frozen at -70^o C. The analyses of blood samples collected at different points in time were performed at the Laboratory of Medical Investigation at University of São Paulo.

Modular (Roche, Basel, Switzerland) was used for determining hs-CRP levels. Measurements of interleukin, metalloproteinase, TNF-α and VEGF were performed with the Luminex methodology (Milipore Corporation, Billerica, MA, USA). Detection limits were the following: IL-1β<0.1 pg/mL; IL-4 <0.01 pg/mL; IL-6<0.08 pg/mL; IL-8<0.4 pg/mL; IL 10<0.01 pg/mL; TNF-α < 0.2 pg/mL and VEGF<0.04 pg/mL.

Measuring biomarkers in the carotid plaque and histological study

During CEA, the plaque was removed from within the lumen as a single specimen. The carotid plaque was divided into two similar segments of 5-mm thickness along the longitudinal axis. One portion was preserved in 10% formaldehyde for histological analyzes, while the other portion was stored at -70° C for measurement of cytokine levels.

The determination of tissue concentrations of IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α, VEGF, MMP-8, and MMP-9 was performed using the Luminex methodology (Millipore Corporation). For the histogical study,

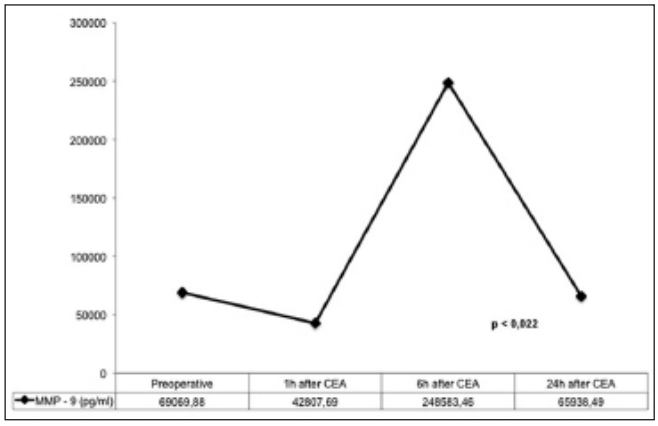


Figure 1.—Inflammatory response curves of MMP-9.

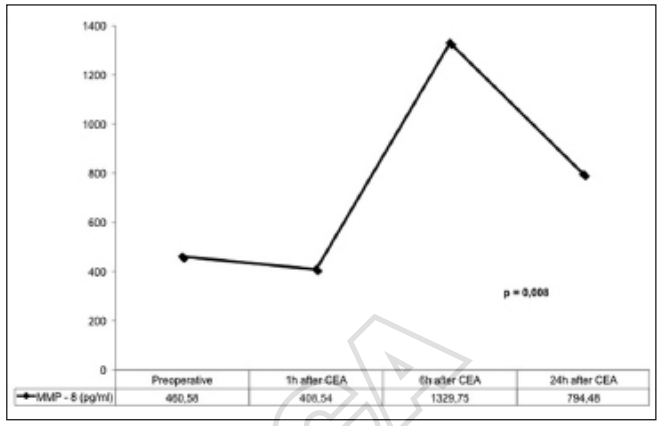


Figure 2.—Inflammatory response curves of MMP-8.

the surgical specimens were sectioned (5μm), fixed in paraffin and stained with hematoxylin and eosin stain. After the specimens were prepared for histologic analysis, they were examined by a pathologist blinded to the patient's clinical status.

Morphologic characteristics of the carotid plaque were established according to the classification of the American Heart Association (AHA) for advanced lesions.[7] The plaques were subdivided in complicated and stable plaques. In the complicated plaques, the pathologist described the following pathologic characteristics: macrophage, neutrophil and lymphocyte infiltration; ulceration; intraplaque hemorrhage; and fresh thrombus.

Statistical analysis

Data were analyzed with the Statistical Package for Social Sciences software, version 21.0. Values of continuous variables are expressed as mean±standard deviation and percentages. The Friedman Test was used for conducting the inferential analyses of the four data points collected over time among themselves for a same variable. This test was complemented by the Wilcoxon Test, adjusted by the Bonferroni correction, when there were significant statistical differences as indicated by the Friedman Test. The Mann-Whitney Test was applied to verify differences between symptomatic and asymptomatic patients for serum and carotid plaque biomarkers. Comparisons among quantitative variables were made with the help of Spearman's correlation coefficient. Values of $P<0.05$ were considered statistically significant.

Results

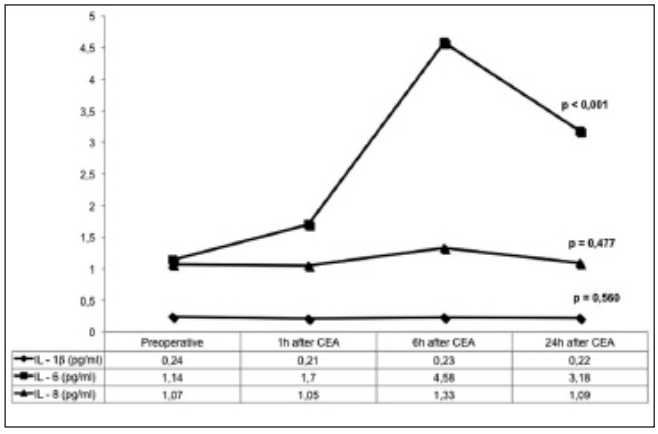
Measurements of serum biomarkers

Among the biomarkers analyzed, MMP-9 and MMP-8 were the cytokines that exhibited the highest inflammatory activity in the preoperative period. Serum values of MMP-9 were high both in the preoperative and postoperative periods. Table II depicts the inflammatory activity of serum biomarkers at the four moments of analysis.

The concentration of MMP-9 was higher than that of MMP-8, however the inflammatory response patterns of these two cytokines were similar to each other following CEA. A reduction in their concentrations was observed 1 hour after CEA, with inflammatory peak values recorded after 6 hours (248,583.46 pg/mL and 1,329.75 pg/mL, respectively), and a decrease in their values after 24 hours. MMP-9 ($P=0.022$) and MMP-8 ($P=0.008$) inflammatory response curves were statistically significant (Figures 1, 2).

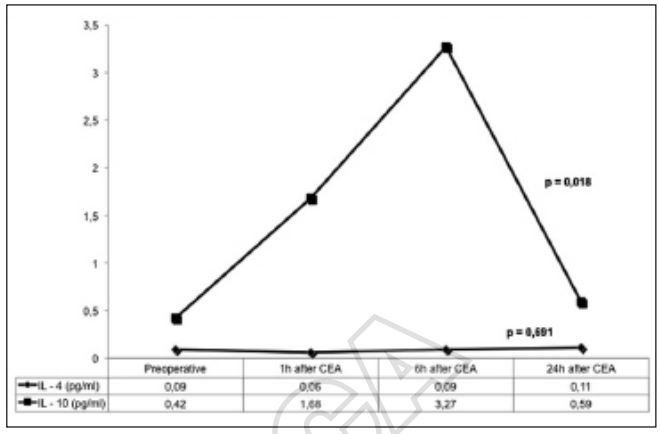
In the group of interleukins, IL-1β, IL-4, and IL-8 showed no significant variation in their concentrations during the periods analyzed. Moreover, IL-6 and IL-10 exhibited inflammatory response patterns similar to each other following CEA. There was an increase in their levels 1 hour after CEA, inflammatory peak values were recorded after 6 hours (4.58 pg/mL and 3.27 pg/mL, respectively), and then there was a reduction in their levels 24 hours after CEA. IL-6 ($P<0.001$) and IL-10 ($P=0.018$) inflammatory response curves were statistically significant (Figures 3, 4).

Serum hs-CRP showed an increase in its inflamma-



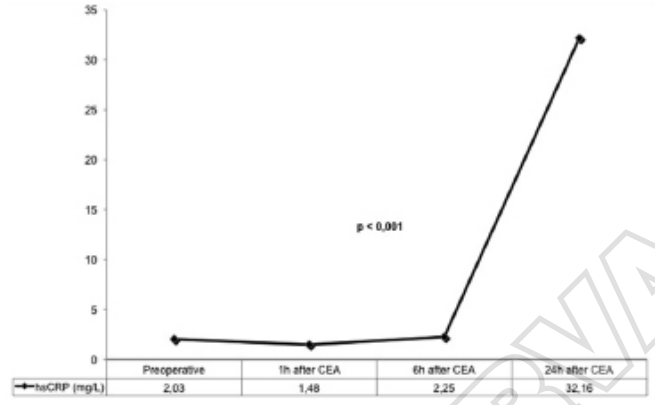
	Preoperative	1h after CEA	6h after CEA	24h after CEA
IL - 1β (pg/ml)	0,24	0,21	0,23	0,22
IL - 6 (pg/ml)	1,14	1,7	4,58	3,18
IL - 8 (pg/ml)	1,07	1,05	1,33	1,09

Figure 3.—Inflammatory response curves of IL–1β, IL-6 and IL-8.



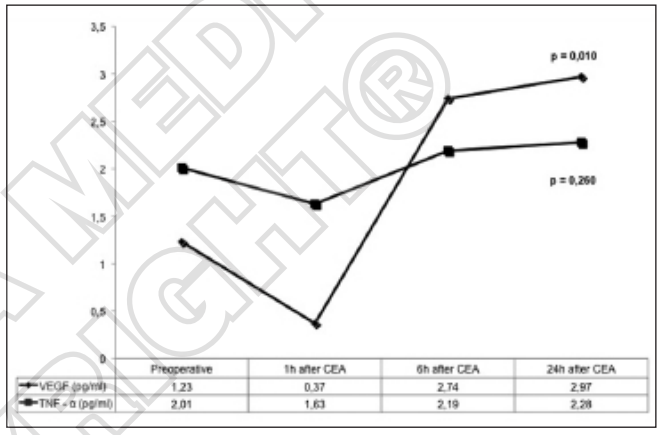
	Preoperative	1h after CEA	6h after CEA	24h after CEA
IL - 4 (pg/ml)	0,09	0,05	0,09	0,11
IL - 10 (pg/ml)	0,42	1,68	3,27	0,59

Figure 4.—Inflammatory response curves of IL-4 and IL-10.



	Preoperative	1h after CEA	6h after CEA	24h after CEA
hsCRP (mg/L)	2,03	1,48	2,25	32,16

Figure 5.—Inflammatory response curves of hsCRP.



	Preoperative	1h after CEA	6h after CEA	24h after CEA
VEGF (pg/ml)	1,23	0,37	2,74	2,97
TNF - α (pg/ml)	2,01	1,63	2,19	2,28

Figure 6.—Inflammatory response curves of VEGF and TNF-α.

tory activity 6 hours after CEA, while its peak value was recorded after 24 hours (32.16 mg/L). VEGF and TNF-α levels, on the other hand, exhibited inflammatory response patterns similar to each other following CEA. There was a reduction in their concentration 1 hour after CEA, an increase in their inflammatory activity after 6 hours, and inflammatory peak values 24 hours after CEA (2.97 pg/mL and 2.28 pg/mL, respectively). hs-CRP (P<0.001) and VEGF (P=0.010) inflammatory response curves were statistically significant (Figures 5, 6).

Differences in serum biomarkers between symptomatic and asymptomatic patients

Symptomatic patients exhibited higher concentrations of MMP-8, MMP-9, IL-1β, IL-4, IL-8, hs-CRP and TNF-α than asymptomatic patients during the periods analyzed. Significant statistical differences were found between symptomatic and asymptomatic patients in IL-4 levels at 1 hour (0.10 pg/mL and 0.02 pg/mL, respectively; P=0.027) and 6 hours (0.14 pg/mL and 0.03 pg/mL, respectively; P=0.035) after CEA and in TNF-α values at 6 hours (3.09 pg/mL and 1.10 pg/mL, respectively; P=0.010) and 24 hours (3.19 pg/mL and 1.20 pg/mL, respectively; P=0.045) after CEA.

Asymptomatic patients, on the other hand, showed higher concentrations of IL-10 during the periods analyzed; IL-6 at 24 hours after CEA and VEGF at 1 hour and 24 hours after CEA, in comparison to symptomatic patients. No significant statistical differences were observed for these biomarkers between these two groups.

Table III depicts the inflammatory activity of serum biomarkers between symptomatic and asymptomatic patients at the four moments of analysis.

TABLE III.—*Inflammatory activity of serum biomarkers between symptomatic and asymptomatic patients.*

		Symptomatic	Asymptomatic	p
IL-1β (pg/mL)	Preoperative	0.27±0.18	0.20±0.10	0.714
	1h	0.25±0.15	0.16±0.07	0.271
	6h	0.26±0.09	0.18±0.07	0.119
	24h	0.27±0.10	0.17±0.08	0.119
IL-4 (pg/mL)	Preoperative	0.09±0.13	0.10±0.12	0.921
	1h	0.10±0.05	0.02±0.02	0.027
	6h	0.14±0.08	0.03±0.05	0.035
	24h	0.16±0.11	0.06±0.08	0.063
IL-6 (pg/mL)	Preoperative	1.31±0.70	0.95±0.27	0.313
	1h	1.85±0.71	1.52±0.57	0.360
	6h	4.62±2.51	4.53±2.93	>0.999
	24h	2.64±1.30	3.84±4.87	0.272
IL-8 (pg/mL)	Preoperative	1.08±0.74	1.06±0.48	0.784
	1h	1.09±0.84	1.01±0.45	0.854
	6h	1.56±0.78	1.07±0.58	0.314
	24h	1.22±0.71	0.94±0.47	0.409
IL-10 (pg/mL)	Preoperative	0.43±0.23	0.42±0.28	0.927
	1h	0.68±0.92	2.87±4.31	0.521
	6h	3.30±5.22	3.24±5.54	0.784
	24h	0.48±0.25	0.72±0.35	0.522
MMP-8 (pg/mL)	Preoperative	621.83±355.76	267.08±113.57	0.201
	1h	466.42±376.86	339.09±302.64	0.361
	6h	1625.22±1025.44	975.19±1140.96	0.465
	24h	983.88±663.43	567.20±407.95	0.273
MMP-9 (pg/mL)	Preoperative	97564.42±65483.60	34876.43±13252.10	0.045
	1h	54751.75±32769.94	28474.82±19764.07	0.068
	6h	335931.02±263337.80	143766.38±189076.18	0.100
	24h	79929.31±20569.79	49149.51±46105.68	0.100
hsCRP (mg/L)	Preoperative	2.38±1.26	1.60±0.90	0.273
	1h	1.75±0.96	1.16±0.69	0.313
	6h	2.68±2.08	1.74±1.16	0.272
	24h	38.85±24.66	24.14±22.59	0.273
TNF- α (pg/mL)	Preoperative	2.37±2.60	1.58±1.78	0.647
	1h	2.47±1.68	0.64±0.61	0.052
	6h	3.09±0.47	1.10±1.01	0.010
	24h	3.19±1.60	1.20±1.17	0.045
VEGF (pg/mL)	Preoperative	0.88±0.65	1.66±1.49	0.465
	1h	0.22±0.42	0.55±0.56	0.443
	6h	3.43±4.92	1.91±1.35	>0.999
	24h	1.20±1.05	5.11±5.26	0.273

IL: interleukin; MMP: metalloproteinase; hsCRP: high sensitive C reactive protein; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor.

Differences in tissue biomarkers between symptomatic and asymptomatic patients

Symptomatic patients exhibited higher tissue concentrations of MMP-8, MMP-9, IL-1β, IL-6 and VEGF than asymptomatic patients. Significant statistical difference was found between symptomatic and asymptomatic patients in tissue IL-6 levels (30.95 pg/mL and 9.33 pg/mL, respectively; P=0.028).

Asymptomatic patients, on the other hand, showed higher tissue concentrations of IL-4, IL-8 and TNF-α, in comparison to symptomatic patients. No significant statistical differences were observed for these biomarkers between these two groups.

Table IV depicts the inflammatory activity of tissue biomarkers between symptomatic and asymptomatic patients.

Correlation between tissue biomarkers and carotid plaque histology

In symptomatic patients, tissue concentration of MMP-8 correlated with intima ulceration (Spearman=0.853;

TABLE IV.—*Inflammatory activity of tissue biomarkers between symptomatic and asymptomatic patients.*

	Symptomatic	Asymptomatic	P
IL-1β (pg/mL)	1.07±0.47	0.78±0.57	0.200
IL-4 (pg/mL)	0.01±0.00	0.02±0.02	0.787
IL-6 (pg/mL)	30.95±28.75	9.33±7.27	0.028
IL-8 (pg/mL)	77.33±75.41	113.01±123.39	0.715
IL-10 (pg/mL)	0.86±0.86	0.86±0.66	0.715
MMP-8 (pg/mL)	1317.36±1889.05	410.78±283.72	0.465
MMP-9 (pg/mL)	6362.56±5901.41	3584.74±4263.05	0.201
TNF-α (pg/mL)	1.29±0.33	1.40±0.46	0.628
VEGF (pg/mL)	11.02±17.85	8.15±13.13	0.715

IL: Interleukin; MMP: metalloproteinase; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor.

P=0.031) and tissue IL-1β correlated with thrombus (Spearman=0.894; P=0.041). The greater the number of macrophages (Spearman=0.971; P=0.001) and neutrophil (Spearman=0.828; P=0.042) in the plaque, the higher the levels of IL-6. In asymptomatic patients, on the other hand, tissue concentrations of IL-6 (Spearman=0.949; P=0.014) and IL-10 (Spearman=0.949; P=0.014) correlated with intima calcification.

Discussion

To the best of our knowledge, this is the first study that features a pattern of systemic inflammatory response associated with CEA and characterizes the acute inflammatory activity of serum biomarkers after CEA between symptomatic and asymptomatic patients. Laffey *et al.*, however, highlighted that the inflammatory response is mainly activated by the ischemia-reperfusion injury to the end organs as a result of arterial cross-clamping.[8] Moreover, restoration of perfusion on release of the arterial cross-clamp is associated with activation of key indices of the inflammatory response.[8]

We also believe that, in addition to carotid cross-clamping, the inflammatory response observed in our patients after CEA was influenced by preoperative morbid conditions, surgical trauma, perioperative hemodynamic factors, general anesthesia and postoperative evolution. In this study, our objective was to investigate the inflammatory response occurred after carotid cross - clamping and because of this we opted to perform CEA with the same surgical technique, type of anesthesia and surgical team to avoid bias. Furthermore, unlike previous publications that analyzed few cytokines, we have measured a broad panel of serum and tissue biomarkers in order to elucidate the inflammatory activity of most biomarkers involved in carotid stenosis and associated with CEA.

In this study, we observed that the systemic inflammatory response following CEA occurred at two main moments: 6 hours and 24 hours after CEA. In the first period, both inflammatory (IL-6, MMP-8 and MMP-9) and anti-inflammatory cytokines (IL-10) showed relevant activity, whereas in the second moment, only inflammatory cytokines (hs-CRP, VEGF and TNF-α) demonstrated inflammatory activity. As observed by Raja *et al.*, the magnitude of the inflammatory response may varies, but the persistence of any degree of inflammation may be considered potentially harmful to the surgical patient.[9] Agreeing with this author, we believe that these two time points of inflammatory response after CEA may represent periods of patient vulnerability to the action of plasma cytokines after carotid cross-clamping. Profumo *et al.* concluded, after investigate the intracellular expression of cytokines in peripheral blood from 43 patients before and after CEA, that the increased expression of TNF-α, interferon-γ, IL-1β, IL-4, IL-6, IL-8 and IL-10 were associated with the onset and progression of contralateral disease following CEA.[10]

Musialek *et al.* found higher serum levels of IL-6, IL-8, MMP-8 and MMP-9 in symptomatic patients and concluded that these circulating biomarkers have been implicated in symptomatic transformation of the atherosclerotic carotid plaque through their association with plaque erosion, rupture, and thrombosis.[11] Puz *et al.*, on the other hand, demonstrated higher serum concentrations of IL-6 and TNF-α in patients with unstable carotid plaque morphology.[12] In our study, symptomatic patients exhibited higher plasma concentrations of MMP-8, MMP-9, IL-1β, IL-4, IL-6, IL-8, hs-CRP and TNF-α during the periods analyzed. These patients also showed

higher tissue levels of MMP-8, MMP-9, IL-1β, IL-6 and VEGF whereas asymptomatic patients expressed higher plaque levels of IL-4, IL-8 and TNF-α. This result alerts us that even asymptomatic patients, which generally exhibit more stable carotid plaques, manifest tissue inflammatory activity that may lead to carotid instability and future cerebral microembolization.

IL-6 is a proinflammatory cytokine and one of the prime mediators in the acute phase response.[13] It has several potential atherothrombotic effects such as procoagulant properties by inducing the production of fibrinogen, promotes the expression of adhesion molecules, stimulates macrophages to secrete monocyte chemoattractant protein-1 and is the principal determinant of the hepatic synthesis of CRP.[13, 14] Liang *et al*, in experimental study with rabbits, observed that tissue levels of IL-6 and TNF-α were significantly increased after common carotid artery injury and the new intima appeared after 7 days of the injury and reached the peak on 28 days, resulting in vascular restenosis.[15]

Despite the known anti-inflammatory properties of IL-4, we observed that symptomatic patients exhibited significant higher serum levels of this biomarker at 1 hour and 6 hours after CEA while asymptomatic patients showed an important reduction of IL-4 levels at hour after CEA. Our results agree with Profumo *et al.*, whose study demonstrated that after CEA, IL-4 expression decreased significantly in patients with cured or stable atherosclerotic disease and increased in those with progressive disease.[10] Profumo *et al.*, in previous study investigating the relationship of serum cytokine expression with the histological type of atherosclerotic plaque removed during CEA, noted a proatherogenic role of IL-4 in promoting the atherosclerotic process and accelerating lesion development.[16]

IL-10, on the other hand, is a powerful anti–inflammatory cytokine that plays a protective role in atherosclerosis, provides protection from ischaemia and reperfusion injury and the same factors causing the secretion of IL-10 are factors that stimulate the production of IL-6.[17] In our study, we observed relevant inflammatory activity of IL-6 and IL-10 at 6 hours after CEA. A possible explanation for this phenomenon consists in a compensatory defense mechanism orchestrated by the immune system in order to control the inflammatory response at this moment of observation, minimizing the postoperative effects of IL-6, MMP-8 and MMP-9 in our patients. At 24 hours after CEA, however, we observed an imbalance between inflammatory and anti-inflammatory biomarkers, characterized by reduced levels of IL-10, reflecting a time in the postoperative period when the patient may be unprotected against the effects of hs-CRP, VEGF and TNF-α.

An increased expression of MMP-8 and MMP-9 stimulate cell migration, infiltration of monocytes and T lymphocytes in the subendothelium, degradation of the fibrous cap and extracellular matrix, arterial remodeling and intraplaque neoangiogenesis.[18, 19] These metalloproteinases also degenerate connective tissue type I, II, III, IV, V, VII, X and XII collagens and elastin, thus favoring carotid plaque rupture and hemorrhage.[18, 19] Molloy *et al.*, after performing transcranial Doppler monitorization in 75 patients submitted to CEA during the dissection phase of the CEA, until the application of carotid clamps, observed that a greater rise in MMP-9 levels was seen in those patients suffering > 2 emboli than those patients suffering 1 or 2 emboli, concluding that the increase in MMP-9 is due to cerebral damage caused by embolisation.[20]

Plasma hs-CRP is a chemoattractant for monocytes, upregulates adhesion molecules, decreases nitric oxide release by endothelial cells and exhibits both proliferative and apoptotic properties on vascular cells.[21] Results from the present study also correspond with results obtained by Alvarez Garcia *et al.*, who observed that elevated hs-CRP concentrations were significantly associated with symptomatic patients and with the presence of macrophages and T lymphocytes in unstable carotid plaques.[22] Unlike our study, that demonstrated an important tendency to increase the levels of hs-CRP at 24 hours after CEA, Dósa *et al.* showed a reduction in its concentration at 5.7 weeks postsurgery.[23]

VEGF is a monocyte chemoattractant, induces the expression of MMP-1 and the synthesis of tissue factor, a prothrombotic factor, and promotes plaque neovascularization.[24] Szabó *et al.*, after studying 82 patients who underwent carotid eversion endarterectomy and were followed up by carotid duplex scan sonography for 14 months, observed that carotid restenosis has occurred with high probability in patients with marked elevation in serum VEGF on day 4 after surgery.[25]

This study has some limitations. Even though our observations suggest that the knowledge of the inflammatory response following CEA may have therapeutic

implications, allowing for the development and testing of specific drugs targeted against the inflammatory process present in carotid stenosis, we don't have enough information about the future implications of this acute inflammatory response in the postoperative evolution of our patients. Longitudinal studies are needed to determine whether a reduction in the inflammatory activity of serum and tissue biomarkers may attenuate carotid vulnerability, the progression of atherosclerotic disorders, and the occurrence of future neurological events.

Conclusions

In conclusion, our data demonstrate that systemic and tissue inflammatory response occurs even after CEA, being observed important activity of inflammatory and anti – inflammatory cytokines at 6 and 24 hours after CEA. Symptomatic patients show higher concentrations of serum and tissue biomarkers in comparison to asymptomatic patients. The individual analysis of cytokines demonstrates variability of their concentration during the postoperative follow-up period.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.
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