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Plasmatic biomarkers of inflammation correlate with ^{18}FDG-PET-CT and microembolic signals in patients with carotid stenosis

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Abstract

Aim: To determine whether plasmatic biomarkers correlate with ^{18}fluoro-2-deoxy-D-glucose (FDG) positron emission tomography/computed tomography (PET-CT) and presence of microembolic signals (MES) detected by transcranial Doppler in patients with carotid stenosis.

Methods: ^{18}FDG-PET-CT and MES detection was performed in consecutive patients with 50% to 99% symptomatic or asymptomatic carotid stenosis. Uptake index was defined by a target to background ratio (TBR) between maximum standardized uptake value of the carotid plaque and the average uptake of the jugular veins. The analysis of biomarkers included adhesion molecules [intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule 1, P-selectin and E-selectin], interleukins (IL-1, IL-6), chemokines (RANTES, monocyte chemoattractant protein 1), cytokines (tumor necrosis factor α), matrix-metalloproteases (MMP), myeloperoxidase, and lipoprotein-associated phospholipase A2.

Results: There were 54 symptomatic and 57 asymptomatic patients. TBR values were significantly higher in the symptomatic compared to the asymptomatic (median 2.1 *vs*. 1.8, $P = 0.002$) and in the MES positive (MES+) compared to the MES negative (MES-) group (MES+, $n = 19$, median 2.3 and MES-, $n = 88$, median 1.8, $P = 0.01$). The best threshold for TBR values was of 1.9. We found a significant correlation between higher ^{18}FDG uptake (TBR $\geq$ 1.9) and the

plasmatic levels of chemokine RANTES (P = 0.03) and higher levels of ICAM-1 in MES+ patients (P = 0.03). Interestingly MMP-2 levels were more important in patients with lower TBR values (P = 0.02) and MMP-3 and P-selectin in those who were MES- (respectively P = 0.001 and P = 0.009).

Conclusion: In the present study, ICAM-1 was associated with the presence of thrombotically active atherosclerotic plaques, while RANTES mainly correlated with the inflammatory process. MMP-2, MMP-3 and P-selectin levels were more important in patients with stable plaques.

Keywords: Carotid plaque, biomarkers of inflammation, microemboli detection, transcranial Doppler, ^{18}FDG-PET-CT

INTRODUCTION

Inflamed carotid plaques play a key role in the occurrence of distal embolic cerebral infarcts[1-3]. Intensive research has been performed in the last few decades aimed at optimizing different imaging modalities with adequate spatial resolution to precisely analyse the arterial wall morphology, plaque composition, and degree of local inflammation[4-6]. Among them, positron emission tomography (PET) using the glucose analogue ^{18}fluoro-2-deoxy-D-glucose (^{18}FDG) as a radiotracer reflecting glycolytic activity has shown promise for non-invasive functional detection of local inflammation in atherosclerotic plaques and is considered as an emergent marker of plaque vulnerability[7-9]. Furthermore, ultrasound-based imaging modalities demonstrated that the presence of microembolic signals (MES) detected by means of transcranial Doppler downstream the stenosis is associated with an increased risk of embolic stroke[10-12].

In the present study we investigated in patients with symptomatic or asymptomatic carotid stenosis the relationship between ^{18}FDG-PET-computed tomography (CT), MES and plasmatic biomarkers of inflammation including adhesion molecules, interleukins (ILs), chemokines, cytokines, matrix-metalloproteases (MMP) and lipoprotein-associated phospholipase A2 (lp-PLA2).

METHODS

We included patients with unilateral symptomatic or asymptomatic carotid disease with 50% to 99% degree of stenosis according to the ECST criteria. Symptomatic stenosis was defined as any recent (< 6 months) neurological or retinal deficit, persisting or transient ischemic attack (TIA), which could be plausibly attributed to the ipsilateral carotid artery. Assessment of clinical parameters was performed upon study inclusion. Symptomatic patients had a complementary work up including cardiac ultrasound examination and long duration electrocardiogram for 7 days in order to exclude a cardio-embolic origin of stroke. Asymptomatic stenosis was defined as no history of recent (within the last 6 months) neurological or retinal deficit and/or presence of ipsilateral ischemic magnetic resonance imaging (MRI) lesions. All patients gave their written consent.

^{18}FDG-PET/CT angiography

All patients underwent ^{18}FDG-PET-CT with contrast angiography within 2-3 days after admission when symptomatic, and within 10 days after assessment of the diagnosis of carotid stenosis when asymptomatic. We used a standard protocol as described previously[13]. Analysis of PET-CT was done by two experienced investigators (JPW, HM) blinded to the clinical and biological data. The target to background ratio (TBR) was assessed by dividing the SUVmax of the carotid plaque wherever highest by the average SUVmean of the jugular veins.

Ultrasound analysis

Standard examination included duplex ultrasound of the carotid arteries (SIEMENS) with assessment of degree of stenosis according to the ECST criteria[14], plaque surface morphology and plaque echogenicity.

For MES detection, bilateral transcranial doppler (TCD) recording was performed during 60 min. In symptomatic patients, this examination was performed within 7 days after stroke onset and within 10 days when asymptomatic. We used a standard protocol as described elsewhere[13]. Embolic signal interpretation was done manually by an experienced ultrasonographer based on the criteria of the International Consensus group on Microembolus Detection[15,16]. Detection of at least 1 MES ipsilateral of the stenosis resulted in a positive exam, and those patients were defined as MES+.

Imaging

Symptomatic patients underwent a CT scan with contrast angiography of vessels of the neck and brain. Additionally, MRI study was performed with T1, T2, diffusion weighted (DWI) and fluid attenuated inversion recovery (FLAIR) sequences.

Plasmatic biomarkers

Venous blood samples taken on the day of ^{18}FDG-PET-CT were analyzed for 111 patients. Plasma levels of MMP-2, -3, -8, -9, IL-1, IL-6, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, P-selectin, E-selectin, tumor necrosis factor α (TNFα), RANTES, monocyte chemoattractant protein (MCP)-1, were performed using a bioplex 200 array reader (Bio-Rad Laboratories; Hercules, CA, USA) with Luminex MAP™ Technology (Luminex Corporation, Austin, TX, USA). MPO levels were measured using the colorimetric enzyme-linked immunosorbent assay (ELISA), a commercial kit purchased from R&D Systems (Minneapolis, MN, USA), according to manufacturer's instructions. The lp-PLA2 concentration (mass) was measured using PLAC Test ELISA Kit.

Statistical analysis

All continuous variables were summarized by mean and median. For testing of significant difference between groups, the *U*-Mann-Whitney test was applied. Receiver operating characteristic (ROC) curve analysis was used to determine the prognostic accuracy of the plasmatic biomarkers with respect to TBR values and presence or absence of MES. For correlation we used Spearman's rho method. Statistical analysis was performed using MedCalc (MedCalc Software, Ostend, Belgium) software.

RESULTS

From 2009 to 2015, 111 patients were analyzed. Fifty-four patients presented with symptomatic and 57 with asymptomatic carotid disease. All demographical data of the patients are summarized in Table 1. The CV risk factor profile was similar in the two groups, for the exception of degree of stenosis which was significantly higher in symptomatic patients. The male gender predominated in both groups.

MES

MES detection could be carried out in 107 patients. In 4 patients the investigation was not possible because of insufficient temporal bone window. Nineteen of 107 (18%) patients presented microembolic signals ipsilaterally during TCD recording. The mean number of emboli was 10 (range 1-50). The proportion of MES+ patients was higher in the symptomatic (26%, n = 14/54) when compared to the asymptomatic group (9%, n = 5/57; P = 0.01) [Table 1].

^{18}FDG-PET

One hundred eleven patients with 111 carotid plaques were analyzed. Hundred-one plaques presented with partial calcification whereas 10 showed exclusively a soft component on CTA. ^{18}FDG uptake was significantly higher in the symptomatic group as compared to the asymptomatic one (TBR: median 2.1 *vs.* 1.8, P = 0.002) [Table 1]. When confronting presence of MES to ^{18}FDG uptake, those plaques producing

Table 1. Baseline characteristics of the whole cohort

	Symptomatic (*n* = 54)	Asymptomatic (*n* = 57)	*P* values
Age	Mean 71.7	Mean 72.1	0.7
Gender (male)	44 (81%)	43 (75%)	0.5
Degree of stenosis	Mean 77.3	Mean 73.2	0.03
High blood pressure	41 (76%)	42 (74%)	0.9
Diabetes	17 (31%)	16 (28%)	0.5
Dyslidemia	25 (46%)	34 (60%)	0.2
Tobacco	30 (56%)	31 (54%)	0.3
Coronary disease	10 (19%)	10 (18%)	0.9
Family history	4 (7.4%)	5 (8.7%)	0.9
Antiplatelet	7 (17%)	4 (3.5%)	0.3
Statins	18 (33%)	26 (48%)	0.2
Stroke	42 (78%)	-	
TIA	12 (22%)	-	
Lesion MRI (ipsilateral to stenosis)	41 (76%)	-	
*MES	14 (19%)	5 (9.2%)	0.02
TBR	Median 2.1	Median 1.8	0.002

*MES detection performed in 107 patients. TIA: transitory ischemic attack; MRI: magnetic resonance imaging; MES: microembolic signal; TBR: target to background ratio

Table 2. Inflammatory plasmatic biomarkers in symptomatic and asymptomatic patients (median values)

	Symptomatic (*n* = 54), pg/mL	Stroke only (*n* = 42), pg/mL	TIA only (*n* = 12), pg/mL	*P* value (stroke *vs*. TIA)	Asymptomatic (*n* = 57), pg/mL	*P* value (sympt *vs*. asympt)
MMP-9	283,302	291,844	186,353	0.07	195,299	0.03
MMP-8	11,292	11,574	9302	0.7	8001	0.03
MMP-3	17,909	18,745	17,485	0.6	24,434	0.18
MMP-2	344,126	326,695	394,951	0.1	345,067	0.26
TNF-α	5.2	5.1	5.8	0.8	5.3	0.94
ICAM-1	277,016	280,808	256,042	0.7	285,594	0.56
VCAM-1	1,024,550	1,024,550	1,014,682	0.8	953,996	0.87
P-selectin	96,102	102,334	87,809	0.9	99,868	0.40
E-selectin	36,714	37,362	34,503	0.3	41,567	0.12
RANTES	45,188	45,992	41,941	0.2	44,278	0.49
MCP-1	296	310	251	0.3	258	0.17
*MPO	107	107	80	0.5	63	0.07
IL-6	1.35	1.3	2.07	0.7	1.35	0.24
IL-1	1943	1946	1840	0.7	1707	0.10
*lp-PLA2	126	118	131	0.4	138	0.25

**n* = 51. MMP: matrix-metalloproteases; TNF: tumor necrosis factor; ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule; MCP: monocyte chemoattractant protein; MPO: myeloperoxidase; IL: interleukin; lp-PLA2: lipoprotein-associated phospholipase; TIA: transitory ischemic attack

emboli, showed also an increased inflammatory activity (TBR: median 2.3 *vs*. 1.8, $P = 0.01$). The best TBR threshold value for the distinction between symptomatic, asymptomatic, MES+ and MES- negative patients was 1.9[13].

Plasmatic biomarkers

Analysis of plasmatic biomarkers was performed in 111 patients. When symptomatic and asymptomatic patients were compared, levels of MMP-8 and MMP-9 were significantly higher in the symptomatic ones ($P = 0.03$ for both) [Table 2]. In a subgroup analysis of the symptomatic patients differentiating between stroke and TIA, only stroke patients maintained a difference of MMP-9, however without reaching significance. MPO showed a trend towards higher levels in stroke patients but did not attain statistical significance [Table 2].

Table 3. Inflammatory plasmatic biomarkers according to TBR values of ^{18}FDG-PET-CT in symptomatic or asymptomatic patients with carotid stenosis (median values)

	TBR ≥ 1.9 (*n* = 56), pg/mL	TBR < 1.9 (*n* = 55), pg/mL	*P* value
MMP-9	227,612	253,273	0.9014
MMP-8	10,263	10,181	0.83
MMP-3	22,183	20,670	0.8273
MMP-2	328,626	365,796	0.0268
TNF-α	4.8600	5.5600	0.1018
ICAM-1	276,728	290,987	0.85
VCAM-1	959,190	1,020,700	0.4242
P-selectin	102,491	87,460	0.5672
E-selectin	38,700	38,744	0.5514
RANTES	51,396	41,367	0.0387
MCP-1	283	283	1.0000
MPO	73	88	0.1969
IL-6	1.3500	1.4900	0.6376
IL-1	1742	1934	0.3514
lp-PLA2	130	125	0.6187

MMP: matrix-metalloproteases; TNF: tumor necrosis factor; ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule; MCP: monocyte chemoattractant protein; MPO: myeloperoxidase; IL: interleukin; lp-PLA2: lipoprotein-associated phospholipase; TBR: target to background ratio; ^{18}FDG-PET-CT: ^{18}Fluorodoeoxyglucose positron emission tomography/computed tomography

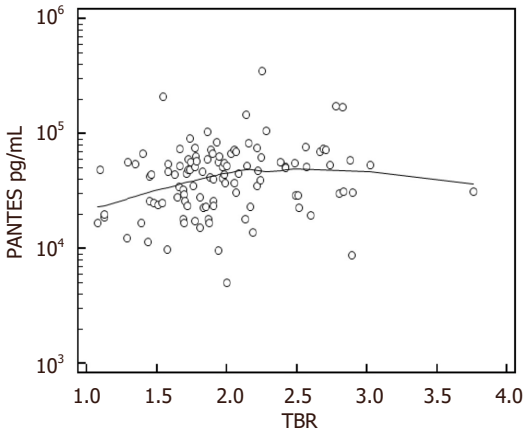

Figure 1. Correlation between RANTES and target to background ratio (TBR) values, rho 0.249 (*P* = 0.008)

We found a significant correlation between higher ^{18}FDG uptake (TBR ≥ 1.9) [Table 3] and the plasmatic levels of chemokine RANTES (*P* = 0.03). The correlation between RANTES and TBR values was of rho 0.249 (*P* = 0.008) [Figure 1].

There were higher levels of ICAM-1 (*P* = 0.03) in MES+ patients [Table 4]. The correlation with the number of MES was of rho = 0.21 (*P* = 0.03).

The predictive values of RANTES and ICAM-1 are shown on Table 5.

MMP-2 levels were more important in patients with lower TBR values (*P* = 0.02) and MMP-3 and P-selectin in those who were MES- (respectively *P* = 0.001 and *P* = 0.009) [Tables 3 and 4]. There was an inverse correlation between number of MES and MMP-3 with rho = -0.319 (*P* = 0.0008) and number of MES and P-selectin with rho = -0.26 (*P* = 0.006).

Table 4. Inflammatory plasmatic biomarkers according to presence or absence of MES

	MES+ (*n* = 19), pg/mL	MES- (*n* = 88), pg/mL	*P* value
MMP-9	257,003	230,582	0.9938
MMP-8	11,885	9698	0.4884
MMP-3	13,022	24,087	0.001
MMP-2	319,239	344,595	0.06
TNF-α	5.8	5.3	0.6712
ICAM-1	304,084	272,250	0.03
VCAM-1	1,250,217	963,976	0.2967
P-selectin	69,370	100,415	0.009
E-selectin	35,766	39,562	0.22
RANTES	40,975	47,131	0.3653
MCP-1	265	270	0.6836
MPO	83	85	0.7081
IL-6	1.3500	1.3500	0.9022
IL-1	1968	1794	0.2006
lp-PLA2	124	139	0.7652
TBR	2.3	1.8	0.01

MMP: matrix-metalloproteases; TNF: tumor necrosis factor; ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule; MCP: monocyte chemoattractant protein; MPO: myeloperoxidase; IL: interleukin; lp-PLA2: lipoprotein-associated phospholipase; TBR: target to background ratio; MES: microembolic signal

Table 5. Sensitivity and specificity of biomarkers to predict high TBR values of ^{18}FDG or presence of MES

	Sensitivity (%)	Specificity (%)	AUC	Criterion	*P* value
RANTES	82	42	0.614	> 27,835 pg/mL	0.03
ICAM-1	90	42	0.615	> 239,642 pg/mL	0.015

AUC: area under the curve; ICAM: intercellular adhesion molecule; TBR: target to background ratio; MES: microembolic signal; ^{18}FDG: ^{18}Fluorodoeoxyglucose

DISCUSSION

The clinical role of plasmatic biomarkers in the setting of carotid atherosclerosis has been extensively studied in these recent years[17-19]. This not only for the assessment of the embolic risk but also for the choice of the best type of carotid intervention[20-22].

The present study has examined the relationship between plasmatic mediators of inflammation, ^{18}FDG uptake in carotid plaques and the presence of MES. We found a significant correlation between higher ^{18}FDG uptake and the plasmatic levels chemokine RANTES [Table 3 and Figure 1]. Furthermore in MES+ patients higher levels of ICAM-1 were present.

In the dal-PLAQUE study, baseline ^{18}FDG uptake positively correlated with blood MPO and IL-6[23,24]. Interestingly, hs-CRP, P-selectin, E-selectin, ICAM-1, MMP-3 and MMP-9 did not correlate with TBR values[23,24]. In an earlier report by Rudd *et al.*[9], ^{18}FDG uptake was significantly associated with serum MMP-9 levels. We did not find a correlation of PET-CT to MMP-9 in our study. One possible explanation is that MMP-9 may be influenced by the burden of ischemic brain lesions and does not only reflect inflammation within the carotid plaque. Supporting this hypothesis, we found a higher level of MMP-9 in stroke in contrast to TIA patients with a trend towards significance [Table 2].

Chemokines coordinate communication between circulating inflammatory cells and endothelium[25,26]. We found that circulating RANTES correlated positively to ^{18}FDG uptake in the carotid plaque, but there was no significant difference between symptomatic and asymptomatic patients. These findings are inline to those of Zaremba *et al.*[27] who found no differences in RANTES levels between the sera of stroke patients and those of

controls. Furthermore, in the present study the analysis of the plasmatic levels of RANTES between patients with and without a lesion on MRI showed no statistical significant difference. Also correlation to TBR persisted even in patients without a stroke lesion. RANTES may therefore be a marker of inflammation in atherosclerosis and be less influenced by ischemic damage in stroke [Table 5]. In the present study, we found higher levels of ICAM-1 in MES+ patients, but not in patients with higher TBR values. These results possibly reflect different plaque components; in fact ^{18}FDG-PET-CT mainly depicts the inflammatory state of the whole carotid plaque whereas presence of MES reflects surface abnormalities such as ulceration and/or plaque thrombus[7,10]. Although a significant correlation between presence of symptoms and MES could be demonstrated in our cohort [Table 1], no difference was found between symptomatic and asymptomatic patients with respect to ICAM-1 levels, again giving more strength to the link between biomarkers and thrombotically active plaques *per se* [Table 5]. Cellular adhesion molecules, including ICAM-1, VCAM-1 and E-selectin promote recruitment of inflammatory cells into the arterial wall where they interact with lipid particles leading subsequently to plaque formation[28]. In the Atherosclerosis Risk In Communities (ARIC) study, the relationship of ICAM-1 and E-selectin with coronary heart disease and carotid artery atherosclerosis was independent of other known risk factors[29]. Cellular adhesion molecules have also been implicated in the destabilisation of atherosclerotic plaques. In a recent study including human carotid endarterectomy (CEA) specimens from asymptomatic (n = 30) and symptomatic (n = 30) patients, expression of VCAM-1 on the endothelium of CEA specimens from symptomatic patients was 2.4-fold greater than that from asymptomatic patients ($P < 0.01$)[30]. In another study performed upon 40 patients undergoing carotid endarterectomy it was possible to determine the influence of surgery on the levels of adhesion molecules. A statistically significant decrease of the ICAM-1 levels 1 h and 6 h after the endarterectomy compared to levels before the operation was found suggesting that decrease of ICAM-1 could be a possible marker of endothelial de-activation after plaque removal[31]. Only very few studies investigated the relationship between biomarkers and presence of MES[32,33]. One study including 104 controls and 118 patients found increased values of CXCL16 in stroke and in MES+ patients[33]. Other studies reported the following biomarker candidates for MES:P-selectin, fibrinogen, high neutrophil count, reduced ratio of CD4+CD25, high regulatory T cells and the C allele of TNF receptor superfamily member[32]. At present ICAM-1 has never been reported in association with MES. However, as this biomarker may be involved in the process of plaque destabilization, its relationship to MES is nevertheless very likely. Interestingly in our cohort MMP-2 levels were significantly more important in patients with lower TBR values and MMP-3 and P-selectin in those who were MES-. There was also a trend of MMP-2 levels to be higher in MES- patients. MMPs are a class of proteases involved in extracellular matrix degradation, which appear to play a key role in the process of vascular remodeling during the course of vascular disease[34,35]. Numerous studies suggest that MMPs and in particular MMP-3, MMP-7, MMP-9 and MMP-12, may be involved in the process of plaque destabilization[36,37]. However there are conflicting results in particular regarding MMP-3. The correlation between MMP-3 blood levels and carotid atherosclerotic disease has been reported in several studies. Lien *et al.*[38] showed in a study including 433 patients that MMP-3 was significantly associated with the presence of higher carotid plaques scores reflecting more unstable plaques. On the other hand, experimental studies show that MMP-3 is required for efficient neointima formation after carotid ligation and for smooth cell migration, supporting the fact that MMP-3 acts on plaque stability[39]. The relationship between MMP-2 and stable plaques has been already described by Sluijter *et al.*[40] who showed in a study including 150 subjects that there was an increased activity of MMP-2 in association with the presence of smooth muscle cells and a fibrous phenotype. This finding suggested that MMP-2 may be considered as a marker of a stable plaque. The correlation between P-selectin and stable plaques has been less well documented. In the study reported by Yin *et al.*[32], P-selectin was increased in MES+ patients. On the opposite, in our cohort there was a significant increase of P-selectin levels in MES- patients with an inverse correlation between the number of MES and the plasmatic levels of P-selectin.

To conclude, in the present study ICAM-1 was associated with the presence of thrombotically active atherosclerotic plaques, while RANTES mainly correlated with the inflammatory process. MMP-2, MMP-3

and P-selectin levels were more important in patients with stable plaques. Further studies combining ^{18}FDG-PET-CT and MES detection are needed in order to confirm our results.

DECLARATIONS

Authors' contributions

Contributed to manuscript redaction: Mueller H
Contributed to patients inclusion: Fisch L, Bonvin C
Helped to define the the appropriate biomarkers: Lalive P, Pagano S, Vuilleumier N
Contributed to the PET-CT protocole and to the interpretation of results: Ratib O, Willy JP, Lovblad K
Contributed to the study design and to manuscript redaction: Sztajzel R

Availability of data and materials

All data were collected by our research study nurse and are available.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The study was approved by Human Central Ethics commission of University Hospital Geneva and all patients gave their written consent.

Consent for publication

Not applicable.

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