Leukocytes and complement in atherosclerosis

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b. Markers of leukocyte activation and mRNA expression in different vascular compartments in patients with coronary artery disease

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ABSTRACT

Introduction: Coronary artery disease (CAD) may reflect generalized inflammation. It is unclear whether inflammation differs in different vascular compartments. We evaluated leukocyte activation in subjects with and without CAD in different vascular compartments. Moreover, the mRNA expression of inflammatory genes as well as at the whole genome level were studied.
Methods: Patients were divided in 2 groups consisting of subjects without CAD (controls; n=25) and patients with multi-vessel CAD (n=52) based on coronary angiography. Blood samples were collected from the peripheral vein of the forearm, the femoral artery, the abdominal aorta and the right and left (main) coronary arteries. Classical cardiovascular risk factors were determined as well as the leukocyte activation markers CD11b, CD66b and cytoplasmatic myeloperoxidase (MPO) by flowcytometry using fluorescent labelled antibodies. Gene expression analysis in these locations was performed using microarrays.
Results: Leukocyte activation based on MPO expression was higher in CAD patients at all sites compared to controls. The other leukocyte activation markers as well as their mRNA expression did not differ. A gradient of inflammation from peripheral vessels to the coronaries was found by differences in MPO in both groups. Other leukocyte activation markers did not show such a gradient. Microarray analysis showed up-regulation of genes linked to endothelial dysfunction, smooth muscle cell proliferation, and especially the macrophage scavenger receptor gene in CAD patients compared to controls.
Conclusions: Despite the fact that there seems to be a generalized inflammatory neutrophil gradient for MPO from peripheral vessels towards the coronaries in patients with and without CAD, the former show a higher degree of inflammation. Moreover, compared to controls, treated CAD patients display an up-regulated mRNA expression in different pathways involved in the atherosclerotic cascade.

INTRODUCTION

Atherosclerosis is considered to be an inflammatory disorder (1). The mechanisms involved comprise activation of the endothelium, leukocytes and complement and the generation of oxidative stress (2). Leukocyte activation has been described as one of the crucial steps in atherogenesis (1). The expression of leukocyte integrins like neutrophil and monocyte CD11b, and neutrophil CD66b have been linked to atherosclerosis (3-8). CD11b (also termed MAC-1 or CR3) is involved in early adhesion of leukocytes to the endothelium (9,10). CD66b (also termed CEACAM8) and cytoplasmatic myeloperoxidase (MPO) are degranulation markers of neutrophils (11). It has been shown that intracellular MPO is reduced in myocardial infarction and unstable angina (12).

An increasing body of evidence suggests that inflammation may also be a localized phenomenon with predilection for specific sites. MPO was identified as a leukocyte activation marker specifically expressed at sites of inflammation in the arterial tree (13). The expression of neutrophil and monocyte CD11b/CD18 was increased in the coronary sinus of subjects with unstable angina compared to blood collected from the aorta (14,15). These data are in some way unexpected because atherosclerosis is considered to be a generalized process and not a localized phenomenon (1).

The aim of the present study was to investigate differences of leukocyte activation between subjects with and without CAD in different vascular compartments in vivo. Moreover, whole genome gene expression analysis was carried out to identify the differences between the groups in genes involved in atherosclerotic pathways.

MATERIALS AND METHODS

Subjects

Subjects who visited the outpatient clinic of the department of Cardiology of the Sint Franciscus Gasthuis and who were scheduled to undergo a diagnostic coronary angiography were invited to participate. Their indication for coronary angiography was the clinical presentation of chest pain and a positive cycle ergometry. These patients were selected consecutively.

Subjects were divided into 2 groups. The first consisted of patients without CAD based on the coronary angiography, nor a history of peripheral or cerebral vascular disease (controls). The second group consisted of subjects with at least wall irregularities in both left and right coronary systems (CAD). The coronary angiographies were evaluated by an independent cardiologist who did not have access to clinical data of the subjects.

Exclusion criteria were: The presence of inflammatory disorders like rheumatoid arthritis, systemic lupus erythematosus and infections, plasma CRP above 10 mg/L, disorders of kidney, liver and thyroid function.
The Institutional Review Board of the St. Franciscus Gasthuis Rotterdam and the regional independent medical ethics committee of the Maasstad Hospital Rotterdam approved the study. All participants gave written informed consent.

Sample collection

Just before angiography, a venous blood sample was obtained from a peripheral vein of the forearm. During coronary angiography, blood samples were obtained from the femoral artery, midway from the abdominal aorta and from each coronary artery. The first 2 mL were discarded in order to avoid contamination with contrast and subsequently, blood samples were collected in tubes containing EDTA (1 mg/mL) and kept on ice until processed for determination of leukocyte activation markers.
For the extraction of RNA needed for the microarray experiments, sampling from three sites was chosen; venous blood sample from a peripheral vein and the left (main) and right coronary arteries. From each site three mL whole blood was drawn into Tempus® blood RNA tubes (Applied Biosystems, Victoria, Australia). After blood extraction, the tubes were handled and mRNA isolation was carried out according to the manufacturer's instructions.

Analytical methods

Parameters for renal and liver function, glucose, C-reactive protein (CRP), total cholesterol, HDL cholesterol (HDL) and triglycerides (TG) were determined using a Synchron LX analyzer (Beckman Coulter, Brea CA, USA) according to standard procedures in our laboratory. LDL cholesterol (LDL) values were calculated using the Friedewald formula. Apolipoprotein (apo) AI and ApoB were determined by rate nephelometry using IMMAGE with kits provided by Beckman (Beckman Coulter, Brea CA, USA). Blood cell counts were determined using the LH analyzer (Beckman Coulter, Miami FL, USA). The leukocyte differentiation was determined as a five-part differentiation on the same instruments.

Leukocyte activation markers

Blood samples for the measurement of leukocyte activation markers CD11b, CD66b and MPO were collected in EDTA and were determined by flowcytometry on the same day essentially as described earlier (6,7,12,13) with minor changes in dilutions. The neutrophil cytoplasmatic marker MPO is, in contrast to CD11b and CD66b, a negative activation marker: a lower content of cytoplasmatic MPO represents higher degree of activation. The fluorescence intensity of

each cell was expressed as the average mean fluorescence intensity (MFI), given in arbitrary units (au).

Microarray data generation and analysis

One-Color Microarray-Based Gene Expression Analysis Protocol (Agilent Technologies, Palo Alto, CA, USA) was used to amplify, label and analyze RNA.
In the analyses we focused on 16 controls and 16 CAD patients, and they were pooled resulting in 4 pools for each group.
Hence, 24 arrays where hybridized and the following comparisons were performed:
1) Differences between the means across sites for controls and CAD patients. 2) Differences across sites in both controls and CAD patients. 3) Differences between controls and CAD patients for each site.

Statistics

Data are given as mean ± SEM in the text and in the Tables. The use of medication, smoking behavior and the occurrence of type 2 diabetes mellitus were tested by Chi-square test. Comparisons between the three groups were done with One-way ANOVA and comparisons between 2 groups by Student's t-test. For comparisons between sampling sites, repeated measures ANOVA with Bonferroni correction and LSD test as post hoc test was carried out. For statistical analysis SPSS version 16.0 was used. P values less than 0.05 (2-tailed) were considered statistically significant.
For the microarray analysis raw signals were thresholded to 1 and quantiles normalization (16) was performed using the software GeneSpring. Data were considered in the log2 scale. Default flags were considered as absent, except saturated spots that were flagged as marginal. From the initial 41081 probes present in the Agilent 4x44K chip, 26644 remained after applying three types of filter (on the 6 condition site*CAD): (i) By expression, we retained those genes with at least 75% of the replicates in range (signal larger than the 20% signal of the whole array) in 3/6 conditions; (ii) by flags, we retained only those genes with at least 75% acceptable values in all conditions and (iii) by error, we retained only those genes with CV<50% within the replicates for every condition. A mixed linear model with two fix covariates (CAD +/- and Site of extraction) and one random effect (pools) was fit to the data and estimates were obtained using methodology specifically designed for the analysis of high-throughput genomic data (17) as implemented in the limma Bioconductor package (18). P-values were adjusted to control the False Discovery Rate (FDR) using Benjamini-Hochberg correction (19). Functional analysis was performed using Ingenuity Pathway Analysis.

We used previous study from our group (20) in order to determine the sample size. Twenty and 25 patients were needed for CD66b and CD11b, respectively, to reach enough power to detect significant differences.

RESULTS

Baseline characteristics (Tables 1 & 2)

In total, 77 subjects were eligible for the study of whom 25 were controls and 52 CAD patients. There were significantly more men than women in the CAD group, compared to controls (73.1% vs. 56.3% respectively, P=0.002).

Table 1 shows the baseline characteristics in the two groups. CAD patients were older and had higher systolic blood pressure than controls. Controls had higher total cholesterol as well as LDL, apoB, HDL and apoAI. A trend was found for higher waist circumference and fasting TG, and lower diastolic blood pressure in CAD patients. There were no differences between the groups for the other parameters listed in Table 1.

There were no differences between the groups for the incidence of type 2 diabetes mellitus, nor for smoking behavior (Table 2). CAD patients used more frequently statins and aspirin than controls. There were no differences between the groups for the use of other drugs listed in Table 2.

Table 1. Baseline characteristics in 25 non-atherosclerotic (controls) and 52 subjects with multi-vessel disease (CAD).

	controls (n=25)	CAD (n=52)	P-value
Age (years)	59.12 (1.99)	66.86 (1.39)	0.002
BMI (kg/m2)	27.59 (1.12)	27.53 (0.58)	0.96
Waist circumference (m)	1.00 (0.04)	1.08 (0.02)	0.09
Systolic BP (mm Hg)	137.0 (3.7)	148.2 (3.2)	0.04
Diastolic BP (mm Hg)	85.3 (2.7)	80.1 (1.4)	0.06
Glucose (mmol/L)	6.65 (0.43)	6.9 (0.2)	0.64
Cholesterol (mmol/L)	5.29 (0.21)	4.57 (0.15)	0.007
HDL-C (mmol/L)	1.37 (0.07)	1.21 (0.04)	0.03
LDL-C (mmol/L)	3.29 (0.18)	2.53 (0.14)	0.002
TG (mmol/L)	1.43 (0.14)	1.83 (0.14)	0.07
ApoAI (g/L)	1.52 (0.06)	1.38 (0.03)	0.03
ApoB (g/L)	1.07 (0.06)	0.92 (0.04)	0.04
CRP (mg/L)	3.12 (0.56)	2.81 (0.31)	0.60
Leukocyte counts (10^9 cells/L)	7.24 (0.43)	7.33 (0.25)	0.86
Monocyte counts (10^9 cells/L)	0.57 (0.03)	0.62 (0.02)	0.19
Neutrophil counts (10^9 cells/L)	4.54 (0.36)	4.53 (0.19)	0.96
Lymphocyte counts (10^9 cells/L)	1.97 (0.13)	1.94 (0.08)	0.85
Platelet counts (10^9 cells/L)	238.3 (12.1)	224.6 (7.8)	0.33

Data are mean (± SEM).

Leukocyte activation markers within and between the groups (Table 3)

In general, intracellular MPO was lower in CAD patients when compared to controls showing statistical significance for venous, abdominal aorta and right coronary artery samples and trends for the femoral and the left main coronary artery samples (Table 3). The rest of the markers did not differ between the groups at different locations.

Table 3 shows the comparisons of leukocyte activation markers at different sites. There were no significant differences between the sampling sites for monocyte and neutrophil CD11b and neutrophil CD66b. Intracellular MPO showed a gradient from venous blood to the coronaries reaching statistical significance in both groups (Table 3A and B).

Upon correction of our data for the use of statins and aspirin (the only drugs differing between the groups) the differences in MPO remained as described above.

Table 2. Use of medication (as %) in 25 non-atherosclerotic (controls) and 52 subjects with multi-vessel disease (CAD).

	Controls (n=25)	CAD (n=52)	P-value
Type 2 diabetes mellitus	24.0	32.7	0.31
Smoking	16.0	15.4	0.59
Statins	40.0	84.6	<0.0001
Ezetimibe	4.0	11.5	0.27
Aspirin	36.0	82.7	<0.0001
Beta blockers	52.0	59.6	0.35
Ace-inhibitors	36.0	34.6	0.55
Angiotensin II receptor blockers	16.0	25.0	0.28
Calcium channel antagonists	20.0	30.8	0.24

Table 3. Leukocyte activation markers in subjects without (controls; Table A) and multi-vessel CAD (CAD; Table B) at different sites.

A.

Controls	Venous	Femoral artery	Abdominal aorta	Left coronary artery	Right coronary artery	P-value
Monocyte CD11b (au)	35.98 (2.45)	33.18 (3.18)	32.43 (2.46)	31.74 (1.84)	32.09 (1.96)	0.22
Neutrophil CD11b (au)	31.84 (2.07)	32.48 (2.68)	33.20 (1.99)	30.41 (1.60)	31.00 (1.13)	0.54
Neutrophil CD66b (au)	6.51 (0.49)	6.68 (0.53)	6.85 (0.47)	6.82 (0.47)	7.06 (0.49)	0.14
Myeloperoxidase (au)	210.2 (11.7)	211.8 (11.5)	194.8 (12.0)	188.8 (14.4)	193.1 (11.9)	0.001

B.

CAD patients	Venous	Femoral artery	Abdominal aorta	Left coronary artery	Right coronary artery	P-value
Monocyte CD11b (au)	33.75 (1.59)	32.58 (1.42)	32.69 (1.57)	33.91 (1.89)	34.79 (1.39)	0.73
Neutrophil CD11b (au)	29.49 (1.59)	32.07 (1.22)	31.92 (1.48)	32.41 (1.55)	33.28 (7.26)	0.77
Neutrophil CD66b (au)	7.30 (0.33)	7.42 (0.32)	7.57 (0.30)	7.76 (0.36)	7.59 (0.37)	0.58
Myeloperoxidase (au)	187.6 (7.3)*	177.6 (6.6)†	165.9 (6.5)*	166.1 (6.1)†	162.8 (6.1)*	0.007

Data are mean (± SEM). *: P<0.05 vs. CAD-. †: P=0.08 vs. CAD-.

Genes differentially expressed between controls and CAD subjects as means of all sites (Table 4)

Microarray-Based Gene Expression Analysis showed that 1964 genes were found differentially expressed between controls and CAD patients at $\alpha \leq 0.05$ after multiple testing corrections to control for false discovery rate. From these genes, 1052 were down-regulated (i.e. more expressed in the controls than in the CAD patients) and 912 were up-regulated.
Table 4 shows the twenty genes that were most significantly differentially expressed between controls and CAD patients as means of all sites. In comparison to controls, the most up-regulated gene in CAD patients was the macrophage scavenger receptor 1 (MSR1).
The most down-regulated gene in the CAD patients compared to controls was the NK2 transcription factor related (NKX2-3) (Table 4).

Genes differentially expressed between sites within controls and CAD patients (Table 5)

In controls, no genes were found to be differentially expressed between different sites. In CAD patients, the expression of several (31) genes was higher in the coronaries than in the venous samples (Table 5). Analyses of these genes did not show any involvement in the atherosclerotic cascade. Both coronaries displayed a similar RNA expression pattern (Table 5).

Differences in gene expression between controls and CAD subjects for each site

Comparison of the gene expression between controls and CAD subjects for each site, showed that six probes corresponding to 4 genes were differentially expressed at all sites between CAD and controls. MARCO (log2 FC 1.04; $P<0.0001$) was up-regulated in all studied sites in CAD patients, and RGPD1 (log2 FC -1,44; $P<0.0001$), GCC2 (log2 FC -1.07; $P<0.0001$) and FMO1 (log2 FC -1.74; $P<0.0001$) were down-regulated in all studies sites in CAD patients compared to controls.

DISCUSSION

The present study evaluated markers of leukocyte activation and mRNA expression of genes linked to atherosclerosis in patients with and without CAD in different vascular compartments. It has been shown that unstable CAD patients display a higher degree of inflammation in the coronaries (13-15,21). We show, for the first time that there is an inflammatory gradient towards the coronaries for MPO, consistently in both groups, and that stable patients with multi-vessel CAD have a higher activation of MPO in all compartments compared to controls. MPO is able to attract neutrophils towards the vascular wall, thereby inducing inflammation and eventually

Table 4. Most significantly differentially expressed between controls and CAD patients by Microarray-Based Gene Expression Analysis

GENE ACRONYM	NAME	FOLD-CHANGE
	UP-REGULATED	
MSR1	macrophage scavenger receptor 1	1,625
SCGB3A1	Secretoglobin 3A	1,583
HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1	1,573
DSP	desmoplakin	1,440
HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	1,294
SLC7A8	solute carrier family 7 (amino acid transporter, L-type), member 8	1,272
TCOF1	Treacher Collins-Franceschetti syndrome 1	1,260
KIF26A	kinesin family member 26A	1,217
RAI2	retinoic acid induced 2	1,203
MRPL43	mitochondrial ribosomal protein L43	1,191
	DOWN-REGULATED	
NKX2-3	NK2 transcription factor related	-2,895
LALBA	lactalbumin, alpha-	-2,853
CDK5R2	cyclin-dependent kinase 5, regulatory subunit 2 (p39)	-2,601
DAB1	disabled homolog 1	-2,555
CRHR1	corticotropin releasing hormone receptor 1	-2,535
PRIMA1	proline rich membrane anchor 1	-2,525
MED24	mediator complex subunit 24	-2,498
PDLIM4	PDZ and LIM domain 4	-2,491
WDTC1	WD and tetratricopeptide repeats 1	-2,469
RARA	retinoic acid receptor, alpha	-2,441

atherogenesis (22). Higher MPO activation in the coronaries represents a situation of local inflammation.

The differences in expression of activation markers were not found for monocyte CD11b, neutrophil CD11b and CD66b, nor for their mRNA expression. As mentioned earlier, leukocyte CD11b and CD66b expression have been associated to atherosclerosis (4-12). Most of these studies were done in patients with unstable CAD (4,13-15), hyperlipidemic (5-7) or hyperglycemic (8) patients. Our data are in line with other studies (23-25). In stable CAD patients, CD11b and CD66b expression may be less sensitive markers than MPO. Alternatively, since CD11b is involved in adhesion, cells with a higher expression may adhere to the endothelium and therefore be absent in the samples. Indeed, angioplasty-induced leukocyte activation is higher on locally obtained cells than on cells in peripheral blood (4,13). Adherence is further enhanced due to atherosclerotic disease. Therefore, we may underestimate the leukocyte activation in CAD, since the 'activated' cells may be attached to the endothelium. Alternatively, the fact that CAD patients had higher MPO activation at all sites and that there was a gradient towards the coronaries, could suggest that the mechanism of activation of MPO differs from that of CD11b and CD66b.

The present study also shows that patients with CAD display highly significant differences in mRNA expression defined as means of all sites in a set of 20 genes which are mostly linked

Table 5. Differences in gene expression between sites in CAD patients.

		Left main coronary vs. Venous		Right coronary artery vs. Venous		Right coronary artery vs. Left main coronary	
Symbol	**refseq**	**logFC**	**adjP**	**LogFC**	**adjp**	**LogFC**	**adjp**
PRAC	NM__032391	0.7212	0.0327	0.8893	0.0043	0.1681	1.0000
BC041996	BC041996	0.6444	0.0327	0.8332	0.0124	0.1888	1.0000
A_23_P78975	A_23_P78975	0.6589	0.0327	0.7620	0.0252	0.1031	1.0000
ATG16L1	AK000897	0.5002	0.0327	0.5490	0.0252	0.0488	1.0000
AK127572	AK127572	0.5473	0.0327	0.5463	0.0254	-0.0010	1.0000
THC2610890	AK127572	0.5181	0.0327	0.5849	0.0252	0.0667	1.0000
AK000313	AK000313	-1.5592	0.0327	-1.6164	0.0254	-0.0572	1.0000
THC2621771	THC2621771	0.4152	0.0573	0.5731	0.0252	0.1579	1.0000
LOC728047	XM_001126928	0.4884	0.0327	0.3754	0.0581	-0.1130	1.0000
ENST00000338772	ENST00000338772	-1.1228	0.0327	-0.8894	0.0581	0.2334	1.0000
AA019203	AA019203	0.8909	0.0327	0.7670	0.0572	-0.1238	1.0000
ZNF443	NM_005815	-0.7727	0.0327	-0.6019	0.0581	0.1708	1.0000
S79672	S79672	0.4074	0.0573	0.5299	0.0254	0.1225	1.0000
A_32_P90468	A_32_P90468	0.5304	0.0506	0.5913	0.0355	0.0609	1.0000
A_32_P102627	A_32_P102627	0.6436	0.0327	0.6029	0.0572	-0.0408	1.0000
C15orf28	AK021784	0.4261	0.0743	0.6167	0.0254	0.1906	1.0000
MGC9712	NM_152689	0.8497	0.0327	0.5893	0.0798	-0.2604	1.0000
AK023954	AK023954	-1.9525	0.0327	-1.7634	0.0572	0.1891	1.0000
WDR52	NM_018338	0.7479	0.0570	-0.0761	0.8743	-0.8240	0.6524
AK026675	AK026675	0.3595	0.0750	0.5181	0.0254	0.1586	1.0000
THC2664215	THC2664215	0.4730	0.0573	0.5301	0.0436	0.0571	1.0000
AF315716	AF315716	-0.9288	0.0327	-0.6599	0.0798	0.2689	1.0000
AK027225	AK027225	0.6078	0.0327	0.3586	0.1205	-0.2492	1.0000
THC2536659	THC2536659	0.3779	0.0573	0.4540	0.0436	0.0761	1.0000
BQ318652	BQ318652	0.5635	0.0327	0.4416	0.0726	-0.1219	1.0000
AK022443	AK022443	0.5329	0.0438	0.5044	0.0572	-0.0285	1.0000
THC2532114	THC2532114	0.5135	0.0327	0.4150	0.0686	-0.0984	1.0000
AFF4	NM_014423	0.5030	0.0327	0.3637	0.0829	-0.1393	1.0000
AK021848	AK021848	0.3973	0.0573	0.4366	0.0572	0.0393	1.0000
VAC14	U25801	0.5622	0.0573	0.6037	0.0572	0.0415	1.0000
SRRM2	NM_016333	0.5333	0.0363	0.4604	0.0581	-0.0730	1.0000
AK024926	AK024926	1.0495	0.0506	1.0127	0.0572	-0.0367	1.0000
RP11-50D16.3	NM_001017370	0.4369	0.0332	0.3626	0.0655	-0.0743	1.0000
BX538090	BX538090	0.7352	0.0327	0.5204	0.0868	-0.2148	1.0000
KIAA0485	AB007954	0.6370	0.0396	0.5544	0.0604	-0.0826	1.0000

Refseq=reference sequence, FC= Fold Change, adjP= adjusted P-value

to the atherosclerotic process. DSP, SLC7A8, CRHR1 and CDK5R2 are involved in endothelial dysfunction, the initial step in the atherosclerotic process (26-29). DSP encodes for Desmoplakin and affects cell-to-cell junctions and endothelium permeability (26). SLC7A8 interacts with ICAM-1, increasing cell adhesion (27). CRHR1, which is associated with the production of adhesion molecules (28), was down-regulated in CAD, potentially increasing permeability

of circulating lipoproteins due to endothelial dysfunction. CDK5R2 which phosphorylates NO synthase and is crucial in arterial vasodilatation (29), was also down-regulated in CAD.

A subsequent key point in the progression of the atherosclerotic lesion is smooth cell proliferation, which in part resembles a tumoral process. In the list of differentially expressed genes there were 4 tumor suppressors. Three of them, LALBA, PDLIM4 and MED24, were indeed down-regulated in patients with CAD. Med24 has been shown to decrease PPAR gamma (30). Additionally, RAI2, an inducer of proliferation, was up-regulated in CAD (31). The NK2 gene, which was down-regulated in CAD, binds to SMCs depending on whether they are normal or neoplastic (31). All these genes are linked to endothelial dysfunction (DSP, SLC7A8, CRHR1 and CDK5R2) (26-29) and smooth muscle cell proliferation (LALBA, PDLIM4, MED24, RAI2 and NK2) (30,31). Particular interest goes out to the macrophage scavenger receptor proteins which are responsible for the uptake of lipoprotein remnants (32), MSR1 being the most up-regulated in CAD and also one of the 4 genes that were differentially expressed between groups and consequently for all sites separately (MARCO) (33). Other genes (RGPD1, GCC2 and FMO1) differentially expressed between the two groups at all sites, do not have an specific role in atherogenesis.

The analyses of the differentially expressed genes between different sites for CAD patients (Supplementary file 1), did not identify block changes that could be associated with any specific process or pathway within atherogenesis.

All the above mentioned changes in gene expression seem to favor a pro-atherogenic situation in CAD patients. The question is which factors are predominantly responsible and negatively influence the atherosclerotic process? While many risk factors were well controlled in CAD patients, atherosclerosis seemed to progress.

Two important considerations regarding these results are that, firstly, these genes were selected merely because they were the most differentially expressed between controls and CAD and not because of a potential function in relation to atherosclerosis and, secondly, these mRNA differences can be detected in circulating cells and seem to discriminate CAD patients and controls as efficiently as MPO and more efficiently than other established markers like CD11b and CD66b.

A confounding factor in this study may have been the use of medication. Statins decrease inflammation (34,35). In a previous study from our laboratory, we did not find an effect of rosuvastatin on the expression of these markers (20). Despite its anti-platelet effects, aspirin does not influence the expression of leukocyte activation marker CD11b (36). After correction for the use of medication, the data remained the same.

We did not find a difference for CRP between the groups. It is well known that statins decrease CRP levels, however, previous studies did not show correlations between CRP and the severity of CAD (37,38), but when unstable CAD patients were included, such a correlation was found (39). It should be underlined that we did not use a high sensitive CRP assay and this may have influenced the results.

In conclusion, this study shows that intracellular MPO, but not CD11b and CD66b expression, is more activated in circulating inflammatory cells in treated stable CAD patients than in controls at all sites, and that there is an inflammatory gradient for MPO towards the coronaries in both controls and CAD patients. Patients with CAD have higher mRNA expression in circulating cells for MSR1 as well as higher expression of genes involved in endothelial dysfunction and smooth muscle cell proliferation compared to controls. The fact that these patients under standard therapy still show a pro-inflammatory situation, may explain why these patients have an elevated risk for new vascular events. These data may help to understand better the inflammatory processes involved in coronary artery disease, which may lead to targeted individual interventions in the future.

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Disclosures
None declared.

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