Published in final edited form as:

J Surg Res. 2012 October ; 177(2): 373–381. doi:10.1016/j.jss.2012.06.021.

Monocytic Adhesion Molecule Expression and Monocyte-Endothelial Cell Dysfunction is Increased in Patients with Peripheral Vascular Disease versus Patients with Abdominal Aortic Aneurysms

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Abstract

Objective—Statin therapy is utilized in the medical management of patients with peripheral vascular disease (PVD) and abdominal aortic aneurysm (AAA) for the pleiotropic and anti-inflammatory benefits. We hypothesize that the inflammatory mechanisms of monocyte-endothelial cell interactions in endothelial barrier dysfunction is more significant in patients with

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AUTHOR CONTRIBUTIONS

Conception and design: EL, MW, SY

Analysis and interpretation: EL, EVS, KC, RP

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Writing the article: EL, EVS, KC

Critical revision of the article: EL

Final approval of the article: EL

Statistical analysis: EL

Obtained funding: EL, MW, SY

Overall responsibility: EL

PVD compared to AAA. The purpose of this study is to assess patient peripheral blood monocyte adhesion molecules by flow cytometry and monocyte-induced endothelial barrier dysfunction by using an in vitro endothelial cell layer and electric cell-substrate impedance sensing system (ECIS).

Methods—Peripheral blood was collected from patients with either PVD (ABI<0.9, toe-arm index <0.8, or required lower extremity vascular intervention) or AAA (aortic diameter >3.0 cm). Monocytes were isolated from fresh whole blood using an accuspinn-histopaque technique. The separated monocytes underwent flow cytometry analysis to evaluate expression levels of the cell membrane adhesion molecules: CD18, CD11a/b/c, and VLA-4. Endothelial cell function was assessed by adding monocytes to an endothelial monolayer on ECIS arrays and co-culturing overnight. Peak changes in trans-endothelial resistance were measured and compared between patient groups.

Results—Twenty-eight monocyte samples were analyzed for adhesion molecules (PVD: 19, AAA: 9) via flow cytometry and 11 patients were evaluated for endothelial dysfunction (PVD: 7, AAA: 4) via ECIS. There was no significant difference between risk factors among PVD and AAA patients except for age, where AAA patients were significantly older than PVD patients in both flow cytometry and ECIS groups ($P=0.02$, 0.01 respectively). There were significantly higher levels of adhesion molecules CD11a, CD18, and CD11c (averaged MFI P -values: 0.047 , 0.038 , 0.014 , respectively) in PVD patients compared to AAA patients. No significant difference was found for CD11b and VLA-4 expression ($P=0.21$, 0.15 respectively). There was significantly more monocyte-endothelial cell dysfunction as a result of monocytes obtained from patients with PVD than from AAA, with a maximal effect seen at 15 hours after monocyte addition ($P=0.032$).

Conclusions—Patients with PVD have increased expression levels of certain monocyte adhesion molecules and greater monocyte-induced endothelial layer dysfunction when compared to patients with AAA. This may lead to other methods of targeted therapy to improve outcomes of these vascular patients.

Introduction

Statin therapy has long been established as beneficial for nonsurgical populations by lowering cholesterol levels to prevent adverse cardiovascular outcomes (1). Increasingly, secondary effects of the benefits of statin therapy, other than cholesterol lowering, have been realized (2). Favorable vascular remodeling in the carotid arteries (3) and improved angiographic appearance of coronary arteries (4) are just a few of the determined “pleiotropic effects” of statin therapy. More recently, statins administered to patients undergoing non-cardiac vascular surgery have shown a significant decrease in myocardial ischemia and death from cardiovascular causes (5).

Biologically, statins have been hypothesized to have several plaque-stabilizing effects, such as increasing the expression of endothelial nitric oxide synthase, decreasing the thrombogenic profile, and reducing inflammation through extravasion of monocytic macrophages (6). These proposed mechanisms support the theory that atherosclerosis is an inflammatory process (7).

In determining monocytic interaction and inflammation in vascular surgical patients, we sought to evaluate two models of vascular disease that share similar yet distinct pathogenesis. Although inflammation plays an integral role in both disease states, AAA patients have a distinct long term prognosis and survival. AAA patients are reported to have an estimated 11 year life expectancy after diagnosis (8), whereas PVD patients can have a lower life expectancy of 6 months to around 5 years (9). Recent evidence suggests that AAA patients may live longer due to better clinical management of risk factors, including an increase in statin use, and decrease in smoking prevalence in the >65 years age group (8, 10, 11). We hypothesize that the cellular mechanisms of monocyte-endothelium interactions associated with vessel barrier dysfunction is more severe in patients with PVD compared to AAA due to increased monocyte adhesion molecule expression. The purpose of this study is to compare AAA and PVD patient peripheral monocyte adhesion molecule expression via flow cytometry and monocyte-induced endothelial barrier dysfunction in vitro.

Materials and Methods

Human study methods

A prospective evaluation of AAA and PVD patients' whole blood that underwent monocyte isolation, flow cytometry and electric cell-impedance sensing (ECIS) analysis was performed under Institutional Review Board approval at the Veterans Affairs Northern California Health Care System. All patients were enrolled and monitored at the Sacramento Veterans Affairs Medical Center.

Clinical data and risk factors

A patient history and physical examination was performed and pertinent medical history was documented. Diagnosis, surgical procedure, age, aneurysm diameter in cm (for AAA patients), ankle-brachial index score (for PVD patients), serum white blood cell (WBC) in K/cm, total cholesterol, statin use, and history of diabetes mellitus, hypertension, coronary artery disease, cerebral vascular accident, chronic obstructive pulmonary disease, cardiovascular disease, and tobacco use were recorded. PVD patients were defined as having ABIs less than 0.9, had a significant drop in ABIs (> 0.15) after treadmill exercise, a toe-arm index of less than 0.8 for patients that had non-compressive vessels, or patients who required documented vascular intervention to improve peripheral blood flow. AAA patients were defined as having an abdominal aortic diameter greater than 3.0 cm.

Monocyte Isolation from human adult peripheral blood

After consent was obtained, 20 mL of blood was collected from patients during surgical intervention for AAA or PVD. The blood was immediately placed in four 5-mL polypropylene heparinized tubes and kept on a rocker until processed. In all instances, the blood was used within 2 hours of removal from the patients. Monocytes were isolated as described by Sun and associates (12) with slight modification to the protocol described. Briefly, 4 mL of blood was overlaid over 4 mL of Fycoll Hypaque density gradient (1.077 g/mL; BD Biosciences, Bedford, MA) in 4–15 mL Falcon tubes (BD, Bedford, MA) and centrifuged for 30 minutes at 400 g. After separation, the buffy white layer of mononuclear cells was transferred via pipette into two sterile 15 mL Falcon tubes and rinsed with Hanks

Buffered Salt Solution (Sigma, St. Louis, MO), then centrifuged at 250 g for 10 minutes. After aspiration, the pellet was resuspended in 2 mL Red Blood Cell Lysis Buffer (Boston Bioproducts, Boston, MI) and allowed to sit for 5 minutes. 2 mL of Phosphate Buffered Saline (pH 7.4) wash buffer solution (containing 0.5 mM EDTA (EMD chemicals, Cincinnati, OH), and 2% Bovine Serum Albumin (US Biochem Corp, Cleveland, OH) was then added and the cell suspension was centrifuged at 250g for 10 minutes. After aspiration, the pellet was resuspended in 500uL of the PBS wash buffer and an EasySep monocyte isolation kit (Stem Cell, Vancouver, BC) was used following manufacturer's instructions in order to enumerate and magnetically isolate monocytes by negative selection without CD16 depletion.

HUVEC Cell Culture

Human umbilical vein endothelial cells (HUVEC) (Clonetics, San Diego, CA) were seeded at a density of 5×10^5 on tissue-culture treated petri dishes (Cellstar, Santa Clara, CA) treated with 1.5% porcine gelatin (Sigma, Saint Louis, MO). The cells were grown in Endothelial basal medium (EBM) (Lonza, Walkersville, MD) supplemented with EGM-2-MV SingleQuot (5% fetal bovine serum in EBM with 12 ug/ml bovine brain extract, 1 ug/ml hydrocortisone, and 1 ug/ml GA-1000) (Lonza, Walkersville, MD). The cells were routinely maintained according to the manufacturer's instruction and media was changed every 2–3 days. ECIS experiments only utilized HUVECs at low (4–5) passages.

Flow Cytometry for monocyte adhesion molecules

Monocytes were isolated as described above and resuspended in 0.5 mM EDTA, 2% BSA, PBS (pH 7.4) wash buffer solution at a density of 1×10^5 for a total volume of 300 uL per 5 mL centrifuge tube (BD, Bedford, MA). Four tubes were prepared for data collection: 1) 10 uL of FITC-conjugated anti-CD18 and PECy5-conjugated anti-CD11c, 2) 10 uL of PE-conjugated anti-CD11a and APC-conjugated anti-CD11b, 3) 10 uL of PECy5-conjugated anti-VLA-4, and 4) 10 uL of each antibody's respective isotype control. All antibodies used were obtained from BD Biosciences (Bedford, MA). Four tubes containing each antibody were prepared and used to adjust voltage and compensation on each channel of the flow cytometer before the sample data was collected. After a 20 minute incubation period, each tube was rinsed with 2 mL of PBS wash buffer and centrifuged at 1400 rpm for 5 minutes, then resuspended in 300 uL of PBS wash buffer. Approximately 5×10^4 unstained cells were added to each compensation tube before collecting data on an Accuri C6 cytometer (Accuri, Ann Arbor, MI). 10,000 events per trial were collected and analyzed using CFlow software (Accuri, Ann Arbor, MI).

Transendothelial Electrical Resistance

The effect of monocytes isolated from PVD versus AAA patients on endothelial barrier permeability was evaluated by measuring transendothelial electrical resistance (TER) as previously described (13, 14) (Fig. 1). Briefly, HUVECs were maintained with EGM-2-MV media, detached with a trypsin-EDTA solution (Cellgro, Manassas, VA), and passaged 3–4 times before being seeded onto ECIS electrode arrays (Applied Biophysics, Troy, NY) and incubated overnight in EBM media in a 5% CO₂ incubator at 37°C. At the start of the experiment, isolated monocytes were resuspended in EBM media at a concentration of $6 \times$

10⁶ cells/mL and added to half the electrode compartments, while the other half served as controls. Barrier function was measured by determining the electrical impedance of the cell-covered electrodes on the bottom of the ECIS arrays. A constant-current source was applied via a 1-V, 4,000-Hz alternating current signal sent through a 1-M Ω resistor. The resistance and capacitive resistance were measured and analyzed with ECMS 1.0 software (CET, Coralville, IA). Endothelial barrier function was indirectly assessed through TER measurements normalized to baseline after addition of monocytes. The values measured at each time point for patient group were averaged and presented as means $\pm$ standard deviation. Only endothelial monolayers with a baseline TER of 5,000 Ω or higher were used for data in this study to ensure uniform monolayer confluency and electrode viability.

Statistical analysis

Results are reported as mean $\pm$ standard deviation. Microsoft Excel (Microsoft Inc, Redmond, WA) was used to perform a two-tailed *t*-test to compare groups for continuous variables and a χ^2 analysis for proportions for clinical risk factors such as the presence or absence of diabetes, hypertension, coronary artery disease, cerebral vascular accident, chronic obstructive pulmonary disease, cardiovascular disease, statin use, and tobacco use. A difference with a value of $P < 0.05$ was considered significant.

Results

Of the 28 patients that underwent flow cytometry analysis, 9 were diagnosed with AAA (age, 75.4 $\pm$ 10.3 years; mean diameter, 6.1 $\pm$ 1.4 cm) and 19 were diagnosed with PVD (67.2 $\pm$ 6.5 years; ABI score 0.56 $\pm$ 0.34). Of the 11 patients that underwent ECIS analysis, 7 were diagnosed with AAA (age, 75.3 $\pm$ 7.6 years; mean diameter, 5.8 $\pm$ 1.4 cm) and 4 were diagnosed with PVD (age, 62.5 $\pm$ 2.5 years; ABI score 0.66 $\pm$ 0.25). AAA patients were found to be significantly older than PVD patients in both flow cytometry and ECIS groups ($P = 0.02$ and 0.01 respectively). There were no significant differences between measured risk factors in both flow cytometry and ECIS groups (Table 1 and 2). There were 3 PVD patients in the flow cytometry group and 1 PVD patient in the ECIS group that had an ABI > 0.9 . Two PVD patients in the flow cytometry group had non-compressive vessels that had falsely elevated ABIs, however both patients had a toe-arm index < 0.8 . The other PVD patient in this group was diagnosed with acute limb ischemia (pre-surgical ABI = 0) that required vascular intervention (balloon angioplasty) to improve blood flow to the lower extremities. The PVD patient in the ECIS group with a normal ABI had peripheral occlusive disease that also required vascular intervention to improve lower extremity blood flow.

A cardiovascular history of patients as defined by previous coronary intervention such as coronary artery bypass graft surgery, percutaneous coronary intervention, or abnormal cardiac stress test were taken into account to ensure our results were not skewed from underlying cardiovascular disease. However, we found no significant differences between PVD and AAA groups for cardiovascular disease. In addition, there were a total of 6 patients that had both disease processes. In the flow cytometry group, 2 patients with AAA also had PVD and 2 patients with PVD group also had an aortic aneurysm. In the ECIS group, only 2 patients with AAA also had PVD.

Flow cytometry analysis of monocytic adhesion molecules from PVD patients (n= 19) and AAA patients (n= 9) show that there is a statistically significant elevation of CD18, CD11a, and CD11c expression displayed in monocytes from PVD patients compared to AAA patients. Purified monocytes were stained with antibodies against CD18, CD11a, CD11b, CD11c and VLA-4 as well as the respective antibody isotype controls. The Mean fluorescent Intensity (MFI) was determined as previously described (Fig. 2), and used as a proxy for monocyte adhesion molecule expression. Flow cytometry analysis showed statistically significant elevated levels of CD11a (P= 0.047) (Fig. 3, A), CD18 (P= 0.038) (Fig. 3, B), and CD11c (P= 0.014) (Fig. 3, C) in PVD patients compared to AAA patients. Increased VLA-4 expression was also noted in PVD versus AAA patients (Fig. 3, E); however the difference was not statistically significant (P= 0.15). There were no significant differences in CD11b monocyte adhesion molecules between patient groups.

Electric cell-substrate impedance sensing (ECIS) analysis of HUVEC monolayer transendothelial resistance was performed at various time points after addition of monocytes from PVD or AAA patients to compare monocytic activity. The results show that monocytes from PVD patients induced a substantial increase in endothelial monolayer permeability (proportional to the measured decrease in TER) compared to monocytes from AAA patients (Fig. 4). Maximal decrease in TER was observed at 15 hours after monocyte addition (P= 0.032). Measurements at further time points, i.e. 15, 16 and 17 hours after monocyte addition, continued to exhibit a significant increase in barrier permeability (P= 0.013, 0.014, 0.016, respectively) (data not shown).

Discussion

This is the first study to our knowledge directly comparing monocytic adhesive and infiltrative ability between PVD and AAA patients. The β_1 and β_2 integrin receptors VLA-4, CD11a/CD18, CD11b/CD18, and CD11c/CD18, which are upregulated on activated monocytes during inflammation, play a crucial role in the pathogenesis of atherosclerosis and other inflammatory diseases (6). Our findings illustrate a significant upregulation of expression for the CD11a, CD11c, and CD18 integrins found on the surface of monocytes from PVD patients compared to AAA patients, indicating a more aggressive monocytic activation in the PVD disease state. The evaluation of these adhesion molecules may be a useful biomarker to titrate statin therapy in preventing cardiovascular events. The CD18 integrin is an especially attractive target, due to its ability to dimerize with multiple members of the β_2 integrin family of adhesion molecules which regulate monocyte diapedesis through vessel endothelium (15). Although, exactly how to pursue modulating the levels of CD18 expression and activity will still require further study.

Typically, monocyte adhesion expression normally increases with age due to age-dependent cytokine dysfunction (16, 17). However, our data suggests that age may not contribute to the differences in monocyte activity observed between PVD and AAA patients. The AAA patients were found to be significantly older and they exhibited lower monocyte adhesion expression levels than PVD patients. There was also a lower incidence of coronary artery disease in the AAA than the PVD group. This finding suggests that age is not a major factor of monocyte behavior in the progression of atherosclerosis usually seen in PVD patients.

Additional studies that stratify PVD patients by age and their monocyte adhesion expression are needed.

Although monocyte adhesion molecule expression is a preliminary indicator of monocyte activity, little can be deduced from these results in regards to monocyte interaction with endothelial monolayers. A key step in the development of inflammatory dysregulation is extensive endothelial barrier permeability (7). In this study, monocyte-induced endothelial permeability was measured in vitro using an ECIS system, in which transendothelial resistance (TER) was used as a proxy for endothelial barrier dysfunction (permeability). Our results indicated that monocytes from PVD patients induced greater endothelial barrier dysfunction compared to monocytes from AAA patients. These results can be explained by the severity of inflammation contributing to both disease states. For instance, a study by Simons and associates measured carotid intima media thickness (IMT), an indicator of atherosclerosis, between PVD and AAA patients and found that PVD patients had a greater carotid IMT thickening compared to AAA patients (18). These results, like our own, support the hypothesis that inflammation plays a more critical role in the pathogenesis of PVD, while only partially contributing to the development of AAA. However, the specific inflammatory mechanisms that cause greater endothelial dysfunction in PVD versus AAA patients remain unclear. It was suggested that hypoxia, especially with the prevalence of ischemia in PVD patients, may have a greater inducing effect on monocyte and macrophage behavior than proinflammatory cytokines alone (19). Furthermore, it has been shown that hypoxic environments greatly affect monocyte proinflammatory responses by modulating the expression of genes for angiogenic factors and inflammatory cytokines (20). Future in vitro studies are needed to confirm the actual potency of a hypoxic versus a proinflammatory environment on monocyte adhesion and endothelial dysfunction.

Statins could be used as a novel therapy to reduce monocyte-induced endothelial dysfunction seen in PVD and AAA patients. Statins have been known to induce anti-inflammatory effects by inhibiting the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) pathway (21, 22). Statins inhibit the HMG-CoA pathway by reducing C-reactive protein-induced chemokine secretion, ICAM-1 upregulation and chemotaxis, which consequently results in decreased monocyte adhesion (22). Other studies have shown the effect of statins in reducing the expression of adhesion molecules in circulating monocytes from hypercholesterolemic patients (23). However, further studies should be performed to evaluate the effects of statins on patient monocytes at escalating doses to see an effect on adhesion molecule expression and the interaction with an endothelial cell layer, especially to understand the extent of the HMG-CoA pathway involvement in the PVD and AAA disease states.

Although analyzing monocyte adhesion molecules by flow cytometry provides an important preliminary insight, there are a few weaknesses to this approach. Patient monocyte yields varied from 3–5 million cells per 20 mL blood obtained. A clinical issue which may have affected our results is the variability within the patient cohorts in regards to medications, diet, and other present disease states. Also, the setting (outpatient, intra-procedural, inpatient) in which the blood collections took place may have had an effect on monocyte number and adhesion molecule expression.

Our study was limited by the use of HUVECs because both AAA and PVD are mainly arterial, not venous, pathologies. Application of monocytes to human aortic endothelial cells (HAEC) and their effect upon endothelial dysfunction would have been a stronger model for the monocyte-endothelial interaction in these disease states. Thus, these experiments should be repeated to confirm that the findings in HUVEC are replicated in an aortic endothelial cell layer.

Patients' age was not controlled for between PVD and AAA groups. Our samples were obtained during vascular interventions directly associated with the disease states. Coincidentally, AAA patients undergoing repair were generally older than PVD patients. This finding is likely the result of a combination of factors, including the overall aggressiveness of the disease and the necessity for intervention, which may contribute to the overall greater life expectancy in AAA patients compared to PVD patients. Although we did not control for age, this was a preliminary study that can serve as a foundation for future studies, in which age-matched control patients should be seriously considered.

Despite the limitations, these findings are important despite having a small patient sample size with multiple technical and clinical variables, there is a significant elevation in monocyte adhesion molecule expression and increased monocyte-induced endothelial barrier dysfunction in patients with PVD versus AAA. These results are consistent with the inflammatory model of monocyte activity for atherosclerosis (7). These findings should also be used to guide further investigations exploring the mechanisms behind other various vascular pathologies.

In conclusion, monocytes obtained from PVD patients have significantly higher expression of adhesion molecules and induce greater endothelial barrier dysfunction when co-cultured with endothelial cells in vitro than AAA patients.

ACKNOWLEDGEMENTS

This work is supported by VA Merit Career Development Award (CDA-2) IK2CX000521 and NIH Grants, HL096640, HL061507, HL070752.

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Figure 1.

Diagram illustrating the ECIS set-up used to compare transendothelial resistance (TER) induced by the addition of monocytes from AAA and PVD patients. A $1\text{ M}\Omega$ resistor applies a 1 V, 4000 Hz alternating current to an electrode underlying a confluent endothelial cell monolayer. This results in a potential voltage (V) across the electrode which is measured by the ECIS instrument. The impedance (Z), which is a direct measurement of resistance, is determined by Ohm's law $Z = V/I$, where I is the current applied. The impedance produced by the monolayer overlaying the electrode is modulated by changes in cell morphology and monolayer integrity.

Figure 2.

Representative figure showing gating technique used to analyze Mean Fluorescence intensity (MFI) of monocytic adhesion molecules. (A) Scattered cytogram and (B) cytohistogram. On scattered cytogram, side scatter detects cell size, and forward scatter detects cell inner structure. The monocyte population can be distinguished from other cell populations by its low forward scatter (FSC) and medium side scatter (SSC) characteristics. (A) Monocytes were gated based on their FSC vs SCC characteristics. (B) MFI of gated monocytes were expressed on a cytohistogram and the MFI for each isotype was subtracted. 10,000 events were collected per trial.

Figure 3.

Flow cytometry analysis showing increased expression of (A) CD11a ($P=0.047$), (B) CD18 ($P=0.038$), (C) CD11c ($P=0.014$), (D) CD11b ($P=0.20$), and (E) VLA-4 ($P=0.15$) in monocytes isolated from PVD versus AAA patients. Data is expressed as the averaged MFI for each patient disease state after subtraction of respective isotype control MFIs. Error bars show the standard deviation for CD11a (PVD SD= 6459, AAA SD= 587); CD18 (PVD SD=7592, AAA SD= 6518); CD11c (PVD SD= 5621, AAA SD= 4821); CD11b (PVD SD= 2910, AAA SD= 2725); and VLA-4 (PVD SD= 3098, AAA SD= 2538).

Figure 4.

Representative figure showing transendothelial resistance of HUVEC monolayers measured throughout the time course of ECIS assays after the addition of isolated monocytes from (A) AAA and (B) PVD patients. EBM media alone was used as a control. Note that in both cases, monolayer resistance decreases upon adding monocytes, however, the decrease is much greater with the addition of monocytes from PVD patients.

Figure 5.

Maximal decrease in transendothelial resistance (TER) of HUVEC monolayers measured at 15 hours after addition of isolated monocytes from AAA or PVD patients ($P=0.032$). Error bars show the standard deviation (PVD SD= 0.12; AAA SD=0.05)

Table 1

Clinical data for all patients that underwent flow cytometry analysis

Pt	Dx	Procedure	Age	AAA Size (cm)	WBC (K/cmm)	DM	HTN	CAD	CVA	COPD	Chol	Statin Use	Tob
1	AAA	Open AAA Repair	64	9.5	11.6	Y	Y	Y	N	N	134	Y	N
2	AAA	EVAR	85	5.6	6	N	Y	Y	Y	N	83	Y	N
3	AAA	EVAR	86	6.2	9.7	N	Y	N	N	Y	134	Y	N
4	AAA	EVAR	68	5.5	6.8	N	N	N	N	N	193	N	N
5	AAA	EVAR	82	6.4	5.4	N	Y	Y	N	N	121	Y	N
6	AAA	EVAR	69	4.2	7.9	N	Y	N	N	N	130	Y	Y
7	AAA	EVAR	72	5.8	7.3	N	Y	N	N	N	305	Y	N
8	AAA	EVAR	90	6.2	3.7	N	Y	N	N	N	151	N	N
9	AAA	Open AAA Repair	63	5.4	7.5	N	Y	N	N	N	161	Y	Y
Mean $\pm$ SD or %				75.4 $\pm$ 10.3	6.1 $\pm$ 1.4	7.3 $\pm$ 2.3	11%	89%	33%	11%	157 $\pm$ 63	78%	22%
Pt	Dx	Procedure	Age	ABI	WBC	DM	HTN	CAD	CVA	COPD	Chol	Statin Use	Tob
10	PVD	Endovascular Intervention	75	0.4	9.3	Y	Y	Y	N	Y	104	Y	Y
11	PVD	Endovascular Intervention	66	0.26	6.5	N	Y	Y	N	N	136	Y	N
12	PVD	Femoral Bypass	55	0.4	6.7	N	Y	Y	N	N	214	N	N
13	PVD	Endovascular Intervention	66	0.66	6	Y	Y	Y	N	N	309	N	N
14	PVD	Femoral Endarterectomy	72	0.24	5.7	N	Y	N	N	N	147	Y	N
15	PVD	Endovascular Intervention	58	0.81	12.8	N	N	N	N	Y	218	Y	Y
16	PVD	Femoral Endarterectomy	68	1.42	4.2	Y	Y	Y	N	N	108	Y	N
17	PVD	Femoral Bypass	61	0.65	5.7	N	Y	N	N	N	138	Y	Y
18	PVD	Femoral Bypass	65	0.51	8.3	N	Y	Y	N	Y	189	Y	Y
19	PVD	Femoral Endarterectomy	67	0.17	11.8	N	Y	N	N	N	191	Y	Y
20	PVD	Femoral Bypass	61	0.42	7.4	N	Y	N	N	N	-	N	N

Pt	Dx	Procedure	Age	AAA Size (cm)	WBC (K/cmm)	DM	HTN	CAD	CVA	COPD	Chol	Statin Use	Tob
21	PVD	Endovascular Intervention	74	0.76	5.7	N	Y	Y	N	N	114	Y	N
22	PVD	Endovascular Intervention	61	0.2	9.4	N	Y	N	N	Y	193	Y	Y
23	PVD	Endovascular Intervention	71	0.7	7.4	Y	Y	Y	N	Y	113	N	N
24	PVD	Endovascular Intervention	73	1.1	5.2	Y	Y	N	N	N	157	Y	N
25	PVD	Endovascular Intervention	69	0.33	11.7	Y	Y	N	N	N	189	Y	N
26	PVD	Endovascular Intervention	64	0.96	10.3	N	Y	N	N	N	117	Y	N
27	PVD	Endovascular Intervention	69	0.56	10.8	N	Y	Y	N	Y	210	Y	Y
28	PVD	Endovascular Intervention	82	0.18	5.1	N	Y	Y	N	Y	141	Y	N
Mean $\pm$ SD or %			67.2 $\pm$ 6.5	0.56 $\pm$ 0.34	7.9 $\pm$ 2.6	32%	95%	53%	0%	37%	166 $\pm$ 53	79%	37%
P-Value			0.02	0.58	0.24	0.57	0.34	0.14	0.16	0.7	0.94	0.44	

AAA, abdominal aortic aneurysm; CAD, coronary artery disease; Chol, total cholesterol; COPD, chronic obstructive pulmonary disease; CVA, cerebral vascular accident; DM, diabetes mellitus; Dx, Diagnosis; EVAR, endovascular aneurysm repair; HTN, Hypertension; PVD, peripheral vascular disease; Tob, tobacco use; WBC, white blood cells

Table 2

Clinical data for all patients whose monocytes underwent ECIS analysis

Pt	Dx	Procedure	Age	AAA Size (cm)	WBC (K/cmm)	DM	HTN	CAD	CVA	COPD	Chol	Statin use	Tob
1	AAA	EVAR	79	5.5	7	Y	Y	Y	N	N	205	Y	N
2	AAA	EVAR	69	4.2	7.9	N	Y	N	N	N	130	Y	Y
3	AAA	EVAR	66	5.3	5.4	N	Y	Y	N	Y	144	Y	N
4	AAA	EVAR	82	5.7	6.5	N	Y	Y	N	N	154	Y	N
5	AAA	EVAR	87	6.1	6.8	N	Y	N	N	Y	142	N	N
6	AAA	EVAR	71	5.3	7.3	N	Y	N	N	Y	131	Y	N
7	AAA	EVAR	73	8.7	7.2	N	Y	N	N	N	-	N	Y
Mean ± SD or %				5.8 ± 1.4	6.9 ± 0.8	14%	100%	43%	0%	43%	151 ± 28	71%	29%
Pt	Dx	Procedure	Age	ABI	WBC (K/cmm)	DM	HTN	CAD	CVA	COPD	Chol	Statin use	Tob
8	PVD	Femoral Bypass	63	0.7	11.1	Y	Y	Y	Y	Y	72	Y	N
9	PVD	Femoral Bypass	65	0.41	10.5	N	Y	Y	N	Y	189	Y	Y
10	PVD	Endovascular Intervention	59	0.53	5.5	N	Y	Y	N	N	152	Y	N
11	PVD	Carotid Endarterectomy	63	0.99	7.8	N	Y	Y	N	N	143	Y	Y
Mean ± SD or %				0.66 ± 0.25	8.7 ± 2.6	25%	100%	100%	25%	50%	139 ± 49	100%	50%
P-Value				0.01	0.1	0.66	1	0.06	0.17	0.82	0.63	0.24	0.48

AAA, abdominal aortic aneurysm; CAD, coronary artery disease; Chol, total cholesterol; COPD, chronic obstructive pulmonary disease; CVA, cerebral vascular accident; DM, diabetes mellitus; Dx, Diagnosis; EVAR, endovascular aneurysm repair; HTN, Hypertension; PVD, peripheral vascular disease; Tob, tobacco use; WBC, white blood cells