

Two C-C Family Chemokines, Eotaxin and RANTES, Are Novel Independent Plasma Biomarkers for Abdominal Aortic Aneurysm

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Background—Inflammation of the aortic wall is recognised as a key pathogenesis of abdominal aortic aneurysm (AAA). This study was undertaken to determine whether inflammatory cytokines could be used as biomarkers for the presence of AAA.

Methods and Results—Tissue profiles of 27 inflammatory cytokine were examined in AAA (n=14) and nonaneurysmal (n=14) aortic tissues. Three cytokines, regulated upon activation normally T-cell expressed and secreted (RANTES), eotaxin, and macrophage inflammatory protein 1 beta (MIP-1b), had increased expression in AAA, particularly within the adventitial layer of the aortic wall. Basic fibroblast growth factor (bFGF) had reduced expression in all layers of the AAA wall. Examination of the circulating plasma profiles of AAA (n=442) and AAA-free controls (n=970) suggested a (risk factor adjusted) AAA-association with eotaxin, RANTES, and high sensitivity C-reactive protein (hsCRP). A plasma inflammatory cytokine score, calculated using these three markers, suggested a strong risk association with AAA (odds ratio, 4.8; 95% CI, 3.5–6.7; $P<0.0001$), independent of age, sex, history of ischemic heart disease, and smoking.

Conclusions—Contrary to reports suggesting a distinct T helper 2-associated inflammatory profile in AAA, this current study suggests a more-generalized pattern of inflammation, albeit with some potentially distinct features, including elevated plasma eotaxin and decreased plasma RANTES. In combination with hsCRP, these markers may have potential utility as AAA biomarkers. (*J Am Heart Assoc.* 2016;5:e002993 doi: 10.1161/JAHA.115.002993)

Key Words: abdominal aortic aneurysm • aorta • C-reactive protein • eotaxin • inflammation • plasma • RANTES

Abdominal aortic aneurysm (AAA [MIM 100070]) is a common condition, particularly in men over the age of 65 years, with a reported prevalence of between 2% and 8%.¹ The demographic risk factor profile for AAA is well described and includes smoking, family history of AAA, older age, male sex, and concurrent atherosclerotic diseases, particularly coronary artery disease (CAD).^{2–4} Though historically considered to be a consequence of atherosclerosis, AAA is now considered to be a distinct clinical entity, albeit one that shares many risk factors with occlusive atherosclerotic vascular disease.^{2,5}

Periaortic inflammation is a key distinguishing feature of AAA,^{6,7} and several studies have suggested an association

between circulating inflammatory cytokines and AAA.^{8–11} Nevertheless, to date only a relatively small number of circulating biomarkers have convincingly been implicated in AAA and none have been translated into AAA-specific clinical practice.

This study aimed to determine infrarenal abdominal aortic tissue inflammatory cytokine profiles in both aneurysm patients and nonaneurysm controls. The resulting AAA tissue profile was then compared with the plasma profile from a large series of well-characterized AAA and ultrasound confirmed nonaneurysmal study participants. As such, this study represents the largest number of samples and simultaneously analyzed inflammatory cytokines published to date.

Materials and Methods

Tissue and Plasma Samples

Infrarenal aortic tissue was obtained from 14 AAA patients undergoing elective open repair at the Vascular Surgery Unit, Dunedin Hospital (Dunedin, New Zealand). These were compared with nonaneurysmal aortic tissues, obtained less than 24 hours postmortem, from 14 age- and sex-matched cadaveric controls (Table 1).

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Table 1. Aortic Tissue Population Demographics

	Controls, n=14	AAA, n=14	P Value
Age, y	68.0±11.4	70.9±6.6	0.3581
Male sex, n (%)	10 (71.4)	10 (71.4)	1.0
Max infrarenal diameter, mm	—	54.3 (47.5–56.6)	—

AAA indicates abdominal aortic aneurysm; Max, maximum.

Plasma samples from 442 AAA patients and 970 AAA-free controls (Table 2) were recruited from the Otago region of New Zealand. AAA patients were either under active aneurysm surveillance or recruited before elective aneurysm surgical repair. All controls were recruited from the same community as part of a local AAA ultrasound screening program (inclusion criteria age greater than 60 years of age and both males and females). Those screened individuals with an infrarenal aorta greater than 25 mm in diameter were excluded from this control group.

Written informed consent was obtained from all participants or relevant next of kin (for material collected post-mortem), and the study was undertaken with the approval of the regional ethics committee.

Tissue and Plasma Preparation

Anterior infrarenal aortic wall biopsies were collected as soon as possible from the operating theatre or postmortem room and immediately transferred into RNA-later solution. Immediately after collection, each (thrombus-free) specimen was divided into 2 equivalent pieces. The first half was divided into

small (≈ 50 mg) full-wall thickness pieces. The second half was carefully microdissected into discrete (≈ 50 mg) adventitial and intima+medial layers specimens for separate processing. Tissue samples were immersed in RNA-later solution (Life Technologies, Carlsbad, CA) for 24 hours at 4°C and then stored at -80°C until processed.

Separate total wall, adventitia, and intima+medial specimens from each individual were thawed and washed in buffer before being homogenized (PT2100; Polytron; Kinetica AG, Lausanne, Switzerland) in ice-cold cell lysis buffer (10 $\mu\text{L}/\text{mg}$ of tissue; Bio-Rad, Hercules, CA). The resulting homogenate was centrifuged at 2000 g for 20 minutes at 4°C. Lysate aliquots were stored at -80°C until assayed.

Case and control EDTA plasma samples were collected, centrifuged, separated into 500- μL aliquots, and stored at -80°C within 30 minutes. The average length of storage time before being assayed was 5.6 years, and despite a lack of significant differences between cases and controls ($P=0.87$), storage times were nevertheless included in the regression analysis to ensure that this was not a confounding factor.

Cytokine Assay

Tissues and plasma inflammatory cytokines levels were determined using the Bio-Plex Pro human cytokine 27-Plex Panel (Bio-Rad), according to the manufacturer's instructions. Data were analyzed using Bio-Plex Manager software (version 4.1; Bio-Rad).

High sensitivity C-reactive protein (hsCRP; Tina-quant high sensitivity assay; Roche Diagnostics, Indianapolis, IN) and nonfasting triglycerides and high-density lipoprotein (HDL; enzymatic-colorimetric method; Roche) levels were also measured for each plasma sample.

Table 2. Blood Plasma Population Demographics

	Controls, n=970	AAA, n=442	P Value
Age, y	68.5±7.6	75.0±7.9	<0.0001
Male sex (%)	741 (76.4)	334 (75.6)	0.76
Max infrarenal diameter, mm	19.7 (18.0–21.2)	52.0 (42.9–60.8)	<0.0001
Male	20.2 (18.6–21.7) n=741	53.0 (44.1–63.2) n=334	
Female	17.9 (16.7–19.1) n=229	46.7 (39.9–56.0) n=108	
Smoking pack-years	1.4 (0–17.0)	22.5 (7.5–42.7)	<0.0001
Hypertension (%)	31.6	59.3	<0.0001
Diabetes (%)	6.8	11.4	0.004
Ischemic heart disease (%)	21.4	36.3	<0.0001
Peripheral artery disease (%)	1.6	16.4	<0.0001

AAA indicates abdominal aortic aneurysm; Max, maximum.

Tissue mRNA Expression

Quantitative polymerase chain reaction (qPCR) probes were used to determine relative expression of eotaxin 1 (chemokine [C-C motif] ligand [CCL]11), eotaxin 2 (CCL24), eotaxin 3 (CCL26), regulated upon activation normally T-cell expressed and secreted (RANTES; CCL5), and macrophage inflammatory protein 1 beta (MIP-1b; CCL4) referenced to GAPDH (Table 3) in 6 AAA and 11 control aortic biopsies. Total RNA was extracted using Norgen Total RNA kits (Norgen Biotek Corporation, Thorold, Ontario, Canada), according to the manufacturers' instructions. RNA quantity and purity were assessed using a NanoDrop spectrophotometer (Implen GmbH, München, Germany). Relative quantification of target gene probe intensities was calculated using the Δ Ct (threshold cycle) method (GAPDH as the housekeeper gene).

Statistical Analysis

StatView software (version 5.01; SAS Institute Inc., Cary, NC) was used to perform statistical analysis. The distribution of continuous variables (kurtosis and skewness) was assessed and analyzed with either the Mann-Whitney *U* test or ANOVA with Fisher's protected least significant difference test. Multiple (step-wise) logistic regression was used to evaluate the interactions between cytokine biomarkers and confounding demographic variables. Network relationships between variables were examined using variable principal component analysis (Omics Explorer 3.1; Qlucore, Lund, Sweden) with log-transformed data and linking each marker with its 2 nearest neighbors in the network (Euclidean distance threshold, 65%). Spearman's rank correlations were used to assess plasma biomarker and aneurysm size correlations.

Receiver operating characteristic (ROC) curves were constructed to determine the optimal binary cut-off value of each

differentially expressed cytokine. This value was calculated using the maximum of the Youden index $J = \max [SE_i + SP_i - 1]$, where SE_i and SP_i are the sensitivity and specificity over all possible threshold values.

Results were expressed as medians and interquartile ranges or mean \pm SD for normally distributed variables. *P* value significance thresholds were conservatively adjusted for multiple testing (Bonferroni correction) to determine statistically significant differentially expressed cytokines in AAA patients.

Results

Aortic Tissue Inflammatory Cytokine Profiles

Tissue inflammatory cytokine profiles were assessed in 14 AAA and 14 control total wall biopsies (Table 4). In the tissue analysis, 90.1% of IL-2 values were below the assay detectable range and therefore this cytokine was excluded from the tissue biomarker analysis. In all 8 cytokines (interleukin [IL]-1b, IL-10, IL-12p70, basic fibroblast factor [bFGF], vascular endothelial growth factor [VEGF], MIP-1a/CCL3, MIP-1b, and RANTES) appear to have suggestive ($P < 0.05$) differential case-control expression in total wall biopsies; however, only bFGF (decreased in AAA compared to controls) and RANTES (increased in AAA) fell below the multiple testing threshold (Table 4). In 12 AAA and 12 control samples, matching isolated intima+media and adventitial specimens were available. When isolated intima+media layers (Table 5) were compared, a similar pattern was observed to that of total wall specimens, but with IL-6 also showing a suggestive association (increased in AAA). While generally matching both total wall and intima+media specimens, adventitial tissue appeared to show the greatest AAA versus control cytokine differences (Table 6). Four markers reached

Table 3. Taqman Probes Used for qPCR

Genes	Type	Sequences/Taqman Probe ID	Control Mean Ct	Case Mean Ct
Eotaxin 1 (CCL11)	SYBR Green	Forward: 5' AAAAGGTCTCCGCAGCACTTCTGT 3' Reverse: 5' AAAAGACAGAAGCTGGCCAGCGA 4'	29.7 (\pm 2.1)	30.2 (\pm 1.2)
Eotaxin 2 (CCL24)	TaqMan	Hs00171082_m1	Undetectable >35	33.0 (\pm 1.0)
Eotaxin 3 (CCL26)	TaqMan	Hs01099415_m1	Undetectable >35	33.6 (\pm 1.4)
MIP-1b (CCL4)	TaqMan	Hs99999148_m1	34.9 (\pm 0.3)	30.0 (\pm 1.5)
MIP-1b (CCL4) alternate probe	SYBR Green	Forward: 5' ACCAATACCATGAAGCTCTGCGTG 3' Reverse: 5' TGCTTCTTTGGTTTGGAAATTTGG 3'	33.7 (\pm 1.2)	30.8 (\pm 1.6)
RANTES (CCL5)	TaqMan	Hs00982282_m1	32.1 (\pm 1.2)	27.5 (\pm 1.6)
GAPDH	TaqMan	Hs02758991_g1	23.2 (\pm 0.6)	24.5 (\pm 1.0)

Taqman probes were used to confirm localized tissue gene expression of the proteins identified in the inflammatory cytokine bioplex experiments. Ct values are means \pm 1 SD. CCL indicates chemokine (C-C motif) ligand; Ct, threshold cycle; MIP-1b, macrophage inflammatory protein 1 beta; qPCR, quantitative polymerase chain reaction; RANTES, regulated on activation, normal T-cell expressed and secreted.

Table 4. Total Aortic Wall Tissue Protein Biomarkers

	Control Total Wall, n=14	AAA Total Wall, n=14	P Value
IL-1b	401 (234–655)	857 (683–1153)	0.0112
IL1-Ra	7836 (5769–10 701)	9737 (6538–12 435)	0.5816
IL-4	311 (206–489)	395 (252–478)	0.9034
IL-5	1755 (878–2517)	2342 (1917–3168)	0.1325
IL-6	3182 (2349–7885)	9479 (5825–16 019)	0.0803
IL-7	1218 (899–1642)	1819 (1270–2553)	0.1573
IL-8/CXCL8	3883 (1963–6904)	6821 (3934–13 158)	0.1415
IL-9	3208 (2336–3328)	2863 (2304–3483)	0.9566
IL-10	2328 (1730–2615)	1467 (1279–1744)	0.0108
IL-12p70	5692 (3565–6891)	3213 (2589–4427)	0.0088
IL-13	792 (677–1298)	798 (656–1513)	0.9566
IL-15 [†]	949 (0–1376)	487 (0–1346)	0.3581
IL17A	2716 (2025–4803)	3601 (2127–4484)	0.8843
bFGF	39 910 (34 115–41 726)	25 941 (20 487–31 659)	0.0006*
VEGF	7335 (5126–9281)	4347 (3308–5161)	0.0101
Eotaxin	2414 (2110–3200)	2694 (2289–4416)	0.1901
G-CSF	2809 (493–6405)	3786 (3076–4303)	0.4347
GM-CSF	3514 (2618–4184)	4264 (3801–5095)	0.0896
IFN- γ	3530 (1689–5483)	4222 (2516–5452)	0.6459
IP-10/CXCL10	29 938 (21 888–38 431)	23 939 (19 724–29 939)	0.1585
MCP-1/CCL2	7104 (3605–8082)	9768 (6284–10 914)	0.1455
MIP-1a/CCL3	113 (56–197)	226 (188–358)	0.0076
MIP-1b/CCL4	2423 (1871–3483)	4989 (3846–6260)	0.0101
PDGF β	5806 (5172–10 346)	8360 (7056–9446)	0.5496
RANTES/CCL5	13 765 (11 557–15 785)	21 280 (18 859–24 370)	0.0010*
TNF- α [†]	1896 (1505–3161)	1593 (0–3411)	0.4146

Aortic wall tissue biomarkers were assessed in total wall biopsies, as well as microdissected (Table 5) intima and media and (Table 6) adventitia. AAA indicates abdominal aortic aneurysm; bFGF, basic fibroblast growth factor; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN- γ , interferon-gamma; IL, interleukin; IP-10, interferon-gamma-induced protein 10; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; PDGF β , platelet-derived growth factor beta; RANTES, regulated on activation, normal T-cell expressed and secreted; TNF- α , tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

*In the total aortic wall samples, only bFGF and RANTES were significantly different after correction for multiple testing.

[†]Some samples recorded IL-15 values below the detectable range of this assay and were assigned a rank order of 0. All inflammatory cytokines are measured in pg/100 mg tissue.

multiple testing significance between adventitial AAA and control specimens. Three cytokines (eotaxin, MIP-1b, and RANTES) were increased, whereas bFGF was decreased, in AAA adventitia.

Plasma Inflammatory Cytokine Profiles

In plasma samples, 15 of the 27 assayed cytokines were significantly (multiple testing threshold, $P < 0.0017$) differentially expressed in AAA patients compared to AAA-free controls (Table 7). In addition, significant differences were also observed in plasma hsCRP, HDL, and the atherogenic index in plasma (AIP; log triglycerides [Trig]/high-density

lipoprotein [HDL]). Plasma IL-15 was below the assay detectable range in the majority of samples (1179 of 1412 [83.5%]), and, although an analysis indicated a potential difference (90th percentile values of 17.1 vs 0.7 pg/mL in cases and controls, respectively; $P = 5.3 \times 10^{-7}$), this cytokine was nevertheless conservatively excluded from further analysis.

Eotaxin, RANTES, and MIP-1b levels were as significantly different between AAA cases and controls in both tissue and plasma samples, although RANTES showed an inverse direction of effect in the two sample types.

Several cytokines that showed suggestive ($P < 0.05$, $P > 0.002$) tissue differences (IL-6, MIP-1a, IL-12p70, and IL-

Table 5. Intima and Media Aortic Wall Tissue Protein

	Control Intima+Media, n=12	AAA Intima+Media, n=12	P Value
IL-1b	526 (234–914)	1685 (1092–1892)	0.0026
IL1-Ra	7848 (4819–12 157)	11 508 (10 568–27 464)	0.0940
IL-4	364 (326–543)	414 (316–535)	0.8525
IL-5	2080 (1537–3098)	2610 (2020–3272)	0.1891
IL-6	4149 (2064–6744)	11 022 (8610–19 028)	0.0092
IL-7	1939 (1319–2273)	1616 (1236–2233)	0.7440
IL-8/CXCL8	6382 (1572–8276)	15 220 (12 050–17 346)	0.0696
IL-9	3116 (2678–4191)	2957 (2561–3728)	0.6924
IL-10	2459 (1968–2682)	2087 (1660–3010)	0.7583
IL-12p70	5873 (4513–6944)	3500 (2728–5681)	0.0671
IL-13	1040 (940–1540)	1378 (980–1748)	0.2184
IL-15 [†]	1225 (0–1599)	702 (0–1159)	0.4386
IL-17A	3331 (2744–5148)	3689 (3166–4367)	0.9738
bFGF	39 529 (34 285–43 898)	20 667 (18 759–26 183)	0.0012*
VEGF	8068 (6942–9679)	5860 (4437–8056)	0.0646
Eotaxin	2893 (2297–3945)	3192 (3096–3975)	0.3258
G-CSF	2401 (493–8789)	3361 (2962–4816)	0.4118
GM-CSF	3858 (3367–4678)	3985 (3431–4305)	0.8603
IFN- γ	2951 (2264–5453)	4344 (2536–4868)	0.9476
IP-10/CXCL10	27 815 (22 667–50 709)	33 094 (26 638–55 247)	0.2623
MCP-1/CCL2	7749 (3236–10 146)	11 087 (8768–11 837)	0.0966
MIP-1a/CCL3	184 (138–493)	559 (370–972)	0.0284
MIP-1b/CCL4	2649 (1484–4500)	7426 (6819–8199)	0.0019
PDGF β	7775 (5199–10 871)	8414 (7558–12 910)	0.3248
RANTES/CCL5	12 592 (10 194–14 215)	25 331 (21 570–29 229)	4.9×10^{-5} *
TNF- α [†]	2076 (0–2410)	2635 (1396–2969)	0.3643

Microdissected intima and media aortic wall tissue biomarkers. AAA indicates abdominal aortic aneurysm; bFGF, basic fibroblast growth factor; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN- γ , interferon-gamma; IL, interleukin; IP-10, interferon-gamma-induced protein 10; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; PDGF β , platelet-derived growth factor beta; RANTES, regulated on activation, normal T-cell expressed and secreted; TNF- α , tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

*As observed in the total wall samples, only bFGF and RANTES were significantly different after correction for multiple testing.

[†]Some samples recorded IL-15 values below the detectable range of this assay and were assigned a rank order of 0. All inflammatory cytokines are measured in pg/100 mg tissue.

10) were also significantly ($P < 0.0017$) different in plasma (although IL-12p70 and IL-10 showed inverse directions of effect in tissue and plasma).

The plasma markers of strongest effect ($P < 5 \times 10^{-6}$), including hsCRP and HDL, were taken forward for logistic regression analysis. The optimal binary cut-off value for each marker was used to calculate odds ratios (ORs) for plasma marker associations with AAA (Table 8). All 13 markers had significant associations as unadjusted variables (Table 9); however, when adjusted for concurrent risk factors, the IL-6, IL-10, and MIP-1b associations became nonsignificant. When the model also included those cytokines that were significantly differentially expressed in both

tissue and plasma, only 5 markers (in order of significance; eotaxin, hsCRP, RANTES, HDL, and IL-8/chemokine [C-X-C motif] ligand [CXCL]-8) remained significantly associated with AAA.

A combined AAA-associated inflammation marker risk score was calculated by assigning 1 point for each of the following: RANTES $< 14\,500$ pg/mL, eotaxin > 125 pg/mL, and hsCRP > 2.5 mg/L, thereby scoring each individual between 0 and 3 (Table 10). The ROC curves for these three markers are shown in Figure 1. In all, 65.0% of AAA patients had a score greater than or equal to 2 compared to 25.9% of controls, with a resulting (adjusted) AAA OR of 4.8. Examination of the control cohort

Table 6. Adventitia Aortic Wall Tissue Protein Biomarkers

	Control Adventitia, n=12	AAA Adventitia, n=12	P Value
IL-1b	356 (196–492)	939 (634–1283)	0.0084
IL1-Ra	7643 (3841–10 426)	9441 (5647–10 677)	0.3559
IL-4	283 (106–443)	290 (122–446)	0.8432
IL-5	2119 (1186–2690)	3153 (2040–3450)	0.0848
IL-6	3465 (1635–5930)	9878 (5662–15 003)	0.0290
IL-7	1599 (685–2128)	1930 (1464–2179)	0.3088
IL-8/CXCL8	2213 (1731–3794)	5104 (2175–6823)	0.1879
IL-9	3208 (2313–3707)	3017 (2469–3626)	0.9439
IL-10	1307 (1073–1556)	1479 (1266–1997)	0.1213
IL-12p70	1847 (180–2670)	2534 (2080–3460)	0.0671
IL-13	1082 (864–1550)	1121 (970–1595)	0.5383
IL-15 [†]	470 (0–916)	533 (0–983)	0.8951
IL-17A	3334 (1970–4056)	2879 (1668–3528)	0.4237
bFGF	43 819 (39 739–48 884)	32 588 (26 826–35 877)	0.0014*
VEGF	4082 (2898–5001)	4493 (3010–4953)	0.7728
Eotaxin	1680 (1182–1869)	3074 (2777–4402)	0.0004*
G-CSF	493 (480–3362)	3903 (2406–4547)	0.0346
GM-CSF	3962 (3445–4358)	4066 (3823–4337)	0.7416
IFN- γ	1443 (1017–3482)	3222 (1935–4537)	0.1131
IP-10/CXCL10	14 629 (13 125–16 341)	16 686 (13 244–29 077)	0.2786
MCP-1/CCL2	4590 (2542–5626)	9095 (6280–11 674)	0.0115
MIP-1a/CCL3	65 (50–133)	248 (158–356)	0.0116
MIP-1b/CCL4	1815 (1511–1999)	5076 (4000–6878)	0.0014*
PDGF β	6514 (5429–7793)	6297 (5552–7633)	0.8951
RANTES/CCL5	11 149 (10 100–14 543)	22 021 (16 661–24 664)	0.0003*
TNF- α [†]	1617 (0–2486)	1821 (0–3169)	0.9461

Microdissected adventitia aortic wall tissue biomarkers. The greatest case/control differences were observed in this layer of the wall, in which eotaxin, MIP-1b, and RANTES were significantly elevated, and bFGF was significantly decreased, in AAA. AAA indicates abdominal aortic aneurysm; bFGF, basic fibroblast growth factor; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN- γ , interferon-gamma; IL, interleukin; IP-10, interferon-gamma-induced protein 10; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; PDGF β , platelet-derived growth factor beta; RANTES, regulated on activation, normal T-cell expressed and secreted; TNF- α , tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

*Markers below the multiple testing threshold ($P < 0.0019$).

[†]Some samples recorded IL-15 values below the detectable range of this assay and were assigned a rank order of 0. All inflammatory cytokines are measured in pg/100 mg tissue.

suggested that several conventional cardiovascular risk factors, including age, sex, smoking, hypertension, and past history of heart disease, were potential confounders of the plasma levels of the putative AAA-associated cytokines (Table 11). Of note, the AAA-associated inflammation marker risk score remained significant when these potentially confounding interactions were also included in the risk model.

In order to determine relatedness between markers, and how this might influence the adjusted logistic regression model, variable principal component analysis (vPCA) was performed. The vPCA indicated that eotaxin, RANTES, MIP-

1b, hsCRP, and HDL were more-remote members of the correlation network (Figure 2A). In contrast, markers such as IL-6, tumor necrosis factor alpha (TNF- α), IL-10, IL-8, IL-4, IL-5, MIP-1a, and IL-1RA were central members of the network, with a higher degree of corelatedness (Figure 2B). Correlations between the three inflammatory cytokines differentially expressed in both tissue and plasma suggested a modest degree of correlation between MIP-1b and both eotaxin and RANTES ($\rho = 0.35$, $P = 6.3 \times 10^{-27}$ and $\rho = 0.46$, $P = 2.5 \times 10^{-42}$, respectively). No significant correlation was observed between eotaxin and RANTES ($\rho = -0.073$). Although a significant correlation between hsCRP and

Table 7. Plasma Protein Biomarkers

	AAA Free Controls, n=970	AAA, n=442	P Value
hsCRP, mg/L	1.6 (0.8–3.1)	3.7 (1.9–8.7)	$2.1 \times 10^{-43*}$
HDL, mmol/L	1.34±0.45	1.19±0.39	$3.8 \times 10^{-10*}$
Triglycerides, mmol/L	1.4 (1.0–2.0)	1.5 (1.1–2.0)	2.4×10^{-3}
AIP (log Trig/HDL)	0.03 (–0.15 to 0.24)	0.13 (–0.07 to 0.33)	$1.5 \times 10^{-7*}$
AIP >1 (% positive)	51.9%	67.1%	$1.3 \times 10^{-6*}$
IL-1b, pg/mL	13.7 (9.4–19.1)	14.0 (9.8–19.8)	0.362
IL1-Ra, pg/mL	448.8 (299.8–791.5)	426.3 (310.2–639.5)	0.113
IL-2, pg/mL	25.2 (6.1–50.6)	36.9 (14.5–63.7)	$1.9 \times 10^{-6*}$
IL-4, pg/mL	14.3 (10.0–21.2)	17.0 (12.5–24.4)	$7.9 \times 10^{-8*}$
IL-5, pg/mL	28.4 (21.1–40.8)	35.6 (24.9–51.1)	$2.9 \times 10^{-11*}$
IL-6, pg/mL	48.9 (34.4–69.8)	53.7 (39.8–80.8)	$4.7 \times 10^{-5*}$
IL-7, pg/mL	43.1 (29.0–61.3)	44.2 (31.8–64.1)	0.131
IL-8/CXCL8, pg/mL	135.2 (91.9–200.4)	174.7 (125.8–253.4)	$4.1 \times 10^{-17*}$
IL-9, pg/mL	41.7 (25.7–66.6)	44.8 (31.0–71.3)	0.024
IL-10, pg/mL	67.3 (42.4–105.4)	80.4 (54.9–125.9)	$1.2 \times 10^{-6*}$
IL-12p70, pg/mL	108.7 (67.7–167.5)	122.7 (72.8–195.2)	$5.8 \times 10^{-4*}$
IL-13, pg/mL	24.0 (17.9–33.1)	26.5 (19.4–35.5)	2.4×10^{-3}
IL-17A, pg/mL	311.4 (224.1–455.5)	338.2 (221.2–496.3)	0.088
bFGF, pg/mL	146.0 (98.8–208.8)	154.0 (108.3–228.0)	0.093
VEGF, pg/mL	36.5 (13.7–77.4)	39.7 (14.7–78.6)	0.505
Eotaxin, pg/mL	93.8 (58.6–137.7)	140.1 (100.1–189.7)	$2.1 \times 10^{-30*†}$
G-CSF, pg/mL	358.0 (263.0–464.3)	349.2 (268.0–443.8)	0.239
GM-CSF, pg/mL	0.87 (0–23.2)	2.9 (0–21.6)	0.902
IFN- γ , pg/mL	421.5 (299.6–598.5)	417.2 (300.0–600.1)	0.856
IP-10/CXCL10, pg/mL	459.0 (354.4–589.3)	577.7 (430.9–776.4)	$3.9 \times 10^{-18*}$
MCP-1/CCL2, pg/mL	85.5 (54.7–132.6)	85.8 (61.7–124.5)	0.566
MIP-1a/CCL3, pg/mL	19.2 (14.1–26.7)	24.1 (17.3–34.6)	$2.1 \times 10^{-15*}$
MIP-1b/CCL4, pg/mL	73.1 (57.4–91.6)	79.7 (62.7–103.2)	$1.6 \times 10^{-5*†}$
PDGF β , pg/mL	1377 (937–2007)	1237 (760–1800)	$2.4 \times 10^{-4*}$
RANTES/CCL5, pg/mL	17 750 (12 279–38 468)	13 940 (10 799–19 020)	$9.5 \times 10^{-17*†}$
TNF- α , pg/mL	288.4 (206.0–422.9)	321.2 (229.9–458.2)	$2.8 \times 10^{-4*}$

AAA indicates abdominal aortic aneurysm; AIP, atherogenic index of plasma; bFGF, basic fibroblast growth factor; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; IFN- γ , interferon-gamma; IL, interleukin; IP-10, interferon-gamma-induced protein 10; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; PDGF β , platelet-derived growth factor beta; RANTES, regulated on activation, normal T-cell expressed and secreted; TNF- α , tumor necrosis factor alpha; Trig, triglycerides; VEGF, vascular endothelial growth factor.

*Markers below the multiple testing threshold ($P < 0.0017$).

†Eotaxin, MIP-1b, and RANTES were significantly different between cases and controls in both tissue and plasma samples.

RANTES has been previously described in 300 individuals,¹² this was not observed in this current investigation ($\rho = -0.02$, $P = 0.56$). Only modest correlations were observed between hsCRP and both eotaxin and MIP-1b ($\rho = 0.12$, $P = 1.6 \times 10^{-6}$ and $\rho = 0.13$, $P = 1.1 \times 10^{-6}$, respectively). HDL was significantly inversely correlated with hsCRP ($\rho = -0.20$,

$P = 2.2 \times 10^{-13}$) and, more modestly, with MIP-1b ($\rho = -0.09$, $P < 0.0017$).

Plasma marker correlations with aneurysm size were determined in the patient group. A relatively weak effect, but statistically significant, correlation was observed for hsCRP ($\rho = 0.278$, $P = 1.2 \times 10^{-8}$) and AAA size. Associations of

Table 8. Plasma Protein Biomarker ROC Curves

Marker	AUC (95% CI)	Binary Cutoff
hsCRP	0.73 (0.71–0.76)	2.5 mg/L
HDL	0.61 (0.57–0.64)	1.25 mmol/L
IL-2	0.58 (0.55–0.61)	30 pg/mL
IL-5	0.61 (0.58–0.64)	32 pg/mL
IL-4	0.59 (0.56–0.62)	14.9 pg/mL
IL-6	0.57 (0.54–0.60)	50 pg/mL
IL-8/CXCL8	0.64 (0.61–0.67)	155 pg/mL
IL-10	0.58 (0.55–0.61)	75 pg/mL
Eotaxin	0.69 (0.66–0.72)	125 pg/mL
IP-10/CXCL10	0.65 (0.62–0.68)	525 pg/mL
MIP-1a/CCL3	0.63 (0.60–0.66)	22 pg/mL
MIP-1b/CCL4	0.57 (0.54–0.60)	75 pg/mL
RANTES/CCL5	0.64 (0.61–0.67)	14 500 pg/mL
Smoking pack-years	0.72 (0.69–0.75)	

Area under the (non-parametric) receiver operating characteristic (ROC) curves (AUC). AUC and the optimal binary cutoff for each significant (unadjusted) plasma biomarker along with smoking pack-years for comparison. CCL indicates chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; IL, interleukin; IP-10, interferon-gamma-induced protein 10; MIP, macrophage inflammatory protein; RANTES, regulated on activation, normal T-cell expressed and secreted.

weaker effect and (suggestive) significance were observed for IL-8 ($\rho = -0.15$, $P = 0.0026$) and eotaxin/CCL11 ($\rho = -0.09$, $P = 0.0574$). RANTES was not associated with AAA size ($\rho = 0.02$, $P = 0.7316$).

mRNA Expression of AAA-Associated Inflammatory Cytokines in Aortic Wall Tissue

To determine whether the differentially expressed inflammatory proteins detected in aortic tissue were produced with the aortic wall, mRNA was extracted from 6 AAA and 11 control aortic wall tissue homogenates.

Transcripts for eotaxin 1/CCL11, RANTES/CCL5, and MIP-1b/CCL4 were detected in all AAA aortic wall tissue, confirming localized expression of these cytokines. Eotaxin 2/CCL24, eotaxin 3/CCL26, and both of the MIP-1b/CCL4 probes had very low or no levels of expression in control tissue. In contrast, eotaxin 1/CCL11 and RANTES/CCL5 had detectable signal in all control samples (Table 3). GAPDH had slightly stronger expression in controls than cases (Ct values 23.2 ± 0.6 and 24.5 ± 1.1 , respectively; $P = 0.02$). Both RANTES/CCL5 and MIP-1b/CCL4 had significantly greater mRNA transcript levels in AAA compared with control aortic wall tissue (Figure 3).

Discussion

Several inflammation-associated proteins have previously been implicated in the pathogenesis of AAA, including hsCRP, HDL-cholesterol, and IL-6.^{8–10,13–15} Perhaps the most comprehensive study examining the relationship between inflammatory cytokines and AAA to date was performed by Middleton et al., who examined 42 cytokines in 10 AAA and 9 control infrarenal aortic biopsies.¹⁶ Notably, they observed increased AAA expression of IL-1b, IL-6, granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein 1 (MCP-1), and RANTES, which was, at least partially, consistent with our current aortic wall (or sublayer) tissue observations.

In this current study, we significantly advanced the investigation of inflammation-associated biomarkers in AAA by including a comparison between tissue and plasma inflammatory cytokine profiles. A particular strength of the circulating marker analysis was its ability to model for confounding interactions between a large number of inflammatory cytokines and other demographic and clinical confounders. In so doing, we identified elevated plasma eotaxin and decreased plasma RANTES, as independent risk markers in AAA. In addition, hsCRP and HDL were also validated as circulating biomarkers for AAA, with hsCRP also having a modest correlation with aneurysm size.

When the plasma markers were analyzed by vPCA, eotaxin, RANTES, hsCRP, and HDL were more-remote members of the network, which may, in part, explain why these markers remained significant in the adjusted regression models. Inflammation has long been broadly implicated in atherosclerosis¹⁷ and with cardiovascular disease risk factors, such as smoking.¹⁸ In the search for disease-specific biomarkers, it is important that candidate circulating cytokines are not significantly confounded by concurrent disease or “conventional” risk factors. These issues were exemplified by IL-6, which, while replicating previous univariate associations with AAA, lost its significance in the multivariate logistic regression models that included concurrent heart disease, smoking, and other AAA-associated inflammatory cytokines. Whereas we were able to replicate previous reports suggesting a strong association between plasma CRP and AAA, its broad association with other inflammatory disorders, as well as cardiovascular risk factors, such as hypertension, and concurrent atherosclerotic disease,¹⁹ may limit this protein’s utility as an AAA-specific biomarker.

Probably the most interesting associations observed in this study were those with eotaxin and RANTES, which showed differential expression in both aortic tissue and plasma. RANTES, is a CC chemokine family T-cell mitogen that has

Table 9. Plasma Protein Biomarker, Logistic Regression

Biomarker/Binary Threshold	Odds Ratio (95% CI, P Value)		
	AAA	Adjusted AAA (Model 1)	Adjusted AAA (Model 2)
hsCRP >2.5 mg/L	4.0 (3.2–5.2), P<0.0001	2.2 (1.6–3.1), P<0.0001	2.1 (1.5–2.9), P<0.0001*
HDL >1.25 mmol/L	0.62 (0.49–0.78), P<0.0001	0.64 (0.47–0.85), P=0.006†	0.67 (0.47–0.95), P=0.026†
IL-2 >30 pg/mL	1.8 (1.4–2.2), P<0.0001	1.9 (1.4–2.6), P=0.0001	1.0 (0.68–1.6), P=0.89
IL-5 >32 pg/mL	2.1 (1.6–2.6), P<0.0001	2.3 (1.7–3.2), P<0.0001	1.7 (1.1–2.6), P=0.0148
IL-4 >14.9 pg/mL	1.7 (1.4–2.2), P<0.0001	1.7 (1.2–2.3), P<0.002	1.2 (0.81–1.8), P=0.35
IL-6 >50 pg/mL	1.4 (1.1–1.8), P=0.002	1.3 (0.9–1.8), P=0.110	0.80 (0.53–1.2) P=0.28
IL-8/CXCL8 >155 pg/mL	2.4 (1.9–3.0), P<0.0001	2.6 (1.8–3.5), P<0.0001	1.6 (1.1–2.5), P=0.0212
IL-10 >75 pg/mL	1.5 (1.2–1.9), P<0.001	1.4 (1.0–1.9), P=0.049	0.84 (0.56–1.3), P=0.39
Eotaxin >125 pg/mL	3.1 (2.4–3.9), P<0.0001	3.4 (2.5–4.8), P<0.0001	3.3 (2.3–4.8), P<0.0001*
IP-10/CXCL10 >525 pg/mL	2.7 (2.1–3.4), P<0.0001	1.8 (1.3–2.5), P=0.0002	1.3 (0.89–1.8), P=0.19
MIP-1a/CCL3 >22 pg/mL	2.2 (1.8–2.8), P<0.0001	2.2 (1.6–3.0), P<0.0001	1.3 (0.83–1.9), P=0.28
MIP-1b/CCL4 >75 pg/mL	1.5 (1.2–1.9) P=0.0003	1.4 (1.0–1.9) P=0.055	1.2 (0.80–1.7), P=0.44
RANTES/CCL5 >14 500 pg/mL	0.55 (0.44–0.69), P<0.0001	0.48 (0.34–0.66), P<0.0001	0.43 (0.30–0.63), P<0.0001*
Ischemic heart disease	2.1 (1.6–2.7), P<0.0001	2.2 (1.6–3.2), P<0.0001	3.0 (2.0–54.6), P<0.0001
Smoking, per 10 pack-years	1.3 (1.2–1.4), P<0.0001	1.4 (1.3–1.5), P<0.0001	1.3 (1.2–1.4), P<0.0001

Adjusted model 1 included age, sex, history of ischemic heart disease (IHD), hypertension, diabetes, atherogenic index in plasma (AIP), smoking (pack-years), and sample storage time. Adjusted model 2 included age, sex, history of IHD, smoking (pack-years), and sample storage time plus the other significant (adjusted) markers (RANTES, eotaxin, MIP-1b, HDL, and hsCRP). AAA indicates abdominal aortic aneurysm; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; IL, interleukin; IP-10, interferon-gamma-induced protein 10; MIP, macrophage inflammatory protein; RANTES, regulated on activation, normal T-cell expressed and secreted.

*Markers with statistical significance below the multiple testing threshold (P<0.0042).

†AIP excluded from the HDL odds ratios adjusted models. When AIP was included, adjusted models for HDL >1.25 mmol/L became nonsignificant (model 2: 0.90 (0.58–1.4); P=0.657).

been implicated in the release of proinflammatory cytokines.^{20,21} The release of RANTES from CD8 T cells has been associated with a wide range of tissue-inflammatory reactions, including allergic reactions, cancers, and gastritis.²² Notably, RANTES can be released by multiple cell types relevant to AAA, including not only T cells, but also smooth

muscle and platelets and has been shown to be associated with CAD risk.¹² In addition, pharmacological inhibition of platelet degranulation reduced the inflammatory cytokine burden (including RANTES) in intramural thrombi and was associated with reduced rupture rates in AAA patients.²³ Consistent with our current study, levels of RANTES mRNA²⁴

Table 10. Percentage Distribution and Logistic Regression for AAA-Associated Inflammation Risks

	AAA-Associated Inflammation Risk Score			
	0	1	2	3
Control (n=970)	25.5%	46.6%	21.5%	4.4%
AAA (n=442)	6.4%	28.6%	44.8%	20.2%
AAA OR	0.43 (0.27–0.68)‡	1	3.5 (2.7–4.7)†	7.8 (5.1–12.1)†
Adjusted OR*	0.36 (0.20–0.64)‡	1	3.5 (2.4–5.1)†	5.3 (3.1–9.1)†
Adjusted OR*	0.36 (0.20–0.64)‡	1	3.8 (2.7–5.4)†	
Adjusted OR*	1		4.8 (3.5–6.7)†	

The combined AAA inflammation marker risk score assigned 1 point for each of the following: hsCRP >2.5 mg/L, RANTES <14 500 pg/mL, and eotaxin >125 pg/mL (scoring each individual between 0 and 3). AAA-associated inflammation risk scores were significantly higher in the AAA group (χ^2 , P<0.0001). Using an AAA inflammation score of 1 (the most common score among controls) as the reference value, logistic regression suggested a protective association for those with a 0 score and a staged positive risk association with higher (2 or 3) scores. AAA indicates abdominal aortic aneurysm; hsCRP, high sensitivity C-reactive protein; OR, odds ratio; RANTES, regulated on activation, normal T-cell expressed and secreted.

*Adjusted for age, sex, history of ischemic heart disease, smoking (pack-years), and sample storage time.

†P<0.0001; ‡P<0.0005.

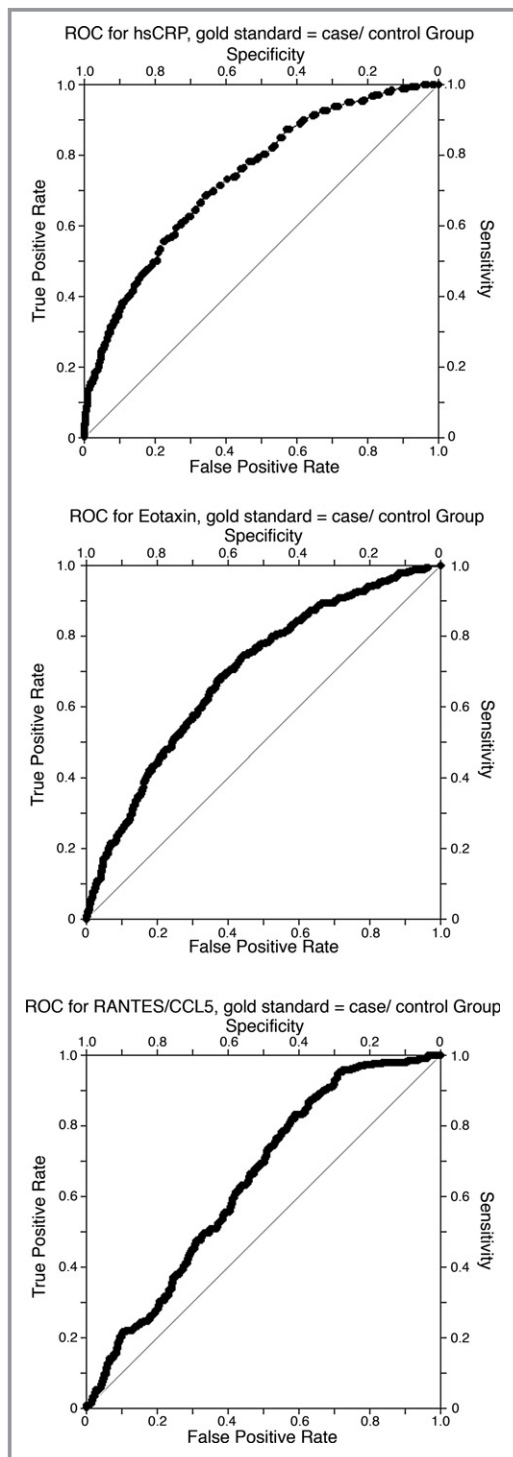


Figure 1. Receiver operating characteristic (ROC) curves for hsCRP, eotaxin, and RANTES. ROC curves were constructed to determine the optimal binary cut-off value of each differentially expressed cytokine. Cytokines were compared case/controls status (group) as the gold standard. CCL5 indicates chemokine (C-C motif) ligand 5; hsCRP, high sensitivity C-reactive protein; RANTES, regulated on activation, normal T-cell expressed and secreted.

and protein¹⁶ have been previously reported to be elevated in AAA aortic wall tissue. In contrast to tissue expression, we observed significantly lower plasma RANTES in AAA patients than controls. Although serum RANTES has been reported as elevated in CAD,¹² there are also contradictory reports²⁵ regarding this relationship. It is also worth noting that, because RANTES is released from platelets during blood clotting, serum measures should be viewed cautiously and direct plasma/serum comparisons avoided altogether. Nevertheless, in our plasma analysis, we observed a strong association between elevated RANTES and a history of ischemic heart disease (IHD) in our nonaneurysmal control group (Table 11). This association was strikingly absent within the AAA group, the reasons for which are unclear; however, we postulate that this may be attributed to the aforementioned relationship between RANTES and platelets. AAA has been previously linked to reduced circulating platelet counts²⁶ and increased platelet activation,²⁷ both of which may, in part, explain increased localized (AAA wall) and decreased circulating levels of platelet-associated factors such as RANTES.

Eotaxin-1 (or CCL11), another member of the CC chemokine family, is known to be a key mediator in asthma.²⁸ Eotaxin was included in our analysis solely on the basis that it formed part of a broad (relatively unbiased) cytokine multiplex panel, and we had no a priori suggestion of a link with AAA. Intriguingly, however, acute asthma has recently been linked to AAA susceptibility and rupture,²⁹ an observation that appears to indirectly validate our observed eotaxin-AAA association.

The antibody used in protein bioplex array could not distinguish between eotaxin -1, -2, and -3; however, our mRNA studies suggested that eotaxin 1/CCL11 was the dominantly expressed variant. Haley et al.³⁰ demonstrated that eotaxin expression could be induced in cultured aortic smooth muscle cells by treatment with TNF- α , and there have been conflicting reports regarding a possible association with atherosclerosis.^{31,32} More-recent studies have also suggested an association with cardiovascular risk factors and the soluble form of the receptor for advanced glycation endproducts.³³ B cells form a significant ($\approx 40\%$) proportion of the adventitial lymphocyte population in AAA,^{15,34} but are rare within the adventitia of aortic occlusive and normal aortae.³⁵ B cells, from patients with inflammatory bowel disease, have been shown to release eotaxin-1, in a disease-associated fashion, a process that has been suggested as a mediating factor in immune cell activation.³⁶ Whether eotaxin acts in a similar fashion in AAA is unclear; however, the elevated levels of eotaxin observed within AAA adventitial tissue certainly warrant further investigation.

In conclusion, contrary to some previous reports suggesting a distinct T helper 2-associated inflammatory (IL-4, -5,

Table 11. Confounding Interactions With Conventional AAA Risk Factors in Nonaneurysmal Controls

Sex	Female, n=229	Male, n=741	P Value
RANTES, pg/mL	19 516 (13 313–53 009)	17 391 (11 968–34 932)	0.013
Eotaxin, pg/mL	78 (48–136)	98 (65–139)	0.0014
MIP-1b, pg/mL	76 (60–93)	72 (57–92)	0.17
hsCRP, mg/L	1.8 (0.8–3.3)	1.5 (0.7–3.1)	0.13
HDL, mmol/L	1.45 (1.20–1.71)	1.23 (1.02–1.48)	<0.0001
Age	<69 years, n=491	>69 years, n=479	P Value
RANTES, pg/mL	21 130 (12 075–50 154)	15 933 (11 634–25 663)	<0.0001
Eotaxin, pg/mL	85 (49–126)	104 (69–148)	<0.0001
MIP-1b, pg/mL	72 (57–92)	74 (59–92)	0.81
hsCRP, mg/L	1.5 (0.7–3.1)	1.7 (0.8–3.2)	0.23
HDL, mmol/L	1.25 (1.03–1.54)	1.29 (1.08–1.55)	0.08
Clinical History of IHD	Without IHD, n=718	IHD, n=196	P Value
RANTES, pg/mL	14 608 (11 060–20 333)	60 755 (49 415–85 193)	<0.0001
Eotaxin, pg/mL	99 (63–144)	76 (44–116)	<0.0001
MIP-1b, pg/mL	68 (54–87)	87 (72–114)	<0.0001
hsCRP, mg/L	1.5 (0.7–2.8)	2.3 (1.2–4.0)	<0.0001
HDL, mmol/L	1.30 (1.08–1.59)	1.15 (0.95–1.39)	<0.0001
Clinical History of Hypertension	Without Hypertension, n=663	Hypertension, n=306	P Value
RANTES, pg/mL	16 956 (11 754–30 660)	20 754 (13 160–52 990)	0.0003
Eotaxin, pg/mL	92 (58–135)	96 (60–144)	0.34
MIP-1b, pg/mL	70 (55–89)	79 (62–101)	<0.0001
hsCRP, mg/L	1.5 (0.7–2.9)	1.9 (1.0–3.4)	<0.0001
Smoking History	Nonsmoker, n=457	>10 Pack-Years, n= 310	P Value
RANTES, pg/mL	16 601 (11 739–32 181)	20 750 (13 371–51 793)	0.0004
Eotaxin, pg/mL	90 (57–138)	102 (66–143)	0.046
MIP-1b, pg/mL	73 (57–93)	75 (60–95)	0.21
hsCRP, mg/L	1.3 (0.7–2.7)	2.1 (1.0–4.0)	<0.0001
HDL, mmol/L	1.29 (1.07–1.54)	1.23 (1.02–1.54)	0.15
AIP	Negative, n=392	Positive, n=458	P Value
RANTES, pg/mL	16 497 (11 669–24 434)	17 889 (12 345–44 462)	0.003
Eotaxin, pg/mL	93 (59–144)	92 (58–132)	0.20
MIP-1b, pg/mL	71 (56–87)	74 (58–92)	0.06
hsCRP, mg/L	1.3 (0.6–2.6)	1.9 (0.9–3.7)	<0.0001

The potential confounding interactions between the lead circulating inflammatory cytokines (RANTES, eotaxin, MIP1b, hsCRP, and HDL) was investigated in the control cohort (to exclude case-control interactions). AAA indicates abdominal aortic aneurysm; AIP, atherogenic index in plasma; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; IHD, ischemic heart disease; MIP, macrophage inflammatory protein; RANTES, regulated on activation, normal T-cell expressed and secreted.

and -10) profile in AAA,³⁷ this current study is more consistent with those studies that have suggested a more-generalized pattern of inflammation.¹⁴ Whereas the plasma inflammatory cytokine profile of AAA patients

contained numerous cytokines with differential expression compared to nonaneurysmal controls, 3 markers (eotaxin, hsCRP, and RANTES) were notable as potentially useful AAA biomarkers.

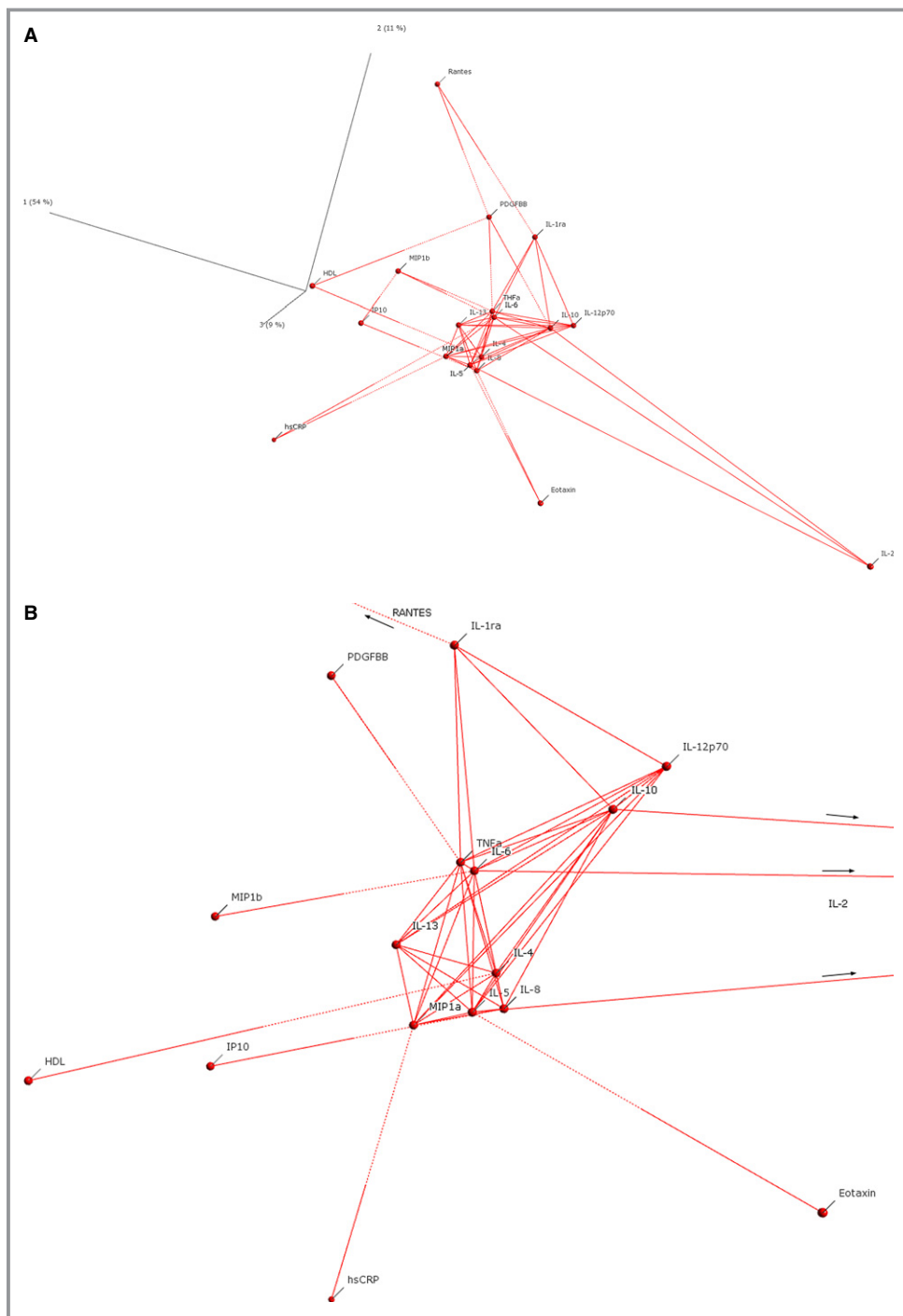


Figure 2. Variable principal component plots of plasma biomarkers. A, Log-transformed AAA-associated markers ($P < 0.05$), with each linked to its nearest two neighbors. Solid lines (Euclidean distance threshold $> 65\%$) indicate those markers with a stronger degree of relatedness. Notice that the AAA-associated markers, which showed independent association in multiple logistic regression (eotaxin, hsCRP, RANTES, and HDL), were more-peripheral members of the network with a lesser degree of relatedness (dotted connection lines) to the central hub of the network. B, Central hub of the network shown in (A), with each linked to its nearest neighbor or displaying a stronger degree of relatedness (Euclidean distance threshold $> 65\%$). Notice that markers such as IL-6, TNF- α , IL-10, IL-8, IL-4, IL-5, IL-12p70, MIP-1a, and IL-1RA were central members of the network. AAA indicates abdominal aortic aneurysm; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; IL, interleukin; MIP-1a, macrophage inflammatory protein 1 alpha; RANTES, regulated on activation, normal T-cell expressed and secreted; TNF- α , tumor necrosis factor alpha.

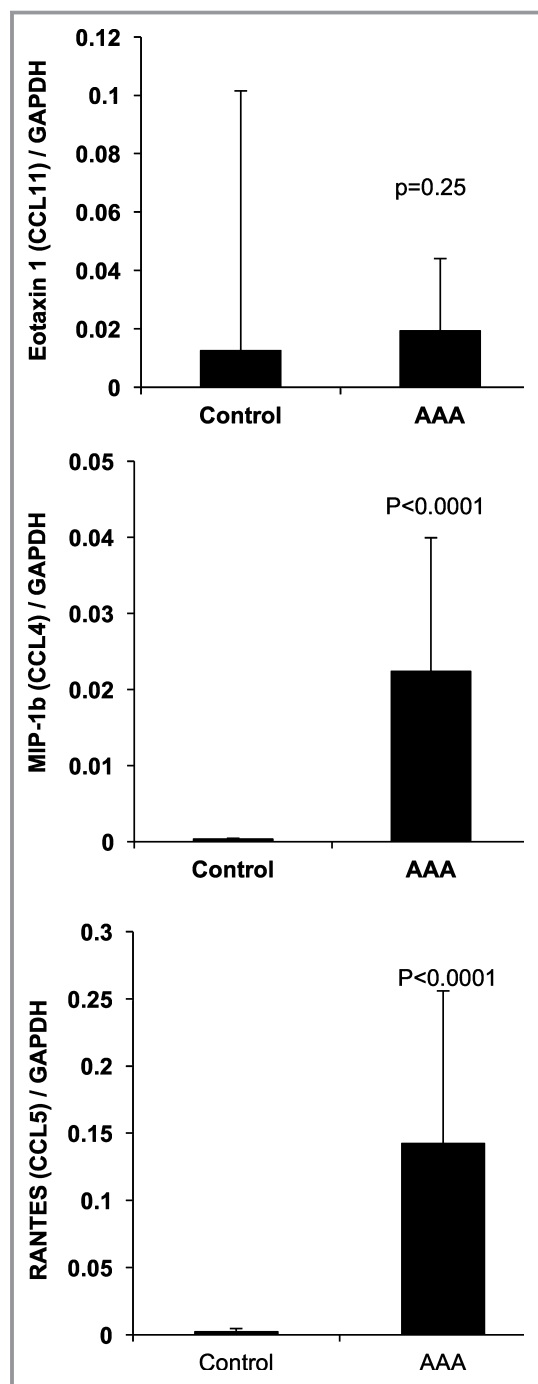


Figure 3. Quantitative PCR results comparing AAA total wall with nonaneurysmal control total wall biopsies for eotaxin 1/CCL11, MIP-1b/CCL4, and RANTES/CCL5. Transcripts for eotaxin 1/CCL11, RANTES/CCL5, and MIP-1b/CCL4 were detected in all AAA aortic wall tissue, confirming localized expression of these cytokines. Data represented as means and 1 SD. AAA indicates abdominal aortic aneurysm; CCL, chemokine (C-C motif) ligand; MIP-1b, macrophage inflammatory protein 1 beta; PCR, polymerase chain reaction; RANTES, regulated on activation, normal T-cell expressed and secreted.

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Disclosures

None.

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