Metformin Prescription Associated with Reduced Abdominal Aortic Aneurysm Growth Rate and Reduced Chemokine Expression in a Swedish Cohort

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Background: Recent reports suggest that the negative association between diabetes mellitus and abdominal aortic aneurysm (AAA) may be driven by metformin, the world's most common anti-diabetic drug rather than diabetes per se. We sought to investigate the association among AAA growth rate, chemokine profile, and metformin prescription in a contemporary Swedish cohort.
Methods: Patients under surveillance for small AAA were identified at 4 Swedish vascular centers with active AAA screening programs. Annual AAA growth rate, medical history, and prescribed medications were recorded for linear regression analysis. In a subset of patients with AAA and control subjects without AAA or diabetes, plasma samples were available and analyzed for 40 inflammatory chemokines.
Results: A total of 526 patients were included for AAA growth analysis: 428 without type 2 diabetes mellitus (T2DM), 65 with T2DM and metformin prescription, and 33 with T2DM but without metformin prescription. Patients were included from 2005 to 2017 with mean follow-up of 3.2 (1.7) years and median annual AAA growth rate 1.6 mm, range −4.8 to 15.4 mm. Mean (standard deviation) annual AAA growth rates were 2.3 (2.2) mm in non-T2DM patients versus 1.1 (1.1) mm in patients with T2DM with metformin prescription and 1.6 (1.4) mm among those with T2DM without metformin prescription. With non-T2DM patients as reference in an unadjusted and 2 adjusted models, metformin prescription was significantly associated with reduced AAA growth rate ($P < 0.001$, $P = 0.005$, and $P = 0.024$, respectively), but not T2DM without metformin prescription ($P = 0.137$, $P = 0.331$, and $P = 0.479$, respectively). Among 240 patients with AAA (152 without T2DM, 51 with T2DM and metformin, and 37 with T2DM without metformin) and 59 without AAA or T2DM, metformin prescription was associated with reduced expression of chemokines representing all classes of leukocytes.
Conclusions: Metformin prescription is associated with reduced AAA growth rate, possibly mediated by broad anti-inflammatory effects. A randomized controlled trial is needed to determine what role metformin may play in AAA disease, particularly in the absence of T2DM.

Conflicts of interest: The authors have no conflicts of interest to declare.

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INTRODUCTION

The natural course of an abdominal aortic aneurysm (AAA) is gradual expansion over several years and eventual rupture. With current AAA practice guidelines[1,2] monitoring of an AAA is recommended until a threshold diameter of 5.5 cm for men or 5.0 cm for women is reached, whereupon prophylactic surgical or endovascular repair is considered. Most AAAs are discovered at an early stage when they are small and risk for rupture is minimal, allowing for a window of opportunity to impede the disease progress. A crucial limitation of contemporary AAA treatment is the lack of medical treatment to reduce AAA growth and risk for rupture.

AAA shares many risk factors with atherosclerotic disease, with diabetes mellitus as a notable exception being associated with reduced AAA incidence[3,4] and growth rate[4–7] but higher operative risks[8] across multiple cohorts. Glycemia is unlikely to be the main driver of this protective effect as glucose levels seem to have a positive correlation with AAA prevalence in patients without diabetes.[9] Recent reports from the United States, Australia, and Taiwan have suggested that the common oral antidiabetic drug metformin may be the principal factor responsible for reduced AAA incidence,[10] growth rate,[11–13] and AAA-related events[14] among patients with diabetes.

Metformin is recommended as first line treatment of type 2 diabetes mellitus (T2DM) in global practice guidelines.[15] It inhibits hepatic glucose production and increases insulin sensitivity, but it is also suggested to have a pleiotropic protective effect of the vascular system over and above glycemic control.[16] Metformin has been proposed to reduce AAA growth by inhibiting key pathologic mechanisms implicated in AAA, including inflammation and extracellular matrix remodeling. Two different rodent models of AAA have found that metformin may reduce AAA growth in euglycemic animals,[11,17] but mechanistic human data are lacking.

The aim of this study was to investigate the potential association of metformin prescription with AAA growth rate and markers of systemic inflammation, measured by chemokine expression, in a contemporary Swedish cohort.

MATERIALS AND METHODS

Patient Selection

Patients under surveillance for AAA were identified at 4 Swedish centers with active AAA screening programs[18]: Uppsala, Malmö, Jönköping, and Linköping. No distinction was made in the patients enrolled in AAA surveillance through screening or otherwise. Inclusion criteria were initial abdominal aortic diameter $\geq$30 mm[1] with a minimum of 2 ultrasonography scans of the abdominal aorta and at least 6 months of follow-up. All patients were followed prospectively from the time of enrollment to AAA surveillance with the first patient included in 2005.

Subjects without AAA or T2DM were recruited from the screening program in Jönköping and Linköping to serve as control subjects for plasma chemokine levels. All participants provided written informed consent to participate in AAA-related research, with approval from local ethical committees at the 4 sites.

Clinical Data

Clinical parameters at baseline were retrieved from prospectively recorded data in respective cohorts and complemented by a review of individual patient records. Age, gender, initial AAA diameter, self-reported smoking habits, and comorbidity were all prospectively recorded.

Definitions of comorbidity were clinical manifestations of either condition such as ongoing antihypertensive treatment for hypertension, history of myocardial infarction or angina for coronary artery disease, history of stroke or transient ischemic attack for cerebrovascular disease, and clinical diagnosis for chronic kidney failure.

Data on prescribed antidiabetic and cardiovascular medication at baseline were retrieved retrospectively from individual patient records and divided into drug classes according to the Anatomical Therapeutic Chemical classification. Classes of drugs prescribed to 10 or more individuals were included in the analysis. Duration, dose, or compliance to prescribed drugs was not recorded.

Ultrasonography scan examinations of the abdominal aorta were performed by experienced operators using the standardized technique of leading-edge-to-leading-edge anteroposterior diameter perpendicular to blood flow. AAA growth rates were calculated by dividing the difference in diameter (mm) between the first and last ultrasonography scan by years of follow-up.

Chemokine Analysis

A subsample of the main cohort and additional subjects without AAA confirmed at AAA screening had ethylenediaminetetraacetic acid (EDTA) plasma samples available from the time of enrollment to surveillance. A panel of inflammatory biomarkers

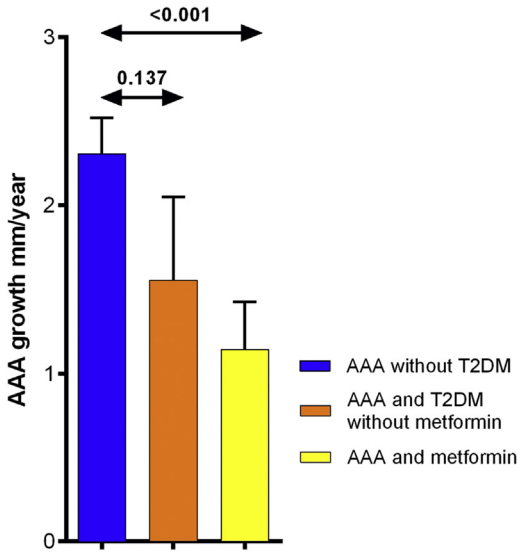

Fig. 1. Mean yearly AAA growth rate with 95% confidence interval for respective group. Significance for unadjusted growth rate.

was analyzed with a commercial kit, Bio-Plex Pro™ Human Chemokine Panel, 40-Plex (Bio-Rad Laboratories, Inc, Hercules, CA) according to the manufacturer's recommendations. The panel includes a variety of cytokines, chemokines (e.g. eotoxins, Gro, and macrophage inflammatory protein family), interleukins (ILs; e.g. IL-1b and IL-6), interferon gamma, and tumor necrosis factor α. An automatic magnetic washer (Magpix) was used during the implementation of the assay. The 96-well microtiter plate was measured with the Luminex 200 system (Luminex Corp, Austin, TX) like the Bio-Plex system. A standard curve was created for each cytokine with 7 points, whereas standard one served as the highest standard. By using the computer software Masterplex (MiraiBio Group of Hitachi Solutions America, Ltd), a 5-parameter logistic curve was generated for each analyte.

Statistical Analysis

The association of metformin prescription and AAA growth rate was assessed with unadjusted and adjusted linear regression models. First all patients with T2DM were compared with non-T2DM patients, then patients with T2DM were divided according to the metformin prescription with non-T2DM patients as reference and finally only patients with T2DM were analyzed. Risk factors suspected to affect AAA growth were included in a first regression model (gender, age, initial AAA diameter, active smoking, and hypertension) along with kidney failure as it was significantly more common in patients with T2DM without metformin prescription. In a second model, prescription of cardiovascular and other antidiabetic drugs was added. Antidiabetic drugs, including metformin, were omitted in the model when T2DM was included as an independent variable because of collinearity. A separate exploratory regression model was performed including initial AAA diameter, age, gender, active smoking, and all drug classes recorded. No formal power calculation was performed before study start. Cases with missing data were excluded listwise. As AAA growth rates were right skewed, growth rate data were log transformed in the regression analysis. Variance inflation factor did not reveal any major collinearity, and best-fit test showed linearity in the regression models. Statistical significance was determined at 2-tailed $P < 0.05$. Statistical analysis was performed using Statistical Product and Service Solutions v24 (IBM, Armonk, NY) and Prism v6 (Graph Pad, San Diego, CA).

RESULTS

Growth Analysis

A total of 526 patients with AAA were included in the growth analysis: 428 without T2DM, 65 with T2DM and metformin prescription, and 33 with T2DM and without metformin prescription. There were no patients with type 1 diabetes mellitus and no patients with metformin prescription without T2DM. Mean (standard deviation) follow-up was 3.2 (1.7) years and mean initial AAA diameter was 38.0 (6.1) mm. Mean annual AAA growth rates were 2.1 (2.1) mm for all 2.3 (2.2) mm in patients without T2DM versus 1.1 (1.1) mm in patients with metformin prescription and 1.6 (1.4) mm in patients with T2DM without prescribed metformin (Fig. 1). In the whole cohort median annual growth was 1.6 mm/year (range −4.8 to 15.4 mm) including 16 with negative growth.

Patients with T2DM had a higher prevalence of hypertension and coronary artery disease along with higher rates of prescription of drugs acting on the cardiovascular system. Kidney failure was most common among those with T2DM without metformin prescription. Baseline characteristics are reported in Table I.

In the first regression model, using AAA patients without T2DM as reference, the whole group of patients with T2DM taken together ($P = 0.006$) and the subgroup with metformin prescription ($P = 0.005$), but not those without metformin prescription ($P = 0.331$) had a significantly reduced

Table I. Clinical characteristics and prescribed medication in 526 patients with AAA at baseline according to T2DM and metformin status

Clinical characteristics	AAA without T2DM ($n = 428$)	AAA and T2DM ($n = 98$)	*P* value	AAA and T2DM without metformin ($n = 33$)	AAA and metformin ($n = 65$)	*P* value
Age (years) (SD)	69.1 (5.4)	69.0 (6.0)	0.194	70.1 (6.9)	68.5 (5.4)	0.196
Female, *n*	22 (5.1%)	8 (8.2%)	0.244	3 (9.1%)	5 (7.7%)	0.811
Former smoker, *n*	233 (57.1%)	62 (63.3%)	0.145	22 (68.8%)	40 (63.5%)	0.611
Active smoker, *n*	123 (30.1%)	28 (28.6%)	0.897	8 (25.0%)	20 (31.7%)	0.495
Initial AAA diameter (mm) (SD)	38.2 (6.1)	36.9 (5.9)	0.959	37.5 (6.0)	36.5 (5.9)	0.441
Hypertension, *n*	282 (66.7%)	85 (86.7%)	<0.001	30 (90.9%)	55 (87.3%)	0.598
Coronary artery disease, *n*	139 (33.0%)	42 (42.9%)	0.039	15 (45.5%)	27 (43.5%)	0.859
Cerebrovascular disease, *n*	56 (13.3%)	16 (16.3%)	0.334	5 (16.1%)	11 (17.5%)	0.872
Kidney failure, *n*	22 (5.2%)	7 (7.1%)	0.403	7 (21.9%)	0 (0%)	<0.001
Medication						
Insulin, *n*	0	32 (32.7%)		10 (30.3%)	22 (33.8%)	0.724
Sulfonylurea, *n*	0	24 (24.5%)		7 (21.2%)	17 (26.2%)	0.591
Dipeptidyl peptidase-4 inhibitor, *n*	0	13 (13.3%)		3 (9%)	10 (15.4%)	0.385
Diuretic, *n*	97 (22.9%)	42 (42.9%)	<0.001	14 (42.4%)	28 (43.1%)	0.951
Beta blocking agent, *n*	175 (41.4%)	61 (62.2%)	<0.001	22 (66.7%)	39 (60%)	0.520
Calcium channel blocker, *n*	134 (31.7%)	52 (53.1%)	<0.001	15 (45.5%)	37 (56.9%)	0.282
RAAS acting agent, *n*	211 (49.9%)	72 (73.5%)	<0.001	22 (66.7%)	50 (76.9%)	0.277
Lipid modifying agent, *n*	275 (65.0%)	86 (87.8%)	<0.001	29 (87.9%)	57 (87.7%)	0.979
Antithrombotic agent, *n*	241 (57.0%)	75 (76.5%)	<0.001	24 (72.7%)	51 (78.5%)	0.527

RAAS, renin-angiotensin-aldosterone system; SD, standard deviation.
Mean with SD or *n* and percentage of those at risk. Significance for unpaired *t*-test or Pearson's chi-squared test.

AAA growth rate. Including only patients with T2DM in the regression analysis, the association between metformin prescription and AAA growth rate did not reach statistical significance ($P = 0.254$). The second regression model showed similar results (Table II). In the exploratory regression model ($n = 502$), metformin ($P = 0.037$) but not insulin ($P = 0.821$), sulfonylurea ($P = 0.733$), or dipeptidyl peptidase 4 inhibitors ($P = 0.716$) were significantly associated with reduced AAA growth rate. Also there were no such associations found for any antidiabetic drug other than metformin in any of the other models used.

Active smoking and initial AAA diameter were positively associated with AAA growth rate across all models except for the adjusted models including only patients with T2DM (data not shown).

Initial AAA diameter was <35 mm in 36.3% of the patients, and 53.3% of those with no or negative AAA growth were found in this group. In a sensitivity analysis including only those with an initial AAA diameter ≥35 mm ($n = 335$), mean AAA growth rate was 2.6 (2.4) mm/year and the association between T2DM status and metformin prescription remained similar (Table III). Excluding outliers with negative AAA growth or AAA growth rate ≥5 mm/year yielded similar results ($n = 462$) (data not shown).

Chemokine Expression

A total of 240 patients with AAA had plasma samples available and were included in the chemokine analysis, including 152 without T2DM, 51 with T2DM and metformin and, 37 with T2DM without metformin. Non-AAA non-T2DM control subjects were all males of similar age, 68.6 (3.0) years, as those in the AAA cohort but had less comorbidity with 15.3% having ischemic heart disease, 39.0% hypertension, and 1.7% renal dysfunction. The percentage of former smokers was 37.3% and of current smokers was 8.5%.

With non-T2DM AAA patients as reference, 30 of 40 chemokines were lower expressed among those with metformin prescription (Table IV). Four of those factors were also lower among patients with T2DM without prescribed metformin. Only 1 factor, CXCL8, was higher expressed in patients with T2DM, irrespective of metformin prescription. Comparing only patients with T2DM, 21 factors were lower expressed in plasma among those prescribed metformin (Table IV). Compared with 59 subjects without AAA or T2DM at screening,

Table II. Association between AAA growth rate and T2DM, T2DM with metformin prescription, T2DM without metformin prescription, and metformin prescription in patients with T2DM

	T2DM versus non-T2DM patients[a]		T2DM with metformin versus non-T2DM patients		T2DM without metformin versus non-T2DM patients		Metformin prescription in patients with T2DM only	
Model	*P* value	*n*	*P* value	*n*	*P* value	*n*	*P* value	*n*
Unadjusted	<0.001	526	<0.001	493	0.137	461	0.127	98
Model 1	0.006	501	0.005	470	0.331	438	0.254	94
Model 2	0.020	500	0.024	469	0.479	437	0.340	94

Adjusted for (1) gender, age, initial AAA diameter, active smoking, kidney failure, and hypertension; (2) gender, age, initial AAA diameter, active smoking, kidney failure, hypertension, diuretics, beta blockers, calcium channel blockers, renin-angiotensin converting drugs, lipid lowering agents, antithrombotic drugs, insulin, sulfonylurea, and DPP4 inhibitors.
[a]Model 2 does not include metformin, insulin, sulfonylurea, or DPP4 inhibitors.

Table III. Sensitivity analysis including only those with initial AAA diameter ≥35 mm. Association between AAA growth rate and T2DM, T2DM with metformin prescription, T2DM without metformin prescription, and metformin prescription in patients with T2DM

	Sensitivity analysis AAA ≥35 mm							
	T2DM versus non-T2DM patients[a]		T2DM with metformin versus non-T2DM patients		T2DM without metformin versus non-T2DM patients		Metformin prescription in patients with T2DM only	
Model	*P* value	*n*	*P* value	*n*	*P* value	*n*	*P* value	*n*
Unadjusted	0.010	335	0.023	315	0.177	300	0.587	55
Model 1	0.020	319	0.029	300	0.305	284	0.965	54
Model 2	0.102	318	0.041	299	0.601	283	0.899	54

Adjusted for (1) gender, age, initial AAA diameter, active smoking, kidney failure, and hypertension; (2) gender, age, initial AAA diameter, active smoking, kidney failure, hypertension, diuretics, beta blockers, calcium channel blockers, renin-angiotensin converting drugs, lipid lowering agents, antithrombotic drugs, insulin, sulfonylurea, and DPP4 inhibitors.
[a]Model 2 does not include metformin, insulin, sulfonylurea, or DPP4 inhibitors.

Table IV. Plasma cytokine expression in patients with AAA according to the diabetes metformin prescription status

	AAA without T2DM ($n = 152$)	AAA and T2DM without metformin ($n = 37$)	AAA and metformin ($n = 51$)	AAA and T2DM with/without metformin	AAA and T2DM with/without metformin
Factor	Mean ± SD (ng/mL)	Mean ± SD (ng/mL)	Mean ± SD (ng/mL)	FC	*P* value
CCL1	91 ± 84.2*†	60 ± 76.1	24 ± 37.5	0.40	0.004
CCL2	25 ± 13.3*	22 ± 10.8	16 ± 8.1	0.73	0.012
CCL3	6 ± 2.9*†	5 ± 2.3	4 ± 2.1	0.80	0.045
CCL7	72 ± 52.3*	55 ± 46.5	36 ± 35.9	0.65	0.031
CCL8	64 ± 55.4*	51 ± 64.8	22 ± 38.0	0.43	0.009
CCL11	87 ± 85.0*	61 ± 81.4	28 ± 69.1	0.46	0.045
CCL13	142 ± 154.1*	112 ± 160.2	45 ± 69.6	0.40	0.009
CCL15	1,440 ± 441.7*	1,345 ± 649.6	997 ± 535.8	0.74	0.007
CCL17	73 ± 96.2	84 ± 70.3	90 ± 134.3	1.07	0.818
CCL19	163 ± 140.5	159 ± 108.9	136 ± 99.4	0.86	0.289
CCL20	11 ± 12.0	12 ± 14.2	13 ± 22.0	1.08	0.925
CCL21	2,831 ± 2,017.9**	2,350 ± 1,566.0	1,966 ± 1,394.8	0.84	0.245
CCL22	506 ± 288.0	489 ± 219.6	426 ± 200.5	0.87	0.163
CCL23	246 ± 114.5	256 ± 113.1	227 ± 110.0	0.89	0.230
CCL24	327 ± 324.9*	238 ± 246.0	135 ± 217.1	0.57	0.041
CCL25	439 ± 325.8*	357 ± 322.5	208 ± 234.0	0.58	0.014
CCL26	56 ± 62.8*	38 ± 50.1	16 ± 29.8	0.42	0.013
CCL27	605 ± 333.9**	597 ± 396.1	444 ± 361.8	0.74	0.063
CXCL1	110 ± 52.5*	92 ± 12.3	63 ± 41.0	0.68	0.012
CXCL2	805 ± 1,334.1***	686 ± 1,232.4	178 ± 372.9	0.26	0.007
CXCL5	520 ± 395.2**	479 ± 319.9	367 ± 241.9	0.77	0.065
CXCL6	31 ± 23.0**	27 ± 19.1	21 ± 11.6	0.78	0.062
CXCL8	6 ± 2.1***‡	8 ± 3.6	7 ± 3.4	0.88	0.723
CXCL9	766 ± 1,890.3	534 ± 935.0	310 ± 1,140.7	0.58	0.329
CXCL10	1,510 ± 2,193.7*	1,057 ± 1,815.5	226 ± 623.0	0.21	0.003
CXCL11	155 ± 270.0*	82 ± 144.1	18 ± 45.7	0.22	0.004
CXCL12	1,845 ± 1,513.2*†	1,246 ± 1,488.9	498 ± 667.9	0.40	0.002
CXCL13	52 ± 79.5**	32 ± 34.6	16 ± 24.3	0.50	0.017
CXCL16	274 ± 118.6***	265 ± 127.0	219 ± 111.6	0.83	0.079
CX3CL1	174 ± 136.1**	155 ± 126.8	108 ± 116.3	0.70	0.076
GM-CSF	27 ± 46.6***	15 ± 11.2	13 ± 12.3	0.87	0.504
IFN-γ	50 ± 39.8**	40 ± 26.8	32 ± 20.8	0.80	0.085
IL-1β	3 ± 0.9	3 ± 1.0	3 ± 1.0	1.00	0.405
IL-2	13 ± 6.9	12 ± 5.6	11 ± 6.2	0.92	0.387
IL-4	15 ± 8.6	13 ± 5.9	13 ± 7.7	1.00	0.961
IL-6	16 ± 12.9*†	12 ± 9.5	8 ± 6.7	0.75	0.029
IL-10	20 ± 13.0***	19 ± 13.0	16 ± 14.8	0.84	0.253
IL-16	315 ± 140.9*	285 ± 179.8	181 ± 125.8	0.64	0.002
MIF	9,623 ± 11,998.7**	8,662 ± 10,652.5	4,829 ± 5,328.0	0.56	0.029
TNF-α	16 ± 7.0*	13 ± 6.4	11 ± 5.6	0.85	0.025

ANOVA, analysis for variance; FC, fold change; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; MIF, macrophage migration inhibitory factor; SD, standard deviation; TNF-α, tumor necrosis factor α.
Mean cytokine values with SD and FC between the groups with *P* value from Students' *t*-test.
Significant difference between AAA (no T2DM2) and metformin treated T2DM with AAA using 1-way ANOVA with Fisher's least significant difference post hoc analysis. *$P < 0.001$; **$P < 0.01$; ***$P < 0.05$.
Significant difference between AAA (no T2DM) and non–metformin-treated AAA with T2DM using 1-way ANOVA with LSD post hoc analysis. †$P < 0.05$; ‡$P < 0.01$.

chemokine expression tended to be lower among those prescribed metformin and higher among those without (Fig. 2). In the whole cohort, chemokine expression correlated poorly with AAA growth rate with only CCL21 having a significant positive correlation ($r = 0.15$, $P = 0.033$ for Pearson's correlation coefficient). Among those without T2DM a negative association was found with AAA growth

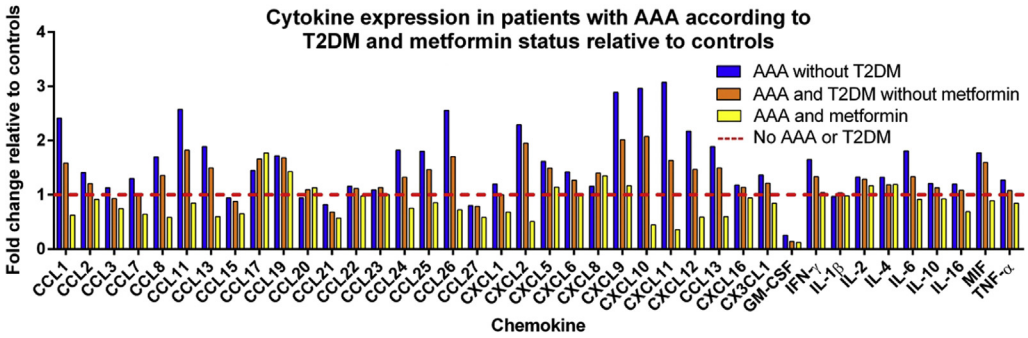

Fig. 2. Chemokines fold change relative to control subjects without AAA or T2DM. GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; MIF, macrophage migration inhibitory factor; TNF-α, tumor necrosis factor α. Significances are reported in Table IV.

rate and CXCL2 ($r = -0.19$, $P = 0.022$) and CCL23 ($r = -0.16$, $P = 0.046$). However, among those with metformin prescription a positive correlation was found between 8 of 40 chemokines and AAA growth rate; IL-1 ($r = 0.32$, $P = 0.024$), IL-6 ($r = 0.32$, $P = 0.024$), IL-8 ($r = 0.51$, $P < 0.001$), IL-16 ($r = 0.30$, $P = 0.036$), CCL3 ($r = 0.34$, $P = 0.016$), CCL15 ($r = 0.42$, $P = 0.02$), CCL20 ($r = 0.56$, $P < 0.001$), and CCL21 ($r = 0.55$, $P < 0.001$). No positive correlation was seen between AAA growth rate and chemokine expression among those with T2DM without metformin prescription. Common risk factors such as active smoking and age correlated poorly with chemokine expression.

DISCUSSION

Growth Data

Across all models, only patients with T2DM prescribed metformin had a significantly reduced AAA growth rate compared with non-T2DM patients. The models included possible confounders such as renal failure and hypertension, which may reflect more advanced T2DM and/or cardiovascular disease, and influence the choice of antidiabetic and cardiovascular medication. There were no indications in any model of an association with any other antidiabetic drug, beside metformin, and AAA growth rate.

Patients with small AAA and T2DM prescribed metformin had a 27% slower AAA growth rate compared with those with T2DM not prescribed metformin and 51% less than those without T2DM. Although differences in growth rates were not significant when only comparing patients with T2DM, the absolute numbers are in agreement with a recent US nationwide analysis of 13,834 Veterans Affairs patients with AAA and diabetes. In that study the reported unadjusted mean AAA growth rate was 1.2 mm/year for AAA patients with T2DM prescribed metformin compared with 1.5 mm/year for those with T2DM not prescribed metformin, corresponding to a 20% reduction in AAA growth rate by metformin prescription.[13]

The present study has a high proportion of metformin prescription of 66.3% among those with T2DM and AAA compared with 39.7% in the US veteran population,[12] 56.7% in 3 cohorts from Australia and New Zealand,[12] and 55.0% in a Taiwanese national diabetes database.[10] The rate of T2DM of 18.6% is similar in this study compared with 18.0% in the cohorts from Australia and New Zealand,[12] but high compared with most historical reports investigating the AAA growth rate reporting diabetes mellitus prevalence of 2.7−20.7%.[5] These differences may reflect both different prescription patterns for antidiabetic medication and different T2DM epidemiology across regions and over time. Nevertheless, T2DM has consistently been associated with reduced AAA incidence and growth rate and when metformin has been evaluated separately, a similar pattern emerges.

These results confirm previous reports of a considerably reduced AAA growth rate among patients prescribed metformin compared with non-T2DM patients. The contrasting lack of a statistically significant association of metformin prescription and AAA growth when analyzing only patients with T2DM is likely a type II statistical error because of insufficient power, only 33 patients with T2DM not prescribed metformin were included for growth analysis.

Chemokine Expression

We found reduced expression of a large number of proinflammatory cytokines in plasma of patients with AAA and metformin prescription. The affected

cytokines are involved in all classes of leukocytes, indicating broad effects on inflammation. This includes interferon gamma, a central immunologic regulator, which may affect vascular remodeling and AAA formation[19,20] and have a role in early AAA development and progression as increased levels have been found in the transition zone between normal aorta and aneurysm.[21] Also, a dramatic reduction was seen in one of its effector molecules, CXCL10, proposed to have direct effects on the vascular wall with relevance for both aneurysmal and atherosclerotic disease.[22,23] IL-6, a proinflammatory acute phase chemokine, rather consistently found increased in cohort studies of patients with AAA[20,24,25] were also lower among those prescribed metformin as were levels of tumor necrosis factor α, another central proinflammatory cytokine associated with AAA.[25] Our results contrast, however, to a recent study reporting no association between a range of proinflammatory and regulatory cytokines and diabetes with or without metformin treatment in a cohort of patients with either confirmed AAA or screened negative for AAA.[26] Although of similar size to our study, the analysis differs to ours as no distinction was made for AAA status, limiting comparability. The roles of individual chemokines in respect to AAA pathophysiology are not well understood, particularly in relation to other concomitant diseases driven by inflammation, such as atherosclerosis, which is prevalent in patients with AAA. Curiously, chemokine expression was more closely linked to the AAA growth rate among those prescribed metformin, with no significant correlation or even negative correlations seen among those without T2DM or T2DM without metformin prescription. However, chemokines expressed lower among those with metformin prescription did not fully overlap those associated with increased growth rate in the same cohort. The correlation between cytokine expression and AAA growth rate among those with metformin prescription along with the large number of cytokines lower expressed suggests a broad effect of metformin to reduce inflammation and extracellular matrix remodeling[11,17] and a possible mechanism whereby metformin may reduce the AAA growth rate.

Limitations

The study has several limitations. By means of design, the study can only show correlation, not causation. Synergistic or additive effects of T2DM and metformin and/or lingering effects from unaccounted previous metformin treatment are possible confounders as well as clinical characteristics not captured in the model. It is also possible that some patients who were prescribed metformin at baseline stopped treatment and vice versa. Duration, dose compliance, or stopped treatment was not recorded for metformin or any other drug. It is not possible to completely delineate what role diabetes may play or establish causality from this or previous reports of the association between metformin and AAA as metformin is almost exclusively used in patients with T2DM. The role of biomarkers in AAA is not clear and by means of study design, the correlations seen with metformin and AAA growth rate might not be causative or reflective of disease progression.

CONCLUSIONS

These results confirm that metformin prescription is associated with a significant reduced AAA growth rate and suggest that this may in part be driven by broad anti-inflammatory effects. A randomized controlled trial is needed to assess the effect of metformin on the AAA growth rate in the absence of T2DM.

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