Remodeling of abdominal aortic aneurysm sac following endovascular aortic repair: association with clinical, surgical, and genetic factors☆,☆☆

Mario D'Oria[1], Filippo Giorgio Di Girolamo[2], Cristiano Calvagna[1], Filippo Gorgatti[1], Nicola Altamura[2], Sandro Lepidi[1], Gianni Biolo[2], Nicola Fiotti[2,*]

[1] *Division of Vascular and Endovascular Surgery, Cardiovascular Department, University Hospital of Trieste, Strada di Fiume, 447 34149 Cattinara, Trieste, Italy*
[2] *Unit of Clinica Medica, Department of Medical, Surgical and Health Sciences of the University of Trieste, Strada di Fiume, 447 34149 Cattinara, Trieste, Italy*

ARTICLE INFO

Article history:
Accepted 22 December 2021

Keywords:
Abdominal aortic aneurysm
Aortic shrinking
Single nucleotide polymorphism
Matrix metalloproteases
Logistic regression analysis

ABSTRACT

After successful endovascular aortic repair (EVAR), abdominal aortic aneurysms (AAA) sac will undergo negative remodeling (i.e., shrinkage) as a measure of successful exclusion. Determinants of shrinkage after EVAR are not fully known.

In 84 post-EVAR patients, time course of AAA diameter after repair and occurrence of endoleaks (ELs) have been correlated with clinical history, medications, anthropometric data, vascular anatomy, and matrix metalloprotease (MMP) genetic variants (namely *MMP-1* rs1799750, *MMP-3* rs35068180, *MMP-9* rs2234681, rs917576, rs917577, *MMP-12* rs652438, and *TIMP1 rs4898*).

During follow-up, 41 ELs were detected in 37 patients (44%, 10.4 events/100 pt./y), accounting for AAA dilation or reduced shrinkage ($P < .001$). High-flow ELs (type 1 and/or 3) occurrence was associated with warfarin use, *MMP9* rs17577 polymorphism, and unfavorable anatomy, while low-flow type 2 ELs occurred more often in *TIMP1* rs4898 non-T carriers. In EL-free patients, AAA diameter decreased for the first three years, (-4, -3 and - 2 mm/year respectively) and remained stable thereafter. Shrinkage between two measurements ($n = 120$) was associated with smaller AAA diameter at the baseline, peripheral arterial disease (PAD), patients' older age at intervention, and G-/G- genotype in MMP1 rs1799750 (binary logistic regression, $P = .0001$).

Aneurysmal sac shrinking occurs for few years after EVAR, only in patients without EL, and is related to older age, PAD, smaller aneurysm size and putative lower MMP1 expression while EL occurrence prevents such a remodeling and is mainly related to local-acting factors like unfavorable anatomy, anticoagulation, and MMP9 and TIMP1 genetic polymorphisms.

1. Introduction

Abdominal aortic aneurysm (AAA) is a degenerative vascular disease characterized by the inflammation and local progressive thinning of the vessel wall; in the absence of any intervention, its natural history is a progressive expansion of the aortic tract involved and an eventual rupture. Nowadays, around 85% of patients with AAA diagnosis and suitable anatomy undergo endovascular aortic repair (EVAR) [1] in accordance with the most recent clinical practice guidelines from the European Society for Vascular Surgery [2]. Following successful EVAR, the expected fate of the AAA sac is a progressive negative remodeling (i.e., shrinkage) as a result of successful exclusion of the aneurysm from the systemic circulation [3]. Pathophysiological elements modulating such a reduction in AAA diameter after EVAR have not been fully investigated, nevertheless, they could mirror those accounting for AAA expansion before EVAR that have been overwhelmed by pulse pressure. Identification of these mechanisms could corroborate or question our knowledge on pathophysiology of AAA expansion, and offer guidance on clinical management and follow-up protocols of patients following EVAR.

Among the complex array of variables involved in AAA remodeling, functional genetic polymorphisms of some matrix metalloproteases (MMPs) or of their natural inhibitors named Tissue Inhibitors of MMPs (TIMPs), might modulate some features of AAA pattern. MMPs are a class of around 26 neutral endoproteases able

☆ The study has been funded by Medtronic Italia, which was not involved in the study design, data collection, data interpretation, article preparation, and the decision to submit the manuscript.
☆☆ All authors report no conflict of interest for the present manuscript.
* Corresponding author: Nicola Fiotti, Unit of Clinica Medica. Ospedale di Cattinara, Strada di Fiume, 447 34149 Cattinara Trieste, Italy. Phone +39 040 3993226.
E-mail address: fiotti@units.it (N. Fiotti).

to degrade all the components of extracellular matrix. MMPs and TIMPs variants have been associated with AAA location [4], thickness of artery wall [5], and speed of growth [6], while limited information is available on AAA negative remodeling after an endograft is implanted [7]. The main complications of EVAR are endoleaks (ELs), i.e., failure of the prostheses to exclude the aneurysmal sac from aortic bloodstream. They are classified as high-flow when the prosthesis does not coalesce on the artery wall (Type I), or have tears allowing communication between aneurysmal sac and bloodstream (Type III) or low flow when arteries stemming from the aneurysmal sac receive blood in a retrograde direction and contribute to sac filling (Type II). ELs can induce continuous pressurization of the sac after stent-graft implantation and also their occurrence could also be modulated by the aforementioned genetic mechanisms.

Aim of the present study is to identify the surgical variants, clinical conditions, and functional genetic polymorphisms of *MMP-1, -3, -9*, -12, and *TIMP-1*, already identified in AAA, associated with a different pattern of remodeling (negative or positive remodeling) of the AAA sac following EVAR as well as those accompanying the occurrence of ELs.

2. Materials and methods

2.1. Study population

All consecutive patients with documented infrarenal asymptomatic AAA and indication for elective repair according to current guidelines (i.e., maximal transverse diameter at Computed Tomography Angiography >5.5.cm in men and >5 cm in women) admitted to the Vascular and Endovascular Surgery Unit of the University Hospital of Trieste for standard EVAR, or follow-up examination from July 15th, 2010 until June 15th, 2013 were considered eligible. Follow-up was carried out until December 31st, 2019.

Each patient received a preoperative Computed Tomography Angiography scan to evaluate for anatomical feasibility for the repair and unfavorable anatomy limiting the sealing of the stent was defined according to previously published evidence [8].

All patients eligible for inclusion were informed on the purpose of the study and, upon agreement to be involved in minimal risk studies, were asked to sign a written informed consent and provide a blood vial for genotyping before the operation or during follow-up visits. Medical personnel reviewed the records (baseline characteristics, procedural details, re-interventions, imaging, and complications) unaware of the genetic status of the patients.

All procedures did not interfere with the good clinical practice in force in the Hospital and were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration and its later amendments or comparable ethical standards.

2.2. Follow-up protocols

Follow-up consisted of clinical examination and imaging evaluation at 1, 6, and 12 months and annually thereafter for the first three years. All patients received Computed Tomography Angiography within 30 days of index EVAR, while subsequent imaging evaluation included Computed Tomography Angiography and/or Computed Tomography without contrast enhancement, with duplex ultrasound of the aorto-iliac territory (with or without contrast enhancement) according to the risk factors for EVAR. After three years of continuous follow-up and in the absence of any suspicious findings or obvious complications, every patient was switched to a less intensive follow-up schedule with plain abdomen x-Ray and ultrasound every 24 months.

Diameter measurements were carried out at the widest portion of the sac, either in cross-sectional imaging on two orthogonal planes (short axis and long axis) with electronic calipers from outer wall to outer wall for ultrasound or using centerline measurements and post-processing software (3Mensio Medical Pie, The Netherlands) for Computed Tomography Angiography. Imaging examinations were assessed independently by two vascular surgeons (M.D. and F.G.) with disagreement to be discussed and resolved by consensus in the presence of a third physician (C.C.). Analysis of determinants of EL-free shrinkage was restricted to patients with no EL at any time. Including EL-free observations from patients who previously or subsequently developed EL was not deemed sufficiently accurate to rule out small ELs due to different and not always optimal sensitivity of different techniques for ELs diagnosis [9].

Following index EVAR, all patients were discharged on lifelong oral single antiplatelet therapy (100 mg acetylsalicylic acid or 75 mg clopidogrel once daily). Anticoagulant drugs were resumed after procedure, when appropriate.

According to standard practice, in the presence of EL1 and/or EL3, secondary endovascular interventions were undertaken if feasible to exclude the EL. In patients who received a repeated endovascular procedure, follow-up was halted at that time for the purpose of this study.

2.3. Polymorphisms analysis

DNA was extracted from a venous blood sample with a suitable extraction kit on Maxwell 16, both products were from Promega Italia and stored at -20°C until further analysis. For *MMP-1* rs1799750, *MMP-3* rs3025058, and *MMP-9* rs12234681variants, genotyping has been carried out according to the methods already published [10]. For assessment of *MMP9* rs17576, *MMP9* rs17577, *MMP-12* rs2276109 and *TIMP-1* rs4898 commercial Taqman technology kits (product number C__11655953_10, C__11655948_1_, C__15880589_10, and C__11175659_10 respectively, Lifetechnologies) have been used in Real-Time Biorad according to the manufacturer instructions.

Patients have been genotyped by expert personnel of the University of Trieste (N.F. and Paola Pitacco) blind from any clinical data. For X-linked *TIMP-1 rs4898*, gender information was required to confirm genotyping, which has been expressed as carrier or not of the T(A) allele, consistent with a previous paper on the same variant [11].

Genetic material has been treated according to the Italian guidelines for treatment of genetic material (General Authorization No. 8/2013 for the Processing of Genetic Data, from the "Garante per la protezione dei dati personali", accessible at http://www.garanteprivacy.it/web/guest/home/docweb/-/docweb-display/docweb/2818993).

2.4. Statistical analysis

Variables were reported as absolute number and prevalence or median and interquartile (IQR) values for categorical or continuous variables, respectively. Comparisons have been carried out with chi square test (and Hazard Ratio, H.R., and 95% Confidence interval) or Mann-Whitney (or Kruskal Wallis test), respectively.

Growth Rate of the AAA (GR), i.e., the actual minus initial aortic diameter divided by the time elapsed between the observations (in mm/month) has been determined and used for comparisons. Such a variable could be positive when the final diameter was greater than the initial, neutral (i.e., 0) when AAA diameter did not change, or negative in the event of AAA shrinkage. Determinants of GR were assessed separately in patients with or without EL. Since some of these determinants were time-related (namely, the age of

the patient at the time of observation, time of EL occurrence, and AAA diameter during follow-up) an assessment of each time periods elapsing between two AAA measurements was preferred to a global "per-patient" GR analysis.

The clinical, surgical, or genetic factors that could identify patients with occurrence of ELs have been investigated considering separately each type of EL and grouping type 1 and 3 as "high-flow Els." When more than one EL occurred in the same patient, these events were considered as independent and underwent separate analysis.

The number of events per 100 patients per year were estimated for the cohort. Independent variables accounting for occurrence of EL were: gender, age, popliteal and/or thoracic aneurysm, smoking habit (never, former, current smoker of more than 3 cigarettes/day), hypercholesterolemia (total cholesterol >200 mg/dL or HDL-cholesterol <40 in women or <35 mg/dL in men), hypertension (systolic and/or diastolic pressure >140/85 mm/Hg on more measurements), diabetes (fasting blood sugar >116 mg/dl), weight, height, body mass index (BMI), occurrence of coronary heart disease, peripheral arterial disease (PAD), use of antiplatelet drugs, anticoagulants, antihypertensive or lipid lowering drugs, unfavorable anatomy, *MMP-1* rs1799750 (1=G−/G−, 2=G−/G+, 3 = G+/G+), *-3* rs35068180 (1 = 5A/5A, 2 = 5A/6A, 3 = 6A/6A), and *-9* rs112290776 (1 =≤21/≤21, 2 =≤21/≥22, 3 =≥22/≥22 CA repeats within a variable number tandem repeat sequence around -90), *MMP9* rs17576 (1= AA, 2= AG, 3= GG), *MMP9* rs17577 (1= GG, 2= AG, 3= AA), *MMP-12* rs652438 (1= AA, 2= AG), *TIMP1* rs4898 T carrier) single nucleotide polymorphisms (SNP) and their interactions. The influence of genotype has been evaluated assuming both an additive and dominant effect of MMPs and TIMP-1. For MMP12 and TIMP1, population was divided according to the condition of being a carrier of A or T allele, respectively.

To observe the time-dependent changes of the AAA size, expressed as ratio between post-surgery and actual AAA diameter, a test for repeated measurements has been used, considering the occurrence of any EL as a "between subjects'" variable.

Event free survival analyses were carried out (Kaplan-Meier curves), and differences among different groups of patients was assessed.

A two tailed *P* value less than .05 has been considered statistically significant. All statistical analysis has been conducted with SPSS 21.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, Il).

3. Results

3.1. Baseline characteristics

Within the time frame considered, 84 patients undergoing elective EVAR for intact AAA met the inclusion criteria and agreed to participate in the study. Median duration of the follow-up was 4.3 years (IQR 1.4-7.2 years) and median number of observations per patient was 4 (IQR 2-7, range 1-18). Overall, the study size consisted of 395 patients/year.

Genotyping was successful in all patients included and the allelic frequencies did not differ between EVAR patients and a group of blood donors and/or those reported in a large database for genetic frequency of SNP available at https://www.ncbi.nlm.nih.gov/snp/.

The demographic, clinical and genetic characteristics of EVAR are reported according to the occurrence of EL (Table 1) considering that when more than one type of EL event occurred in the same patient, the patient was reported on both groups. Comparison of groups showed a different prevalence of patients taking oral anticoagulants (10% in no or type 2 ELs, and 50% in type 1 and 3, $P = .01$) and involvement of inferior mesenteric artery (60 and 50% in type 2 and 3 EL, respectively, $P = .014$).

3.2. Analysis of growth rate in the overall population

The GR was significantly increased in observations when EL events were detected than in those without (2.64 mm/year, IQR 0-7.56 vs 0, IQR - 62-0.8, $P \leq .001$, n = 120 and n = 254 observations, respectively). The ratio between AAA diameter measured at each time point and at surgery changed significantly only in patients without ELs (Fig. 1). Such a result was also confirmed in a "per-patient" analysis: those without EL at any time showed a reduction in AAA diameter, while patients with EL have no such pattern ($P = .002$ for all, .007 for EL effect). Repeated measurements test, in such a comparison, was conducted in patients with data throughout the first four years (7 without and 12 with EL, respectively).

3.3. Predictors of endoleaks occurrence

During follow-up, 41 EL events were observed in 37 patients. This led to a cumulative incidence of (41/395) 10.4 events every 100 pt/y.

High-flow ELs were associated with anticoagulant use ($P = .005$, H.R. 6.4, 95% CI 1.57-26.15), MMP9 rs17577 AA genotype ($P = .033$, HR 5.5 95% CI .99-30.5), and unfavorable anatomy ($P = .051$, H.R. 3.048, 95% CI 0.965-9.62). For type 2 ("low-flow") ELs, a borderline association was found for TIMP-1 rs4849 non-T carriers ($P = .05$, HR 2.6, 95% CI 0.99-6.9). The results were confirmed for the four variables also with event-free survival analysis (Fig. 2) and detailed results, grouped as high-flow or low-flow, are reported in Table 2 (supplementary data).

3.4. Growth rate in patients without endoleaks

During the follow-up, the changes in AAA diameter consisted in 120 intervals with shrinkage, 58 without changes and 73 observations with expansion.

To observe changes in GR according to the time elapsed between EVAR and the observation (Fig. 3), a curve fitting test has been used, comparing linear, logistic, and cubic model. The median (IQR) GR varied during the first three years from -4 (-15-0) to -3 (-7-0) in the second year to -2 (-6-1) mm/year (Kruskal Wallis test $P = .435$). After 3 years, the global GR was 0 (-2-1) without significant changes over the following years.

All linear, logarithmic, quadratic, and cubic curve fittings were significantly associated with data distribution (Table 3 of Supplementary Data and Fig. 3)

Among the 42 EL-free patients with available information on endoprosthesis size, AAA sac reached the prosthesis (±5 mm) in 9 patients, 4 patients during the first 3 years, 3 in the next three years (from fourth to sixth) and other 2 from seventh till ninth.

3.5. Relationship of AAA sac shrinkage with clinical and genetic variables

Considering only observations with more than 5 mm between sac and prosthesis diameter in the final measurement, prevalence of shrinking observations was compared to that of no change or expansion episodes. At the final step, the variables identified as accounting for shrinkage were a smaller AAA diameter at observation, presence of PAD, an older age at intervention, and, in a negative fashion, MMP1 G insertion (G-/G- Vs G+/*). When these variables were dichotomized, AAA diameter lower than 53.4 mm, and age at the time of surgery 75 or older significantly increased

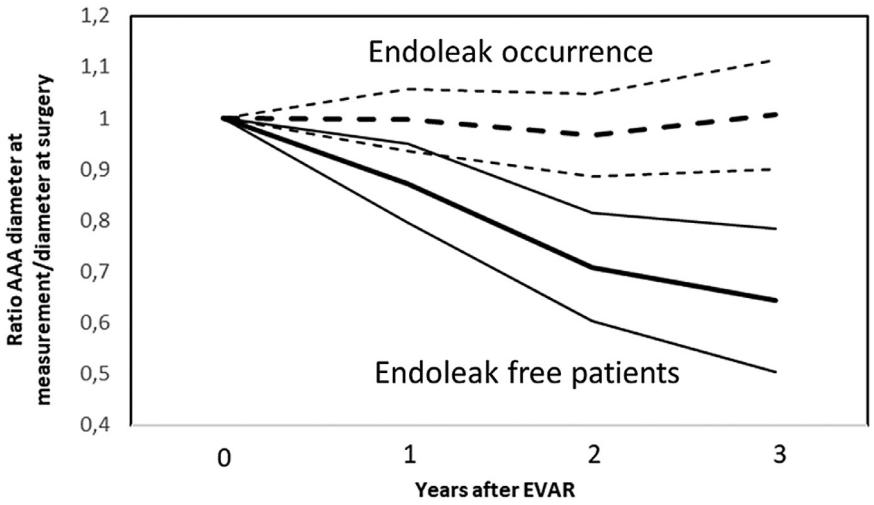

Fig. 1. Time course of the ratio between AAA maximal diameter at the end of each observation/AAA maximal diameter at surgery. Dashed lines: ratio in patients with EL at any time, thick = median value, thin = 95% confidence intervals. Solid lines: ratio in patients without EL at any time, thick = median value, thin = 95% confidence intervals. (GLM $P = .002$ for all, .007 for EL effect).

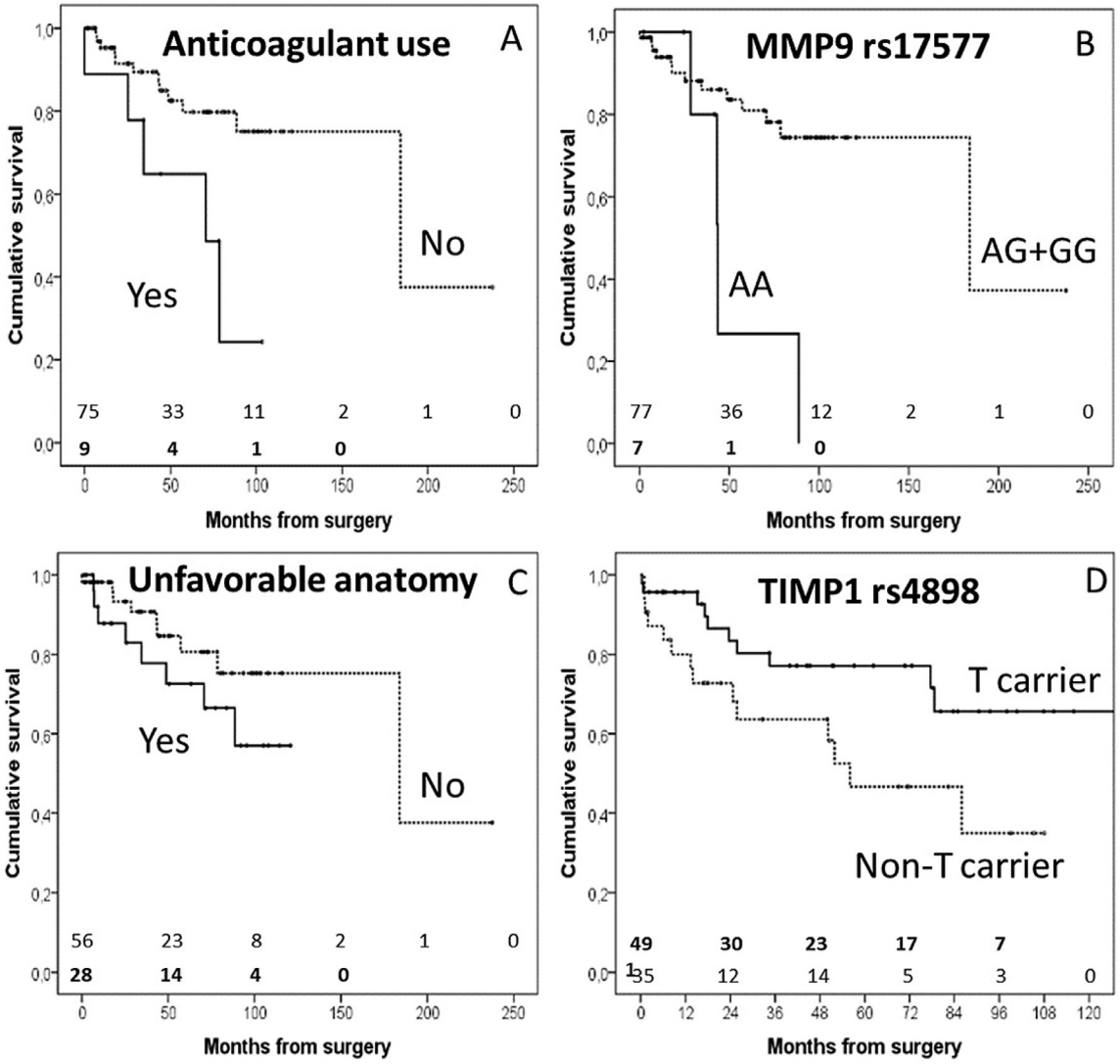

Fig. 2. Kaplan Maier analysis for EL occurrence for high flow ELs (panel A, B, and C) or type 2 EL (panel D). Legend: thick line in panel A is the anticoagulant use ($P = .013$), in panel B is *MMP9* rs17577 AA genotype ($P = .007$), in panel C the unfavorable anatomy ($P = .188$), and (for type 2 EL) in panel D is *TIMP1* rs 4898 T allele carriers ($P = .027$). Above abscissa, in bold and normal, are the numbers of patients at risk for each group.

Table 1
General characteristics of the patients in the study

	No EL (n = 47)	EL 1 (n = 14)	EL 2 (n = 24)	EL 3 (n = 3)	*P*
Follow-up (months)	44.5 (8.5-79.3)	65.6 (21.5-84.4)	77.3 (25.8-101.5)	55.3 (53.5-57.2)	n.s.
Gender (M/F)	42/5	11/3	21/3	3/0	n.s.
Age at intervention	76 (72-79.5)	76 (72-81)	75 (73-78)	80 (75-85)	n.s.
BMI (Kg/m^2)	26.6 (23.8-28.5)	25.4 (22.1-29.4)	26.6 (24.2-28.1)	24.2 (21.6-26.9)	n.s.
Diabetes	8/39	1/13	7/17	1/2	n.s.
Hypertension	38/9	14/1	22/2	3/0	n.s.
Smoking habit (N/F/C)	4/30/13	1/9/4	0/20/4	0/3/0	n.s.
hypercholesterolemia	31/16	10/4	19/5	3/0	n.s.
CVD	20/27	7/7	7/17	1/2	n.s.
PAOD	15/32	2/12	4/20	1/2	n.s.
Aspirin use	34/13	10/4	17/7	3/0	n.s.
Other antiplatelet	7/40	2/12	5/19	0/3	n.s.
All antiplatelet drugs	38/9	11/3	21/3	3/0	n.s.
Anticoagulants	4/43	4/10	3/21	1/2	0.010
Antihypertensive	36/11	12/2	20/4	3/0	n.s.
Lipid-lowering drugs	21/26	10/4	14/10	3/0	n.s.
MMP1 rs1799750	9/23/15	2/8/4	5/16/3	0/3/0	n.s.
MMP3 rs35068180	12/27/5	5/4/5	7/11/6	1/1/1	n.s.
MMP9 rs112290776	22/21/4	5/7/2	11/12/1	2/1/0	n.s.
MMP9 rs17576	22/19/6	5/5/4	10/11/3	0/2/1	n.s.
MMP9 rs17577	33/12/2	7/4/3	12/10/2	1/1/1	n.s.
MMP12 rs652438	41/6/0	13/1/0	22/2/0	2/1/0	n.s.
TIMP1 rs4898 T carrier	31/16	8/6	10/14	2/1	n.s.
Thoracic aneurysm	9	4	6	0	n.s.
Popliteal aneurysm	12	1	10	1	n.s.
Lumbar arteries	0/9/13/4	0/2/3/1	3/7/9/3	0/2/0/0	n.s.
IMA	26	3	16	1	0.014

CHD = coronary heart disease; PAOD = peripheral arterial occlusive disease; N/F/C = Never a smoker/Former smoker/Current smoker, *MMP1 rs1799750 = G+G+/G+G-/G-G-, MMP3 rs35068180 = 5A5A/5A6A/6A6A, MMP9 rs112290776 = <22<22/<22≥22/≥22≥22 CA repeats around -90 position in MMP9 promoter, MMP9 rs17576 = AA/AG/GG, MMP9 rs17577 = GG/GA/AA, MMP12 rs652438 = AA/AG, TIMP1 rs4898 = T carrier/non T carrier, Lumbar arteries = 0/1/2/3 couples of arteries (32 missing), IMA = patients with Inferior Mesenteric Artery included in the EVAR (33 missing), n.s. = not significant (P > .05)*.
When more than one type of EL event occurred in the same patient, the patient was assigned to both groups. Unless otherwise specified, the figures are occurrence /absence of the condition.

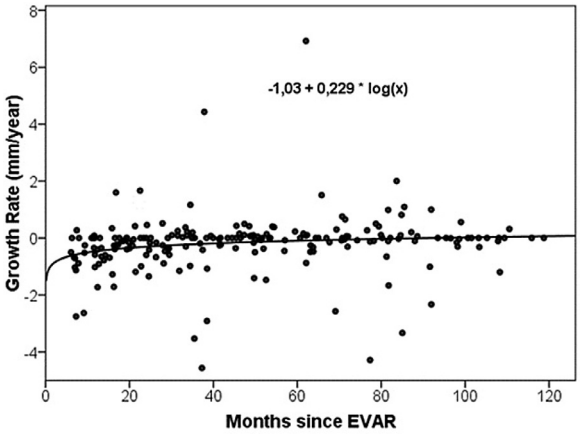

Fig. 3. Relationship between GR and years of follow-up after EVAR. Logarithmic curve equation ($P = .002$) is reported in the inset.

the possibility of having a shrinkage during observation. On the contrary, MMP1 G insertion (G+/*) reduced such a probability. In practical terms, carriers of *MMP1* rs1799750 genotype G-/G- have a yearly median GR of around -5 mm per year, while in patients >75 years at the time of surgery, with PAD or with smaller AAA, the same figures range between -1.2 to -2.5 mm/year. The results, together with the comparison of GR (Mann-Whitney test) for the significant variables in binary logistic regression test, are reported in Table 4 (Supplementary Data).

4. Discussion

The present study shows that positive remodeling of aneurysmal sac or no changes after EVAR are observed in patients with EL occurrence at any time of follow-up, while negative remodeling is mainly observed for few years in those without such a complication. In turn, while negative remodeling observations are chiefly organism-related (genetic, age and comorbidities), EL occurrence has a different pattern of determinants (genetic, therapeutic and anatomical) according to their putative amount of flow.

The peculiar pathophysiology of each EL type required a separate analysis to pinpoint specific determinants: while low-flow type 2 ELs remain the subject of much debate and still represent a controversial entity [12,13], high-flow ELs representing failure of (proximal and/or distal) device sealing or alteration in device integrity, are a more dramatic entity that warrants active treatment. The most striking evidence is that among a wide array of clinical characteristics and surgical features, high-flow EL were associated with anatomical difficulty, *MMP9* rs17577 AA genotype, and anticoagulant therapy, the latter being the most relevant. Such a finding suggests that a clotting film sealing aortic wall and prosthesis or a stable thrombus in the aneurysmal sac are essential in preventing such a complication. Our study is in line with previous ones, identifying a positive association between warfarin use and ELs [14,15], particularly with De Rango et al, who found an increased risk only for type 1 and 3 EL, while the study of Bobadilla reported an association with type 2 EL.

A further variable associated with high-flow ELs is an intron variant accounting for a differential expression of MMP-9. This SNP, in the wild type (G) variant, is a target of hsa-miR-4783-3p miRNA,

and accounts for a 25% reduced expression [16]. The pathophysiology of such an association, remains speculative and needs to be confirmed, although a MMP regulation by exosome-driven miRNA in AAA seems reliable [17], given the extra-aortic origin of cells expressing MMP-9 [18].

Using the same approach, type 2 EL reported a borderline association with a *TIMP-1* functional polymorphism accounting for a reduced protein expression [11]. Consistent with our observation, aneurysmal sac of type 2 EL shows an important thinning of the intima and media, paucity of cells and overexpression of many MMPs [19]. Continuous moderate blood flow and pressure on the aneurysmal sac from communicating vessel, typical of type 2 EL, might be effectively neutralized by normal or higher TIMP1 levels, binding various MMPs and thus preventing or slowing down cell migration [20] and extracellular matrix breakdown.

The most relevant result of the study was to observe the dynamics of remodeling after interruption of pulse pressure in infrarenal aortic aneurysmal tissue. In EL-free patients, the rate of reduction of the AAA sac is, on average, 2 mm/year, such a figure being remarkably close to that of AAA expansion reported in a prior metanalysis [21]. The speed of regression progressively decreases over time even if, in our study, only a minority of aneurysmal sacs shrink enough to reach the prosthesis. On average, before surgery, the AAA expands at a speed of 2-4 mm/year [6], while after EVAR it shrinks at the same pace for the first three years. When the stent fails to protect the aneurysmal wall, i.e., in case of EL occurrence, the aneurism diameter remains stable.

The clinical variables influencing the growth rate have been studied [22–24], although interaction of clinical and anatomical with genetic factors are less well known. The main result of this multivariate analysis is that age at intervention, PAD, aneurysmal sac size and a genetic putative lower MMP-1 expression are almost equivalent in predicting the aneurysmal size reduction.

The epidemiological association between PAD and AAA, which have an opposite pattern, shrinking the former and dilative the latter is apparently counterintuitive [25]. The present study adds some information suggesting that patients with PAD retain a pattern of vascular negative remodeling, which counteracts AAA expansion and might contribute to the shrinking after EVAR. A combination of a higher burden of diagnostic/staging investigation in symptomatic PAD patients and the lower growth rate of aneurysms, widening the time frame for their diagnosis, might account for the higher prevalence of AAA in PAD patients. It has to be considered, also, that less than 50% of AAA are recognized and followed up in the population, rising some doubts on the accuracy of our picture on these two vascular diseases.

Only one paper has investigated the relationship between post EVAR shrinking and age at observation [7], reporting a negative association between shrinking and age at observation. Our study does not confirm Boutrous' findings, but supports the hypothesis that AAA developing and undergoing surgery at older age might have an intrinsic negative remodeling pattern. In our analysis, age at observation and age at surgery might have different meanings, with the first indicating biological age of the patient, and the second, probably, the smoldering evolution of the AAA, and its susceptibility to shrink. Alternatively, it could be postulated that some features, e.g., thrombus presence in preoperative AAA sac, found associated to a faster regression [26], are more prevalent in older patients.

The last finding is the linkage of *MMP1* variant accounting for a low gene expression with a negative remodeling of aneurysmal sac. MMP1 can act mainly as a collagenase in aortic extracellular matrix or be retained within the cells [27] and influence several aspects of cell physiology [28,29,30]. Our finding is therefore consistent with an extracellular matrix degradation hypothesis and with a previous experience of our group [6], where the very same genotype (-/-) had been associated with higher prevalence of growth arrest and accounted for lower GR of AAA before surgery. In MMP1 rs1799750, insertion of guanosine at position -1607 in *MMP1* promoter creates an *ets* binding site which increases by around 8 times the gene expression [31]. *Ets* is a nuclear peptide mediating the proinflammatory effects of angiotensin infusion/hypertension in animal models of vascular [32] and kidney injury [33]. In AAA research, inhibition of the *ets* with decoy DNA binding sequence within the aneurysmal sac of rabbits can completely revert arterial size to the original diameter [34]. The previous work of Adovasio [6] supports such evidence in the aneurysmal expansion rate, while the present shows that the very same variant is associated to vascular negative remodeling when the pulse pressure does not play a role. Such a scenario suggests that MMP1 inhibition could be a therapeutic target to manage aneurysmal growth rate and possibly reduce its size.

MMP/TIMP genetic variants do not play a role in AAA susceptibility, although MMP1 rs1799450 influences the AAA GR and, in this study, it also accounts for negative remodeling. MMP9 genotype with putative high expression is associated with high flow EL, which might be due to positive remodeling after stent positioning. It should be considered that, with the limitation of the small sample size, both type 1 and 3 EL contribute to the statistical association, with the latter occurring independent of the wall reaction to the docking of the stent. The most likely hypothesis, therefore, is that MMP9 affects aneurysmal wall resilience to increased pressure in the sac. At a lesser extent, in the low pressure ELs, TIMP1 could neutralize MMP-guided matrix degradation and positive tissue remodeling. In conclusion, different genetic variants might intervene during EVAR positioning, and influencing vascular resilience. Further studies are required to support these very early results.

4.1. Study limitations

The retrospective study design could have taken away some patients and events to the observation, since it concerned only patients alive during 2010 but excluded those dead before that year. Also, the small number of observations did not allow to obtain a more detailed picture of the relationship with sac remodeling. Plasma values of some of the investigated MMPs as surrogate markers of AAA shrinking, as well as autopsy samples, might have given a more precise picture of MMP pattern during EL and sac evolution. Such information, though, would have been cumbersome requiring several blood collections over the years, while simple genotyping needs to be done only once in life and might provide a better outline of the expression in small organs.

The choice to observe negative remodeling through dichotomization of shrinkage Vs no change or expansion could be seen as an oversimplification, although such an approach avoided the effects of extreme positive or negative growth rate values on the final equation.

The small but existing inconsistencies between Computed Tomography/Angiography and ultrasound imaging might have limited the precision of the estimate of the growth rate, although the choice of a technique derives from recommendations of scientific societies and are not open to changes.

5. Conclusions

Following EVAR, ELs of vascular prosthesis hinder shrinking of aneurysmal sac. Their occurrence is related not only to surgical difficulties, but also to clinical and genetic features. Successful EVAR shows negative remodeling for the first three year of follow-up, which, in turn is associated to clinical features and MMP1 lower expression. Genetic variants of MMPs and TIMP-1 seem to modu-

late the outcome of AAA also after repair, giving hints for the development of a medical approach to aneurysmal disease.

Acknowledgments

We are indebted to Paola Pitacco for her excellent technical support.

References

[1] Gupta AK, Alshaikh HN, Dakour-Aridi H, King RW, Brothers TE, Malas MB. Real-world cost analysis of endovascular repair versus open repair in patients with nonruptured abdominal aortic aneurysms. J Vasc Surg 2020;71(2):432–43.

[2] Wanhainen A, Verzini F, Van Herzeele I, Allaire E, Bown M, Cohnert T, et al. European Society for Vascular Surgery (ESVS) 2019 clinical practice guidelines on the management of abdominal aorto-iliac artery aneurysms. Eur J Vasc Endovasc Surg 2019;57(1):8–93.

[3] Lepidi S, D'Oria M. Long term benefits of EVAR in the modern era: the importance of looking at stent graft durability without forgetting the right pathophysiology. Eur J Vasc Endovasc Surg 2020;60(6):956–7.

[4] Fiotti N, Calvagna C, Sgorlon G, Altamura N, Pitacco P, Zamolo F, et al. Multiple sites of vascular dilation or aneurysmal disease and matrix metalloproteinase genetic variants in patients with abdominal aortic aneurysm. J Vasc Surg 2018;67(6):1727–35.

[5] Liu O, Xie W, Qin Y, Jia L, Zhang J, Xin Y, et al. MMP-2 gene polymorphisms are associated with type A aortic dissection and aortic diameters in patients. Medicine (Baltimore) 2016;95(42):e5175.

[6] Adovasio R, Calvagna C, Sgorlon G, Zamolo F, Mearelli F, Biolo G, et al. Growth rate of small abdominal aortic aneurysms and genetic polymorphisms of matrix metalloproteases-1, -3, and -9. Int J Angiol 2016;25(2):93–8.

[7] Boutrous ML, Peterson BG, Smeds MR. Predictors of aneurysm sac shrinkage utilizing a global registry. Ann Vasc Surg 2021;71:40–7.

[8] Marone EM, Freyrie A, Ruotolo C, Michelagnoli S, Antonello M, Speziale F, et al. Expert opinion on hostile neck definition in endovascular treatment of abdominal aortic aneurysms (a Delphi Consensus). Ann Vasc Surg 2020;62:173–82.

[9] Drelich-Zbroja A, Sojka M, Kuczyńska M, Światłowski Ł, Kuklik E, Sobstyl J, et al. Diagnostic imaging in patients after endovascular aortic aneurysm repair with special focus on ultrasound contrast agents. Pol Arch Intern Med 2018;129(2):80–7.

[10] Fiotti N, Moretti ME, Bussani R, Altamura N, Zamolo F, Gerloni R, et al. Features of vulnerable plaques and clinical outcome of UA/NSTEMI: Relationship with matrix metalloproteinase functional polymorphisms. Atherosclerosis 2011;215(1):153–9.

[11] Lorente L, Martín M, Plasencia F, Solé-Violán J, Blanquer J, Labarta L, et al. The 372 T/C genetic polymorphism of TIMP-1 is associated with serum levels of TIMP-1 and survival in patients with severe sepsis. Crit Care 2013;17(3):R94.

[12] D'Oria M, Mastrorilli D, Ziani B. Natural history, diagnosis, and management of type II endoleaks after endovascular aortic repair: review and update. Ann Vasc Surg 2020;62:420–31.

[13] D'Oria M, Oderich GS, Tenorio ER, Kärkkäinen JM, Mendes BC, Macedo TA, et al. Natural history of isolated type II endoleaks in patients treated by fenestrated-branched endovascular repair for pararenal and thoracoabdominal aortic aneurysms. J Vasc Surg 2020;72(1):44–54.

[14] Bobadilla JL, Hoch JR, Leverson GE, Tefera G. The effect of warfarin therapy on endoleak development after endovascular aneurysm repair (EVAR) of the abdominal aorta. J Vasc Surg 2010;52(2):267–71.

[15] De Rango P, Verzini F, Parlani G, Cieri E, Simonte G, Farchioni L, et al. Safety of chronic anticoagulation therapy after endovascular abdominal aneurysm repair (EVAR). Eur J Vasc Endovasc Surg 2014;47(3):296–303.

[16] Duellman T, Warren C, Yang J. Single nucleotide polymorphism-specific regulation of matrix metalloproteinase-9 by multiple miRNAs targeting the coding exon. Nucleic Acids Res 2014;42(9):5518–31.

[17] Han ZL, Wang HQ, Zhang TS, He YX, Zhou H. Up-regulation of exosomal miR-106a may play a significant role in abdominal aortic aneurysm by inducing vascular smooth muscle cell apoptosis and targeting TIMP-2, an inhibitor of metallopeptidases that suppresses extracellular matrix degradation. Eur Rev Med Pharmacol Sci 2020;24(15):8087–95.

[18] Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter BT. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. J Clin Invest 2002;110(5):625–32.

[19] Menges AL, Busch A, Reutersberg B, Trenner M, Kath P, Chernogubova E, et al. The structural atrophy of the aneurysm wall in secondary expanding aortic aneurysms with endoleak type II. J Vasc Surg 2019;70(4):1318–26.

[20] Fernandez HA, Kallenbach K, Seghezzi G, Grossi E, Colvin S, Schneider R, et al. Inhibition of endothelial cell migration by gene transfer of tissue inhibitor of metalloproteinases-1. J Surg Res 1999;82(2):156–62.

[21] Sweeting MJ, Thompson SG, Brown LC, Powell JTRESCAN collaborators. Meta–analysis of individual patient data to examine factors affecting growth and rupture of small abdominal aortic aneurysms. Br J Surg 2012;99(5):655–65.

[22] Schlösser FJ, Tangelder MJ, Verhagen HJ, van der Heijden GJ, Muhs BE, van der Graaf Y, et al. Growth predictors and prognosis of small abdominal aortic aneurysms. J Vasc Surg 2008;47:1127–33.

[23] Hendy K, Gunnarson R, Golledge J. Growth rates of small abdominal aortic aneurysms assessed by computerised tomography–a systematic literature review. Atherosclerosis 2014;235:182–8.

[24] Powell JT. Genes predisposing to rapid aneurysm growth. Ann N Y Acad Sci 2006;1085:236–41.

[25] Takagi H, Umemoto TALICE (All-Literature Investigation of Cardiovascular Evidence) Group. Association of peripheral artery disease with abdominal aortic aneurysm growth. J Vasc Surg 2016;64(2):506–13.

[26] Tsuyuki Y, Matsushita S, Dohi S, Yamamoto T, Tambara K, Inaba H, et al. Factors for sac size change of abdominal aortic aneurysm after endovascular repair. Ann Thorac Cardiovasc Surg 2014;20(6):1016–20.

[27] Chen YL, Li WY, Hu JJ, Li Y, Liu GM, Jin TC, et al. Nucleus-translocated matrix metalloprotease 1 regulates innate immune response in Pacific abalone (Haliotis discus hannai). Fish Shellfish Immunol 2019;84:290–8.

[28] Conant K, Haughey N, Nath A, St Hillaire C, Gary DS, Pardo CA, et al. Matrix metalloproteinase-1 activates a pertussis toxin-sensitive signaling pathway that stimulates the release of matrix metalloproteinase-9. J Neurochem 2002;82(4):885–93.

[29] Jobin PG, Butler GS. Overall CM. New intracellular activities of matrix metalloproteinases shine in the moonlight. Biochim Biophys Acta Mol Cell Res 2017;1864(11 Pt A):2043–55.

[30] Limb GA, Matter K, Murphy G, Cambrey AD, Bishop PN, Morris GE, et al. Matrix metalloproteinase-1 associates with intracellular organelles and confers resistance to lamin A/C degradation during apoptosis. Am J Pathol 2005;166(5):1555–63.

[31] Rutter JL, Mitchell TI, Butticè G, Meyers J, Gusella JF, Ozelius LJ, et al. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 1998;58(23):5321–5.

[32] Zhan Y, Brown C, Maynard E, Anshelevich A, Ni W, Ho IC, et al. Ets-1 is a critical regulator of Ang II-mediated vascular inflammation and remodeling. J Clin Invest 2005;115(9):2508–16.

[33] Feng W, Chumley P, Hua P, Rezonzew G, Jaimes D, Duckworth MW, et al. Role of the transcription factor erythroblastosis virus E26 oncogen homolog-1 (ETS-1) as mediator of the renal proinflammatory and profibrotic effects of angiotensin II. Hypertension 2012;60(5):1226–33.

[34] Miyake T, Aoki M, Masaki H, Kawasaki T, Oishi M, Kataoka K, et al. Regression of abdominal aortic aneurysms by simultaneous inhibition of nuclear factor kappaB and ets in a rabbit model. Circ Res 2007;101(11):1175–84.