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From clinical observation to genomic study : contribution to the knowledge of the mechanisms of growth and rupture of abdominal aortic aneurysms

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Liège, le 15 février 2005,

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Article 6 de l'Arrêté Royal du 10 mai 1931 appliquant la loi du 21 mai 1929 sur la collation des grades académiques et le programme des examens universitaires : « En aucun cas, les opinions de l'auteur ne peuvent être considérées, par le fait de l'autorisation d'impression de la dissert ation, comme étant celles du Jury ou de l'Université

De l'observation clinique à l'étude génomique : contribution à la connaissance des mécanismes de formation et de rupture des anévrysmes de l'aorte abdominale

Surgical research, like other clinical research, is essential. There is almost certainly not a single living surgeon or other clinician who is satisfied with all aspects of contemporary surgical and clinical management. It follows that surgeons and other clinicians must carry out research.

R.L. CRUESS

A Lysiane,

A Sarah et Elif.

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Résumé

L'anévrysme de l'aorte abdominale (AAA) est une cause importante, et pourtant évitable, de décès chez les personnes âgées. Dans le monde occidental, le taux de mortalité dû à un AAA avoisine 1,3 % de tous les décès des personnes âgées de 65 à 85 ans. Le risque de rupture s'accroît avec l'augmentation du diamètre de l'AAA. C'est la raison pour laquelle nous nous sommes intéressés aux mécanismes impliqués dans la croissance des AAA et aux facteurs qui la déterminent. Les travaux repris dans notre mémoire représentent la suite logique de notre travail de doctorat défendu en 1994.

L'histoire naturelle des AAA (leur vitesse de croissance et leur incidence de rupture) a été retrouvée dans un groupe de 114 patients observés sur une période moyenne de 26 mois. A la fin du suivi, 65 des 114 patients avaient été opérés. Si nous mettons en relation l'incidence d'une chirurgie d'urgence pour rupture, en fonction du diamètre initial de l'anévrysme, nous voyons que, en-dessous de 40 mm, l'incidence de rupture est nulle et qu'elle est de 12 et 22 % dans les groupes de 40-50 et supérieur à 50 mm. Nos observations concordent avec les résultats de différents auteurs : le risque de rupture s'accroît avec la taille de l'AAA. La croissance de l'AAA est exponentielle, mais différente chez chaque individu.

Un groupe de 110 patients hospitalisés pour chirurgie coronaire ou vasculaire périphérique (79 M, 31 F) a été soumis à une mesure systématique du diamètre de l'aorte abdominale infrarénale. Nous avons pu identifier 8 anévrysmes, dont 7 chez des sujets masculins. Dans ce petit groupe de patients, l'incidence d'anévrysme était plus importante chez les sujets admis pour chirurgie coronaire que chez les patients admis pour chirurgie vasculaire périphérique. Une corrélation positive existait entre la présence d'un AAA et le taux de cholestérol sérique, de même que la réduction de l'alpha 1 antitrypsine plasmatique. Ces divers éléments nous ont permis de recommander un dépistage de l'AAA par ultrasonographie chez les sujets masculins de plus de 55 ans présentant une maladie coronarienne ou vasculaire périphérique.

En continuité avec ces travaux, nous avons réalisé un dépistage systématique des AAA dans une population masculine de 65 et 75 ans de la ville de Liège. Durant la période de 1995 à 1996, 1764 hommes nés en 1920 ou 1930 ont été invités à subir un examen ultrasonographique abdominal. Dans cette étude, nous avons constaté que la prévalence de l'AAA (3,8 %) était similaire à celle relevée dans d'autres études où elle se trouve aux alentours de 3 à 4 % dans les populations âgées de 60 ans et plus. En nous basant sur cette étude, il y aurait, actuellement, 14.000 belges âgés de 80 ans ou plus porteurs d'AAA. L'incidence de l'hypertension et de la consommation de tabac était significativement plus importante dans le groupe des AAA (Chapter 2).

Les résultats de la chirurgie prophylactique de l'AAA par mise à plat n'ont cessé de s'améliorer depuis quarante ans. Ils restent cependant grevés d'une lourde morbidité-mortalité opératoire chez les patients âgés. L'étude rétrospective de 138 patients octogénaires, admis entre 1984 et 1996 pour AAA mettent nos résultats de la chirurgie élective dans un classement très favorable. Au total, 52 patients ont bénéficié d'une intervention de mise à plat d'AAA dans des conditions électives résultant en un taux de mortalité opératoire de 5,7% ; 21 patients ont été opérés en urgence pour un AAA douloureux avec une mortalité de 28%, et 41 patients ont été opérés en urgence pour une rupture. Dans le groupe des ruptures, la mortalité opératoire a été de 68%. La mortalité de la chirurgie élective pour AAA chez les octogénaires reste plus élevée par rapport à celle de patients plus jeunes (2,7%). Elle peut, toutefois, être recommandée aux octogénaires porteurs d'AAA importants et dotés d'un bon état général. Notre option est la surveillance armée, chez les patients présentant un risque opératoire majeur (Chapter 3).

La pathogenèse de l'AAA avait déjà largement retenu notre attention au cours du Doctorat. L'élastine et le collagène sont les principales protéines de la matrice extracellulaire qui assurent les propriétés rhéologiques de l'aorte abdominale. Des prélèvements chirurgicaux ont été soumis à des analyses chimiques permettant d'établir une relation entre le diamètre de l'anévrysme et la concentration d'élastine, de même qu'avec l'extractibilité du collagène. L'évolution de ces paramètres est parallèle à l'accroissement de la taille de l'anévrysme pour atteindre un maximum dans les anévrysmes rompus. La perte d'élastine débute dès la phase

précoce de la transformation anévrysmale, tandis que l'altération du collagène, évoquée par l'accroissement de son extractibilité, atteint son paroxysme dans les AAA rompus.

Les mécanismes responsables de ces altérations ont été précisés par l'analyse du rôle des métalloprotéases MMP-2 et MMP-9 (72 kDa et 92 kDa) douées d'activité élastasique. Nous en avons mesuré l'activité dans le sérum, le thrombus pariétal, ou la paroi des anévrysmes. Par comparaison, nous avons également déterminé l'activité de ces élastases dans des parois d'aorte athéromateuse ainsi que dans le sérum d'individus normaux. La paroi aortique anévrysmale contient une proportion plus importante de la gélatinase 92 kDa que celle des contrôles non anévrysmaux. Les formes actives aussi bien des 72 kDa que des 92 kDa sont significativement accrues dans la paroi anévrysmale par rapport aux contrôles et deux fois supérieures à celle observée dans le thrombus pariétal. Ces résultats plaident pour une production in situ des gélatinases plutôt qu'une diffusion des élastases sériques (Chapter 4).

La collaboration de O. Defawe (Laboratoire de Biologie des Tissus Conjonctifs, Prof. B.V. Nusgens) nous a permis de préciser le rôle des différentes métalloprotéinases et de leurs inhibiteurs dans le développement des lésions anévrysmales (AAA) et athérosclérotiques (AOD). Le profil d'expression (ARN-messagers) des gènes des MMPs, des sérines protéinases et de leurs inhibiteurs ont été mesurés par RT-PCR quantitative. Des échantillons provenant de 6 aortes anévrysmales (AAA), de 6 aortes occlusives (AOD) prélevés lors d'interventions, mais aussi de fragments de parois normales d'aortes abdominales et des prélèvements d'aorte thoracique ascendante réalisés lors de pontages aorto-coronaires. En parallèle avec l'expression très accrue des protéases par rapport au tissus normaux dans les deux pathologies, deux de leurs inhibiteurs, TIMP2 et PAI-1, sont réduits dans les AAA mais pas dans les AOD, ce qui introduit une différence significative entre les deux pathologies. Ces données suggèrent qu'un déséquilibre entre les métalloprotéinases, leurs activateurs et leurs inhibiteurs peut expliquer le développement d'une dilatation anévrysmale au niveau de l'aorte abdominale athéromateuse. Le profil d'expression comparatif (AAA, AOD, aorte normale) de multiples gènes potentiellement impliqués dans le développement de l'AAA a dévoilé une importante hétérogénéité des taux d'expression de diverses enzymes considérées comme essentielles par

divers auteurs. Il ne nous a pas été possible, malgré les données de profil d'expression des gènes opérant dans le remodelage de la paroi de l'AAA, de clairement démontrer le mécanisme d'accroissement d'extractibilité du collagène. Il pourrait résulter de l'activation de sa synthèse, le collagène néoformé étant plus extractible, ou d'une dégradation des liaisons intermoléculaires notamment par l'excès de MMP-13 (Chapitre 5).

Nous nous sommes interrogés sur le caractère représentatif des prélèvements tissulaires ponctuels réalisé pendant une intervention pour rupture. Lors de l'intervention, un prélèvement des tissus, juste au niveau du site de rupture, est difficile, à cause de l'hématome dans la paroi, ainsi qu'au niveau de l'espace rétropéritonéal ; en outre, un tel prélèvement est dangereux, en raison d'autres priorités liées à la situation précaire du patient. Un examen postmortem précoce a permis de réaliser des prélèvements de parois aortiques au niveau du site de rupture, et tous les 10mm jusqu'à une distance de 50 mm. Dans ces échantillons, au site de rupture, les MMP-2, -9, -12, -13 sont fortement accrues de même que leurs inhibiteurs. A distance de la rupture, l'hétérogénéité d'expression des MMP-3, -8 et -11 est manifeste. L'expression de TIMP2 ainsi que PAI-1, plus importante au niveau du site de rupture se réduit de façon irrégulière à distance. L'étude histologique du site de rupture et à distance démontre également un infiltrat inflammatoire diffus qui devient focal à distance, et une forte hétérogénéité de destruction de la média (Chapter 6).

Pour démontrer, in vivo, la topographie de ces activités enzymatiques présentes au sein des macrophages de l'infiltrat inflammatoire dans la paroi aortique anévrysmale, nous avons utilisé le PET-scan. Le 18FDG, analogue du glucose marqué par un isotope du fluor visible en résonance magnétique, permet l'évaluation du métabolisme glucidique régional. Cette technique est largement utilisée pour démontrer, notamment, la présence d'un infiltrat néoplasique mais également les réactions inflammatoires granulomateuses et les tissus infiltrés par des macrophages. Nous avons réalisé un PET-scan utilisant le 18FDG chez 26 patients, porteurs d'un AAA. Les 10 patients dont le PET-scan était positif, étaient caractérisés par une augmentation récente de la taille de l'AAA, des signes de fissuration, voire de rupture imminente. La positivité de la paroi aortique lors de l'examen PET- scan chez

un patient référé pour anévrysme de l'aorte abdominale représente dès lors pour nous, un facteur renforçant l'indication opératoire. (Chapter 7).

En outre, nous avons étudié la corrélation entre la captation de FDG et les données histologiques dans une paroi aortique anévrysmale. Les analyses histologiques de tous les échantillons montrent une réaction inflammatoire surtout importante au niveau de l'adventice, et beaucoup moins marquée au niveau de la média. La zone luminale du thrombus contient également des cellules inflammatoires. L'immunohistologie confirme la présence principalement des macrophages. Leur localisation préférentielle est cependant l'adventice de la paroi aortique anévrysmale. Nous avons constaté une corrélation significative entre la positivité du PET-scan et la présence des leucocytes en surface du thrombus et au niveau de la paroi aortique, plus intense au niveau de l'adventice (Chapter 8).

Comme les métalloprotéinases sont libérées par les leucocytes activés, notamment par stress oxydatif, nous avons mesuré le taux sanguin de la vitamine E comme un reflet indirect de l'intensité de ce stress. La vitamine E plasmatique des patients anévrysmaux est significativement réduite, de même que son rapport avec les lipides totaux. Cette observation suggère l'intérêt potentiel d'un monitorage de la vitamine E sérique chez les patients à risque de développement d'un AAA de taille critique (Chapter 9).

Comme les AAA surviennent tard dans la vie, le caractère familial de l'affection a été, en général, sous-estimé. Lors d'analyse de ségrégation, la transmission génétique de l'AAA se ferait sur un modèle autosomal récessif. En 1995, sur un échantillon plus large nous avons ainsi retrouvé 276 cas sporadiques et 81 cas dans un contexte d'atteinte familiale (76 sujets masculins et 5 sujets féminins). Nous avons également constaté quelques différences entre cas familiaux et sporadiques, notamment l'âge au moment de la rupture qui est inférieur dans le groupe familial (65,4 ±6,6) à celui du groupe sporadique (75,2 ±1,6). Le pourcentage de rupture est, par ailleurs, de 32,4 % pour les cas familiaux et 8,7 % pour les cas sporadiques. Nous avons pu déterminer que le risque relatif d'AAA pour les frères d'un sujet mâle porteur d'un AAA est de 18. En testant l'hypothèse d'un modèle mixte, l'explication la plus vraisemblable pour la survenue d'anévrysmes familiaux est la présence d'un gène unique, présentant un caractère dominant (Chapter 10).

Après la publication de nos travaux concernant l'aspect familial et génétique des anévrysmes, nous avons été invités par le groupe de Helena Kuivaniemi (Center for Molecular Medicine and Genetics, Wayne States University Detroit, USA) à participer à une étude multicentrique. Dès leur admission pour chirurgie de l'AAA, les patients sont interrogés sur leurs antécédents familiaux. Les membres des familles qui ne présentent pas d'AAA connu sont invités à subir une échographie et un prélèvement sanguin pour analyse d'ADN. Notre contribution à cette étude a été de pourvoir 59 familles sur les 234 qu'elle comporte. Par la suite, 420 nouveaux patients ont été découverts porteurs d'un anévrysme de l'aorte abdominale. Contrairement aux hypothèses antérieures, l'étude génétique a montré que dans 72 % des familles le mode de transmission est autosomal récessif, et que chez 25 % il est autosomal dominant.

Nous avons participé à l'analyse génomique des patients souffrant d'AAA comparés à des sujets indemmes de l'affection. Cette étude a permis de montrer que les inhibiteurs des métalloprotéinases (TIMP I et 2) présentent un polymorphisme au sein d'une séquence codante chez les patients porteurs d'un AAA, mais également chez les sujets sains. Il s'agit donc d'un polymorphisme neutre.

La poursuite de cette étude multicentrique a mis en évidence une hétérogénéité génétique et la présence de deux régions susceptibles pour l'AAA au niveau des chromosomes 19 et 4. Ces deux régions contiennent plusieurs gènes candidats plausibles. Il s'agit des gènes codant pour l'interleukine 15, la GRB2 Associated binding protein I, le récepteur de type 1 à l'Endothéline, la LDL receptor related protéin III, la trans-membrane protease serin I, le programmed cell -dead 5 et les gènes de peptidase D (Chapter11).

Au terme d'un travail de plus de 15 ans qui s'est intéressé successivement à l'observation clinique des patients porteurs d'AAA, parfois jusqu'à leur rupture, aux modifications de la matrice extracellulaire de la paroi anévrysmale et aux mécanismes physiopathologiques qui en sont responsables, à l'utilisation de nouvelles méthodes d'imagerie (PET-scan), nous avons également coopéré à l'étude multicentrique du génome du patient porteur d'AAA, ou menacé de l'être.

Depuis l'opération princeps de Dubost en 1953, 50 années ont été nécessaires au raffinement des techniques et des indications opératoires, de la réanimation, et des modes de diagnostic. Le but jusqu'à présent a été de prévenir le décès du patient par rupture de son AAA grâce à une mise à plat chirurgicale ou prothèse endovasculaire prophylactiques. Le travail continue. Les prochaines étapes consisteront en l'utilisation de moyens pharmacologiques susceptibles de réduire ou supprimer le développement des AAA chez l'individu génétiquement enclin à cette affection. La tâche de la communauté scientifique sera de prévenir, non plus seulement la rupture de l'anévrysme, mais plus fondamentalement sa survenue et sa croissance.

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TABLE OF ABBREVIATIONS

AAA	Abdominal aortic aneurysm
AOD	Aorto-occlusive disease
APMA	Aminophenylmercuric acetate
ApoE.....	Apolipoprotein E
ARP	Affected relative pair
ASP.....	Affected sib pair
CAA	Control abdominal aorta
COPD	Chronic obstructive pulmonary disease
CT.....	Computerized tomography
CTA.....	Control thoracic aorta
EDTA	Ethylene-diamine-tetra acetic acid
FDG.....	Fluorodeoxyglucose
HPN.....	Transmembrane protease serine 1
HSMC.....	Human smooth muscle cells
ICAM-1	Intercellular adhesion molecule-1
IL-1	Interleukine-1
kDa	KiloDalton
LDL	Low density lipoprotein
LOD.....	Logarithm of odds
LRP.....	Low density lipoprotein receptor-related protein 3
MCP	Monocyte chemotactic protein

MMPs	Matrix metalloproteinase
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
PAI-1	Plasminogen activator inhibitor
PCR	Polymerase chain reaction
PEPD3	Peptidase D3
PET	Positron Emission Tomography
PMNs.....	Polymorphonuclear neutrophil
rAAA	Ruptured abdominal aortic aneurysm
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase-polymerase chain reaction
SDS.....	Sodium dodecylsulfate
SMC	Smooth muscle cells
SPIO	Supra paramagnetic iron oxide
sRNA	Synthetic ribonucleic acid
TIMP	Tissue inhibitor of metalloproteinases
t-PA	Tissue plasminogen activator
u-PA	Urokinase-type plasminogen activator
US.....	Ultrasound
Vit E	Vitamin E

CHAPTER I

GENERAL INTRODUCTION AND AIM OF THE WORK

1. Generalities

The abdominal aorta originates from the thoracic aorta at the level of the diaphragmatic hiatus. It courses on the left anterior flank of the spine in the retroperitoneal space and ends at the aorto-iliac bifurcation at the level of the lower lumbar rachis facing the left anterior side of L4. The infrarenal abdominal aorta is limited to the distal aortic segment starting below the origin of the renal arteries, situated at the level of the lower third of L1 or of the upper side of L2. The skin projection of the bifurcation corresponds to a point located on the abdominal wall, one or two centimeters above the navel. Thus, on average, the infrarenal abdominal aorta has a length of 10 cm, a dimension that can vary according to the exact position of the bifurcation. Its diameter varies according to the age, sex and constitution of the subject. In normal Man, there is a progressive narrowing of the aortic diameter from the supravalvular origin to the bifurcation. For example, it is generally observed that the diameter of the infrarenal aorta is 2 mm less than that of the suprarenal aorta (Steinberg et al., 1965).

An aneurysm can be defined as a permanent irreversible localized dilatation of a vessel. This “abnormal” dilatation involves the three layers of the vascular tunic: the intima, the media, and the adventitia. This definition differentiates an aneurysm sensu stricto from a false aneurysm, which corresponds to a perivascular pulsatile hematoma secondary to a vessel rupture. In this latter instance, however, the capsule is devoid of any residual vascular structure: the external limit of this pulsatile dilatation is made of an amorphous fibrous material. Similarly, the infiltration of blood within the vascular wall, associated to

enlargement of the diameter of the artery (like in aortic dissection) is not an aneurysm in the strict meaning of the term.

In terms of morphology, reviewed in details by Slaney (Slaney, 1990), two types of dilatation involving all the layers of the vessel wall can be recognized. The "fusiform" one has been described since the beginning of medical science. In this type of lesion, the parietal weakening concerns the whole circumference of the artery and participates in the aneurysmal dilatation. In contrast, an aneurysm is designed "saccular" if it involves only a part of the circumference.

If these definitions are straightforward for large aneurysms, there are ambiguous for smaller aneurysms. In fact, the term of "abnormal dilatation" used in the definition is purely qualitative. However, the definition should be initially precise, since it is important to decide what is considered or rejected as an abdominal aortic aneurysm (AAA) in the infrarenal position. In fact, this aspect may have unsuspected effects on epidemiological studies and segregation analyses where an infrarenal dilatation is always considered with respect to the diameter of the normal aorta, that is variable for the gender and the height of the patient, as mentioned previously.

Our interest has been centered on the clinical and experimental investigations of AAA. The abdominal aortic aneurysm (AAA) is indeed important cause of preventable deaths in old patients. In fact, the mortality rate due to AAA is about 1,3% of all deaths among men aged between 65 and 85 years in the western world (Law et al., 1994). Among these, many lives could be saved if rupture of AAA can be prevented. On the other hand, the increasing number of patients presenting an aneurysm of the abdominal aorta represents a burden for the population and a source of expenditure for the Health Care System. Better knowledges of the pathogenesis and of the natural history aimed at a better treatment of aortic aneurysm should be an important goal for physicians. In this approach, our initial goals were to analyze the prevalence of AAA in high-risk patients and also to determine both rate of growing and risk of rupture. It also rapidly became evident that specific molecular mechanisms (matrix elaboration and remodeling) were implicated in the development of AAA. Therefore, our ultimate goal is to identify molecular markers that would be useful in the diagnosis and

treatment of aortic aneurysm, so that patients at risk for developing AAA could be identified before the rupture occurs and operated on electively.

2. Summary of doctoral thesis

Our investigations started in 1989 and have been conducted in the departement of the Cardio vascular Surgery (Professor Limet) and in the Laboratory of Connective Tissu Biology (Professor Lapiere and Nusgens) to result in the publication of our doctoral thesis in 1994. At the beginning of our clinical and experimental study, the majority of studies examining the epidemiology of AAA were based on selected groups of individuals often poorly defined in terms of age, sex, and other risk factors. In addition, most of them have been carried out retrospectively.

At this time, the results of most screening studies, which intended to determine the prevalence in high risk populations differed substantially (Allardice et al., 1988, Allen et al., 1987, Allen, Tudway & Goldman, 1987, Bengtsson et al., 1991, Bengtsson, et al., 1989, Bengtsson, Norrgard et al., 1989, Collin et al.1988, Nevelsteen et al., 1991, Lederle et al., 1988, Lerderleet al., 1994, Scott et al., 1988). The prevalence observed in these groups was usually much higher than that found in the general population according to ultrasound investigation. A question raised up concerning the benefit and the cost-effectiveness of screening by ultrasonograph, to detect unknown AAA in high-risk population.

2.1.Study of prevalence in high-risks patients

Thus we decided to set up a screening program in order to detect AAA in 110 consecutive patients admitted to our department (Sakalihasan et al., 1992), for coronary artery (n = 72) and/or peripheral vascular (n = 38) disease (Table I).

	Coronary artery disease		Peripheral vascular disease		
	n	Mean age ± SD (years)	n	Mean age ± SD (years)	Total
Male	53	62.7 ± 4.7	26	66.9 ± 4.9	79
Female	19	64.1 ± 4.3	12	61.3 ± 4.4	31
Total	72		38		110

Table I. Characteristics of study population screened for AAA.

Except for those presenting disease, age ranging from 55 to 74 years was the only other criterion used for patients' inclusion. On admission all patients were submitted to physical examination, standard laboratory testing, routine lung function test and abdominal ultrasound examination. We defined AAA as a local infrarenal aortic dilatation with diameter equal to 30 mm or larger. Eight aortic dilatations equal to or greater than 30 mm were evidenced, 7 in males (12.3%), and 1 in female (3.1%) (Table II). All patients with AAA were aged 60 or older and the observed prevalence of AAA was higher in groups of patients suffering from coronary artery disease (15% male patients) versus 5.9% in peripheral vascular disease.

	Coronary artery disease			Peripheral vascular disease		
	n	AAA	%	n	AAA	%
Male	40	6	15.0	25	1	4.4
Female	17	1	5.9	7	0	0.0
Total	57	7	12.3	32	1	3.1

Table II. Prevalence of AAA according to risk groups patients aged equal or more than 60 years.

We also observed a positive relation between abnormally elevated cholesterol levels, decreased α_1-antitrypsin plasmatic levels and the occurrence of AAA (TableIII). Therefore we suggested monitorizing α_1-antitrypsin, an inhibitor of leukocyte elastase, in patients at risk of

developing AAA. According to our results, we recommended the screening of AAA by ultrasound examination in selected high-risk patients aged 60 or more.

	Patients without AAA			Patients with AAA			Patients with incipient AAA		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
n patients	66	29	95	7	1	8	6	1	7
Cholesterol *	17	4	21	4	1	5	3	1	4
α_1-antitrypsine **	5	2	7	3	-	3	2	-	2
Hypertension	17	14	31	4	1	5	2	1	3
Diabetes mellitus	4	8	12	2	-	2	1	-	1
COPD	16	4	20	3	1	4	2	-	2
Current smoker	48	17	65	6	-	6	6	1	7
Mean age (years)	63.9 ± 5.7	63.3 ± 4.5		64.9 ± 4.8	61		63.8 ± 5.6	55	
Mean weight (kg)	74.9 ± 9.8	67.3 ± 11.3		77.1 ± 7.7	75		74.7 ± 6.4	80	
Mean height (cm)	172.1 ± 6.2	161.9 ± 6		168.9 ± 7.4	170		174.7 ± 6.5	167	

* χ^2 . $p < 0.005$ odds ratio = 5.286

** χ^2 . $p < 0.01$ odds ratio = 6.286

Table III. Characteristics of patients screened for AAA.

The discovery of non ruptured AAA, notably by screening examination, led us to the problem of treatment choice: is it necessary to operate on all patients prophylactically, or must

we reserve prophylactic surgery only for a determined subgroup where factors indicative of a probable rupture could be identified? On the other hand, until early 1980s, the expansion rate and incidence of rupture of AAAs with respect to their size was a source of controversies. Most studies have shown that the speed of change in size increases (slope) as the aneurysm gets larger, indicating a non-linear relationship (Bernstein et al., 1984, Bernstein et al., 1976, Cronenwett et al., 1985, Cronenwett et al., 1990). We therefore investigated the possibility of modeling the speed of expansion of AAA by an exponential rather than by a linear function. This would have the additional advantage of predicting the future evolution of the aneurysm from two single ultrasound examinations performed at any moment during the time course of the disease.

2.2.Study of the growth rate and risk of rupture

We focused therefore on aneurysm growth in a population of 114 patients who had not been operated on initially, because of various reasons (patients' refusal, high surgical risk, or small diameter as assessed by CT-scan and ultrasonography) (Limet et al., 1991). At the end of the follow-up period of 26,8 months (range 3-132), we studied the risk of rupture according to the size of AAA (TableIV and V).

Diameter group (mm)	First examination n of patients (%)	Final examination n of patients (%)	Average observation period (± SD)
(I) 30-39	49 (43%)	12 (10%)	31.8 ± 3.62
(II) 40-49	41 (36%)	34 (30%)	26.8 ± 2.41
(III) ≥ 50	24 (21%)	68 (60%)	16.7 ± 2.33
Total	114 (100%)	114 (100%)	26.8 ± 2.10

Table IV. Distribution of 114 patients with AAA based on their initial and final diameter values (mm) ; average observation periods (months) are also given.

Diameter group (mm)	Final examination n of patients	n of patients operate on	n of ruptured aneurysms
(I) 30-39	12	2 (17%)	0 (0%)
(II) 40-49	34	13 (38%)	4 (12%)
(III) ≥ 50	68	50 (74%)	15* (22%)
Total	114	65 (57%)	19 (17%)

* Includes the patients who died of rupture before surgery

Table V. Distribution of 114 patients with AAA based on their initial and final diameter (mm) ; average observation periods (months) are also given.

Using individual serial measurements, we determined the linear growth rate (λ) and the exponential growth rate (α) for each of our patients (Tables VI and VII).

Initial diameter group (mm)	Total patient population (n = 114)		Restricted set (n = 101)*	
	n	Mean (± SEM)	n	Mean (± SEM)
(I) 30-39	49	5.30 ± 0.588	46	5.65 ± 0.592
(II) 40-49	41	6.87 ± 0.905	36	7.82 ± 0.924
(III) ≥ 50	24	7.45 ± 1.254	19	9.41 ± 1.230
		F = 1.72 (2 and 111 df) p = 0.1838		F = 4.58 (2 and 98 df) p = 0.0125

* 13 patients showed no increase in size ($\lambda = 0$)

Table VI. Mean (± SEM) linear expansion rate λ (mm/year) of AAA according to initial diameter size in total patient population and in patients with positive λ values.

Initial diameter group (mm)	Total patient population (n = 114)		Restricted set (n = 101)*	
	n	Mean (± SEM)	n	Mean (± SEM)
(I) 30-39	49	0.133 ± 0.0138	46	0.142 ± 0.0138
(II) 40-49	41	0.134 ± 0.0176	36	0.152 ± 0.0180
(III) ≥ 50	24	0.114 ± 0.0185	19	0.144 ± 0.0177
		F = 0.35 (2 and 111 df) p = 0.7055		F = 0.14 (2 and 98 df) p = 0.8695

* 13 patients showed no increase in size ($\alpha = 0$)

Table VII. Mean (± SEM) exponential expansion rate α (year-1) of AAA according to initial diameter size in total patient population and in patients with strictly positive α values.

Our data provided sufficient and statistically significant evidence that the enlargement rate of AAA was exponential. We concluded that :

1. Change in size of an aneurysm increases with time as a function of the initially observed size, thus suggesting a non-linear evolution of the diameter.

2. The exponential expansion rate model was dependent on the initial diameter size.

3. In patients who underwent three or more consecutive examinations, the use of an exponential model was significantly superior that of a linear model, as measured by the percentage of variance applied to the regression line.

4. Our study clearly indicated that the evolution of the disease process could be adequately described by an exponential model, once it strongly suggested that "exponential" rather than the classical "linear" expansion rate should be calculated to assess relative change in size of an aneurysm.

5. Finally our data revealed that rupture of aneurysms is related not only to their size, but also to their expansion rate.

2.3. Initial study on molecular mechanisms

One of the most consistent observations in AAA is the disorganization and distribution of elastin lamellae, collagen and other matrix components of the vessel wall by blood born cells (macrophages and lymphocytes) (Ghorpade & Baxter, 1996, Sakalihasan et al., 1993). Matrix metalloproteinases (MMPs) displaying an elastase activity have been involved in aortic wall degeneration, both in human and experimental animals. The MMPs' activity is further controlled by physiological inhibitors, the tissue inhibitors metalloproteinases (TIMPs) (Sternlicht & Werb, 2001, Wojowicz et al., 1997).

Despite several recent publications, the mechanism underlying the development of aortic aneurysm remained unclear. A consistent finding was a substantial loss of elastin, demonstrated both mechanically and histochemically (Campa et al., 1987, Rizzo et al., 1989, Sumner et al., 1970), but the collagen content has been reported to be reduced, unaltered or increased (Dubick et al., 1988, Menashi et al., 1987, Sumner et al., 1970). A relation between the size of the aneurysm, index of its evolution and changes in the composition of the main extracellular matrix proteins of the aortic wall had never been described. Therefore we have studied a potential relationship between the aortic diameter and the collagen and elastin concentration in the wall of resected aneurysmal aortas (Sakalihasan et al., 1993) (Table VIII).

Group	Collagen (%)		Elastin (%)	
Control	28.4 ± 6.1	(n = 8)	15.3 ± 6.3	(n = 8)
I (< 50 mm)	25.5 ± 7.8	(n = 4)	6.8 ± 3.9*	(n = 6)
II (50-75 mm)	34.8 ± 10.0	(n = 6)	4.4 ± 3.5*	(n = 10)
III (> 75 mm)	34,8 ± 6.9	(n = 7)	4.6 ± 1.5*	(n = 7)
IV (ruptured)	32.7 ± 6.6	(n = 6)	3.4 ± 1.6*	(n = 6)

Significantly different from control group with $p < 0.05$*

Table VIII. Collagen and elastin concentration(in % of defatted dry weight) in normal and aneurysmal aortas.

We observed that, when individual values of elastin concentration were plotted against the respective diameter of aortas, the elastin loss occured mainly during the early phases of the aneurysmal development (Figure1). However, the extractable collagene content was unchanged in small aneurysms (< 50 mm) and was higher in large and ruptured A (Figure 2).

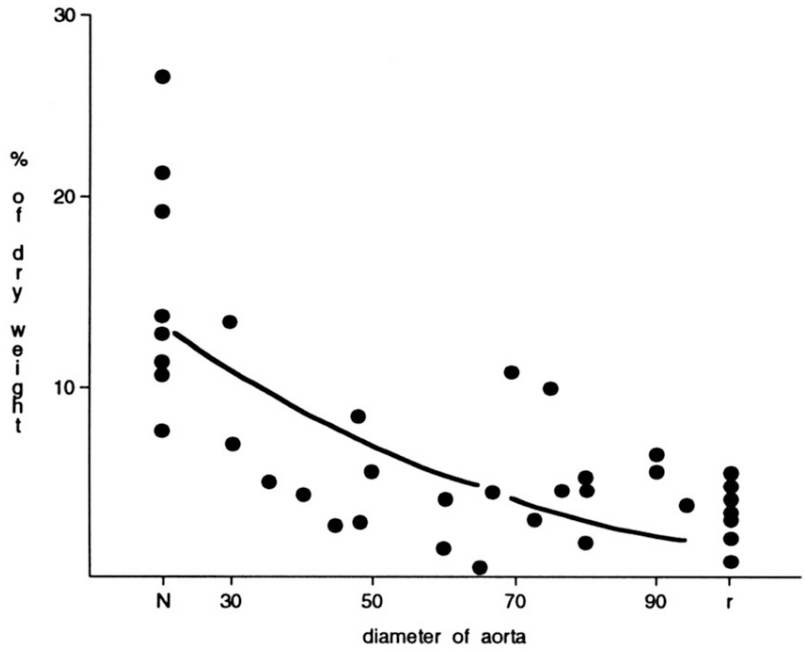


Figure 1. Significant quadratic relationship between the elastin concentration (in % of defatted dry weight) and the individual values of the diameter of the normal aortas (N), AAA of increasing size (mm) and ruptured AAA (r).

According to our findings, we concluded that early degradation of elastin, followed by modification of the collagen polymers, supported the suggestions of Dobrin (Dobrin et al., 1984) that elastin plays a role in dilatation, while subsequent collagen alteration could lead to rupture.

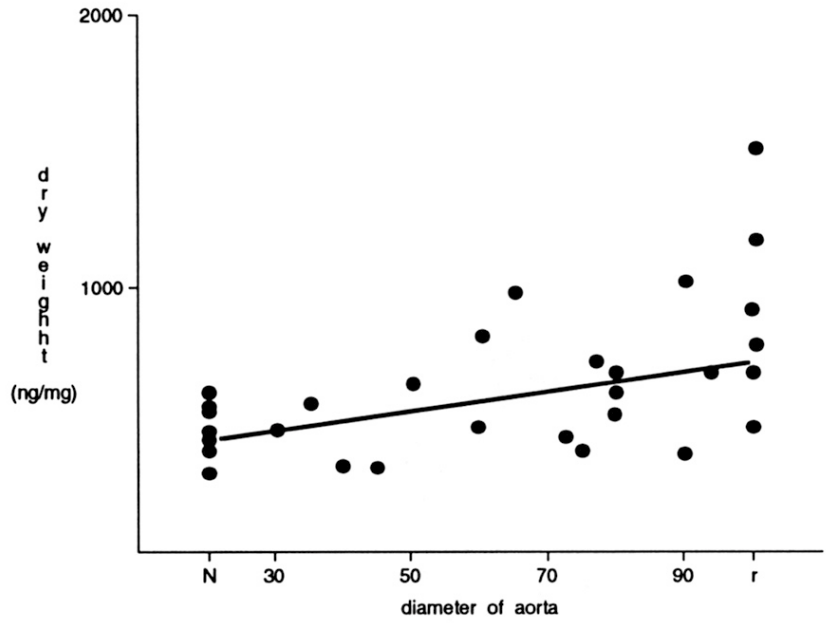


Figure 2. Significant positive linear correlation between the collagen extractability (dry wright ng/mg) and the individual values of the diameter of the normal aortas (N), AAA of increasing size (mm) and ruptured AAA (r).

These results led us to develop some hypotheses, that will be tested by new investigations followed our doctoral thesis (1994).

Aim of the study

Whatever the safety of procedures to treat AAAs before rupture, none of them is performed without a certain mortality (at best less than 2%), morbity, or consequences on health costs. A better knowdlege of the prevalence of AAA in an ageing occidental population, the demonstration of a genetic mecanism and the elucidation of the biochemical pathways depending on heredity and risk factors will provide a gobal approach of AAAs, thus considered as a pathological continuum from birth until death. These knowledges will perhaps allow, for the present, a better therapeutic classification for surgery, but also in the future, will perhaps lead to true prophylactic measures (i.e. chemotherapy along with other interventions). Such are the general goals of our study.

Our work plan answers to the following questions:

1) Is the prevalence of AAA in Belgium similar to the prevalence observed in other countries? Is routine screening with ultrasound examination a cost effective method? Are there any correlations between AAA disease and classical risk factors for atherosclerosis?

Abdominal aortic aneurysm is a common pathology, with an estimate incidence of 20 to 40 cases /100,000 persons per year, and its prevalence lies between 3 and 4% in men aged 60 years or older. All population-based screening studies performed at the beginning of the last decade were performed in American, British, and Scandinavian countries. Therefore, we started a population-based study in Liège among men aged between 65 and 75 years old. (Chapter 2)

2) Based on previous results, the incidence of AAAs in octogenerians is far to be negligeable. So, what are the results of surgical treatment of AAA in octogenarians? (Chapter 3)

3) As shown in our doctoral thesis, the presence of an inflammatory infiltrate in the wall of AAA and degradations of elastin and collagen are early features in the development of AAA. So do metalloproteases play a significant role in the development of AAA? Thus, specific metalloproteinases displaying elastolytic contents (as the 72 and 92 kDa gelatinases) were

identified and quantified in the serum, in the thrombus and in the wall of the blood vessels. (Chapter 4)

4) The involvement of proteases/antiproteases in AAA and AOD (aortic occlusive disease) already documented in aneurysmal progression and plaque instability (Knox et al., 1997) is most often restricted to one or a few potential factors. The following question arise from this conflicts: are the respective implications of MMPs, TIMPs and repair process similar in aneurysmal aorta and AOD? (Chapter 5)

5) Imbalance between MMPs and their inhibitors (TIMPs) in the wall of aneurysmal abdominal aorta is implicated in the expansion of ruptured AAA (Baxter et al., 2002, Knox et al., 1997). Can informations gathered from the samples collected during surgery for ruptured AAA reflect a local activity at the precise site of rupture. (Chapter 6)

6) Although the size of the aneurysm still remains the most accepted predictor of rupture, small AAA may also rupture. So, are there any marker or functional imaging techniques such PET scan able to monitor the development and evolutivity of AAA? (Chapter 7)

7) Since we found a positive relationships between the clinical status of AAA and the functional imaging (chapter 7), a potential correlation was searched between FDG uptake and histological findings in the wall of AAA. (Chapter 8)

8) If our findings favored the local production and/or activation of the MMP-2 and MMP-9 in aortic wall by polymorphonuclear leucocytes (PMNs), does the activation of PMNs result in a systemic oxidative stress ? In order to answer this question, the concentration of a major antioxidant (vitamin E) was determined in the plasma of the patients with AAA. (Chapter 9)

9) Published observations suggest a familial incidence of AAA. (Bengtsson et al., 1996, Powel & Greenhalgh, 1987, Tilson & Seashore, 1984, Webster, St Jean et al., 1991). If AAA is really a familial disease, what is the genetic basis? In order to disclose a possible familial incidence and genetic predisposition, we started a familial enquiry of AAA carriers in the family of AAA patients recruited between 1986 and 1991. (Chapter 10)

10) Some genetic pattern associated to development of AAA in the belgian population were identified (chapter 10). So, may these pattern be transposed to other countries and populations and is it possible to identify a specific genetic locus? Are these genetic predisposition correlated to changes in activities of MMPs and TIMPS? To answer these questions, a multicentric European and North-American study was initiated. (Chapter 11)

CHAPTER 2

EPIDEMIOLOGY OF AAA: A POPULATION-BASED STUDY (APPENDIX 1)

INTRODUCTION

Epidemiology deals with the frequency and distribution of disease within the population. Epidemiological methodology may be helpful in identifying possible causes of disease. The basic estimates of the presence of a disease in the population are the prevalence and the incidence. The prevalence describes the number of patients having a disease at a certain time. The incidence describes how many new individuals will develop the disease during a certain period of time.

According to the definition set up by the World Health Organization, screening means medical investigation that does not arise from patient's request for advice because of specific complaints. Certain prerequisites are necessary before to start screening procedures. The disease must be potentially dangerous and should be asymptomatic. It must be possible to detect the disease before symptoms occur and finally some form of treatment must be available. In the case of AAA all these premises are at hand. Abdominal aortic aneurysm (AAA) is a serious medical problem that affects a significant proportion (± 4%) of the more than 60 year-old (± 3500 men in Liège). It can be foreseen that the prevention of AAA will further be needed in the next decades with the life expectancy progress.

Epidemiology of AAA has been poorly studied in the past. The disease is mostly asymptomatic (until rupture) and previous diagnostic methods, such as conventional X-rays and abdominal palpation, present obvious limitations in detecting aneurysms (Collin et al., 1988, Lederle et al., 1988). With the introduction of new non-invasive diagnostic methods such as computerized tomography (CT) and ultrasonography (US), it has become possible to investigate, with a high diagnostic accuracy, population for the presence of clinical undetected

AAA (Erikson et al., 1980, Gomes et al., 1977) and screening possibilities for AAA diagnosis (Collin, 1985) have led to increase interest for epidemiological knowledge.

The prevalence of AAA seems to vary in the different geographical regions. Fowkes compared information from the United States (Lilienfeld et al., 1987) with data from England and Wales (Fowkes et al., 1989), and found six times higher mortality in Great Britain. Population-based studies from Sweden and Great Britain for ruptured AAA have shown incidence rates from 6 to14 per 100,000 inhabitants (Ingoldby et al., 1986, Johanson & Swedenborg, 1986, Thomas & Stewart, 1988). These differences could result from geographical and racial characteristicsbut there are also possible methodological explanations.

Until 1995 the majority of published ultrasound screening studies has been carried out in Great Britain and in Scandinavian countries (Bengtsson et al., 1996, Chichester Aneurysm Screening Group et al., 2001, Chosky et al., 1999, Collin et al., 1988, Lindholt, 2001, Scott et al., 1988). Therefore we decided to perform the first Belgian population-based study in Liège[1].

MATERIAL AND MEDHODS

Between December 14, 1995 and November 24, 1996 a personalized letter offering the opportunity to undergo free ultrasound screening for AAA was sent to 1764 men born in 1920 and 1930. Selection was based on birth records for the city of Liege. Ultrasound examinations were performed by a radiologist and a surgeon from the Medical Imaging and Cardiovascular Departments of the University of Liège (for more details, see Material and Methods in Appendix 1). The incidence of AAA and the cost of the diagnosis (calculated by dividing the overall cost by the number of AAA discovered) were determined.

RESULTS

As comparison of number of participants with previous AAA screening studies performed in Sweden and Great Britain, compliance in our study was lower (only 41%).

Ultrasound examination was feasible in 98.5% of cases. Anteroposterior diameter was > 29 mm in 33 subjects (4.5 %). The prevalence of AAA defined as largest aortic diameter ≥ 30 mm was 3.8% (Table IX). On the basis of epidemiological data collected, a high-risk population for AAA was identified. The incidence of hypertension and smoking habits were significantly higher in AAA group as compared with non-AAA group (Table X).

Reference	Compliance (%)	Number of subjects	Age (years)	Incidence of AAA (%) *
Smith FCT et al.	76,3	2664	65-75	8,4
Collin J.	51.7	497	65-74	42
Bebgtsson H et al.	75/0	364	74	8.5
This study	41.0	727	65 and 75	4.53

* Defined as maximum transverse diameter >29 mm

Table IX : Data from previous screening studies for AAA

	Without AAA		With AAA		
	n	%	n	%	p
Dyslipidemia	172	24.92	13	35.13	NS
Diabetes	78	11.30	7	18.91	NS
Hypertension	188	27.24	17	45.94	< 0.05
Smoking	156	21.73	13	35.13	< 0.06

NS, non-significant

Table X : Risk factors for cardiovascular disease in the study population (n = 727) without (n = 694) or with (n = 33) AAA

[1] *It was supported by a "Fonds de la Direction Générale de la Santé , Ministère de la Culture et des Affaires Sociales, Communauté Française de Belgique" – Allocation de base 12.20, Division organique 21*

The overall cost of screening was 18,175 €. The cost of diagnosis of each AAA was 551,00 €.

DISCUSSION

Ultrasound screening is considered the method of choice for AAA screening (Bengtsson et al., 1996, Akkersdijk et al., 1991). In addition to being feasible in 98.5% of cases, the sensitivity of ultrasound is over 95% and its specificity is 100%. Other advantages of ultrasound include non-invasiveness, good reproducibility, and cost-effectiveness (seven-fold less expensive than an abdominal CT scan).

The definition of AAA is of great importance in epidemiological studies since the prevalence of the disease will vary substantially simply because of variations in the criteria for the diagnosis. There is as yet no definite consensus on the definition of abdominal aortic aneurysm. However for practical reasons, McGregor's (McGregor et al., 1975) definition is still the most widely used in clinical practice, as well as in research.

In 1965, Steinberg (Steinberg et al., 1965) established normal standards for the aortic diameter by using angiography. Based on the findings of Steinberg, in 1975 McGregor (McGregor et al., 1975) proposed the definition of an AAA as the largest measured infrarenal diameter being ≥ 30 mm. Twelve years later, Sterpetti (Sterpetti et al., 1987) suggested that abdominal aorta is considered as aneurysmal when infrarenal aortic diameter exceeds suprarenal diameter of, at least, 50 %. A few months later, Collin (Collin et al., 1988), who designed one of the first large population screening program in Oxford, proposed that the infrarenal diameter should be ≥ 40 mm or exceed suprarenal diameter by at least 0.5 cm. In 1991, the SVS/ISCVS Ad Hoc Committee (Johnston et al., 1991) proposed the definition of infrarenal diameter being 1,5 times the expected normal diameter.

AAA occurs more commonly among men. Melton (Melton et al., 1984) in Rochester, Minnesota, showed that the incidence rate is at least two times higher in men and that the prevalence of AAA increases markedly with age in both sexes. The prevalence of AAA is between 2 and 3% of males over the age of 65 (Collin et al., 1988, Scott et al., 1988). In an

autopsy study performed by Bengtsson (Bengtsson et al., 1992), based on 45,838 autopsies between 1958 and 1986, the frequency of AAA was 4.3% among men and 2.1% among women. The age-specific frequency of AAA increased markedly after 55 years in men and 70 years in women. The male frequency reached a peak of 5.9% at 80 years and the female 4.5% at 90 years and above. Therefore, although recent English population-based studies show an increasing prevalence of AAA in females (Brown & Powell, 1999), all screening studies unsurprisingly show a higher prevalence of AAA in men than in women. One of the most consistent risks factors for AAA is smoking. According to Janet Powell (Powell, 2003) « the epidemic of aortic aneurysms follows some 40 years after an epidemic of smoking. Since 1950 there have been an increasing proportion of women who smoke, and this may alter future sex ratios for the prevalence of AAA ».

In our study, anteroposterior aortic diameter > 30 mm was observed in 28 male subjects (mean diameter 34.7 mm) and the incidence of AAA was 3.8%. Anteroposterior aortic diameter > 29 mm was observed in 33 cases (mean diameter 30.9 mm) and the incidence of AAA was 4.5%. This incidence of AAA is comparable with previous reports (Collin, 1985, Bengtsson et al., 1996, Smith et al., 1993, Akkersdijk et al., 1991).

In the present study, we targeted 65- and 75-year-old men for routine ultrasound screening. The highest incidence of AAA is in subject over 80 years, the optimal effect on mortality in function of age at diagnosis is observed between 70 and 75 years, and the lowest operative mortality is observed between the ages of 55 and 65 years. Thus, since the detection rate is lower and the cost of screening per case diagnosed is higher (Smith et al., 1993, Collin et al., 1990) in these cases, the best age group for screening in terms of cost effectiveness is that between 65 and 75 years (Bengtsson et al., 1996, Smith et al., 1993, Collin, 1990). In the city of Liège, there were 9344 men between the ages of 65 and 75 years. We choose to study 65-years old men (born in 1930) and 75-years-old men (born in 1920) to obtain an easily useable sample for statistical analysis.

The reliability of population-based studies depends on compliance. The percent of compliance in previous AAA screening studies in English-speaking and Scandinavian countries ranges from 51 to 81% (Bengtsson et al., 1996, Akkersdijk et al., 1991). In our

study, compliance is only 41.1%. It can be considered too low to allow comparison. The effectiveness of screening studies could be enhanced by specific targeting of high-risk groups. The incidence of AAA in subjects over the age of 65 with hypertension was 12% in the study of Allen et al. (Allen et al., 1987) and 9% in the study of Lederle et al. (Lederle et al., 1988). In our department, screening for AAA was carried out on 110 patients scheduled to undergo surgery for either coronary artery disease (n = 72) or peripheral vascular disease (n = 38). The incidence of AAA was 9.7% in patients with coronary artery disease and 2.6% in patients with peripheral vascular disease (Sakalihasan et al., 1992, Sakalihasan & Limet, 1994).

On the basis of epidemiological data obtained, we attempted to define a high-risk population for AAA. The incidence of hypertension was 45.9% in the AAA group as compared with 27.2% in the general population ($p < 0.05$) and the incidence of smoking was 35.1% in the AAA group as compared with 21.7% in the general population ($p < 0.06$). The higher incidence of CABG [18.1% in the AAA group versus 55% in the general population ($p < 0.01$)] could be the result of a bias as patients who have undergone previous surgery may be more aware of the risks of cardiovascular disease and thus more likely to take part in screening.

The cost of each screening examination for AAA was calculated on the basis the officially established rate for ultrasound study : $25.00 /procedure. The cost of diagnosis of each AAA was $551.00, calculated by dividing the overall cost by the number of AAA discovered.

The effects of screening of the incidence of ruptured AAA were recently investigated with a stepped wedge study design in the screening program ongoing in the Huntingdon district (Chosky et al., 2001). This study concluded that screening 65-80 year-old males for AAA could reduce the incidence rate of ruptured AAA by 49%.Similarly randomized clinical trial involving 65-75 year-old men in Chichester (Chichester Aneurysm Group et al., 2001) showed a 55% reduction in the incidence of ruptured AAA by screening as well as the reduction of Health Care burden. The Cost evaluation for elective and emergent AAA repair in our institution is represented in Table XI. For the 20 electively operated patients the hospital stay averaged 14 days (extremes 2 and 31) versus 30 days (extremes 1 and 65) for

ruptured AAA. Mortality reached 5% (1/20) for elective repair and 65% (13/20) for emergent repair (unpublished data). Evaluation of the costs and benefits of randomized clinical trials with respect to AAA presently ongoing in the UK, Australia, and Denmark, involving more than 100,000 65-79 year-old men. These studies are expected to answer the remaining question before systematic screening for AAA can be advocated.

	Ruptured AAA (R1) (n = 20) (7 survived)	Elective AAA repair (R2) (n = 20) (19 survived)	Ratio R1 / R2
Hospitalization	474,854*	138,990*	3.4
Pharmaceutics	197,699	36,871	5.4
Transfusion products	70,879	7,104	10.0
Medical fees	480,598	163,456	2,9
Overall	1,226,404	348,236	3.5
* costs are in Belgian Francs			

Table XI. Cost-effectiveness (charges for elective (n = 20) and emergent repair (n = 20) (1990-1996) divided by number of survivors) (UNPUBLISHED DATA)

CONCLUSION

Even if the compliance rate observed in Liège was low, we showed the feasibility of population-based study on AAA by ultrasound examination and observed similar prevalence with the other countries. The results of our study demonstrate that ultrasound is a cost-effective method for routine screening in men older than 65 years.

The analysis of the cost and effect of a screening program must be performed before any largepopulation screening is planned. However, the benefits of screening must outweigh

the costs. Finally we observed relationshep between AAA and hypertension and smoking as a risk factors.

CHAPTER 3

ABDOMINAL AORTIC ANEURYSMS REPAIR IN ELDERLY (APPENDIX 2)

INTRODUCTION

Currently, about 3.5% of the Belgians are 80 years or older. As a consequence, approximately 14,000 Belgian octogenarians could be expected to have an AAA of 30 mm. In the official Belgian mortality statistics of 1991, aortic aneurysm is recorded as the main cause of death in 0.55% of deaths among octogenarians. AAA also ranks fifteenth among all causes of death (Institut d'Hygiène et d'Epidémiologie 1991). In addition, a dramatic increase in elective surgery for AAA does not decrease the incidence of total mortality of ruptured AAA (rAAA) (Johansson & Swedenborg, 1994). Currently, the key guide to decide on elective AAA repair is the aneurysm diameter. However most AAA patients die from causes unrelated to aneurysmal disease (Bengtsson & Bergqvist, 1993, Jones et al., 1998). Thus, the decision on whether to operate or not abdominal aortic aneurysm in elderly should also depend on the relative risk of the operation versus the natural course of unoperated AAA (Limet et al., 1991, Sakalihasan & Limet, 2003|).

MATERIAL AND METHODS

The medical records of all octogenarians, referred to our department from January 1984 to December 1996, for infrarenal AAA of 40 mm or more in diameter, were retrospectively reviewed. With the computerized vascular registry of our department, we analysed demographic data on the diameter and aspect of the AAA, on comorbid conditions,

on relevant medical history, and on the management of the AAA (conservative versus repair). Follow-up data were collected from the out-patient clinic (control visit last 6 months) in only 10% of cases, from a questionnaire send to the family physician in 75% and from telephone contact with relatives in 15%. An estimate of quality of life after discharge from the hospital (mean delay 6 months) was made by ascertaining the degree of independence and mobility, using a score relating the ability to perform daily activities.

RESULTS

A total of 138 patients, aged 80 years and older, were referred to our department with diagnosed AAA. This represents 8.9% of all (n = 1552) AAA referred during the same period. The major patient's characteristics are presented in Table XII. At referral, 95 patients presented an asymptomatic AAA (mean diameter 64 ± 15 mm) (Group A), 15 were admitted with a painful AAA suggestive for impending rupture (mean diameter 70 ± 15 mm) (Group B), and 28 with ruptured AAA (mean diameter 78 ± 16 mm) (Group C).

Number	138 (21 F ; 117 M)
Mean age	82.9 yr (80-94)
Active smoking	35%
Hypertension	61%
Creatinine > 20 mg/l	26%
COPD	42%
FEVI < 1000 ml/s	14%
Previous myocardial infarction	45%
Congestive heart failure	11%
Previous myocardial revascularization	8%
Previous stroke	6%

Table XII. Patient characteristics

Rather than 30-day mortality, operative mortality is defined as in-hospital mortality (only patients discharged from hospital are considered as operative survivors). The results were compared to the results achieved in octogenerians between 1984 and 1989.

For 58 patients with asymptomatic AAA, surgery was denied at referral because of transverse diameter less than 50 mm (n = 21), patient refusal (n = 10) or unacceptable operative risk or poor general condition (n = 27). Thirty-four of these latter patients were ultimately operated within a mean delay of 41 months because of aneurysm enlargement (n = 15), aneurysm tenderness (n = 6) or rupture (n = 13). Globally, 52 patients had early (n = 37) or delayed (n = 15) elective repair of their AAA. Urgent operation was performed for 21 patients, including the 15 group B patients and the 6 group A patients developing aneurysm tenderness during surveillance. Emergent repair was performed for 41 ruptured AAA, including the 28 group C patients and 13 in group A patients. In this latter group, rupture concerned an AAA previously diagnosed and for which operation was initially decided. The operative outcome of the elective, urgent or emergent repair is summarized in Table XIII.

	Elective repair (n = 52)	Urgent repair (n = 21)	Emergent repair (n = 41)
Operative morbidity			
• Myocardial infarction	3 (6%)	3 (14%)	7 (17%)
• Respiratory failure	9 (17%)	9 (43%)	26 (63%)
(necessitating tracheotomy)	-	2 (9.5%)	4 (10%)
• Renal insufficiency	4 (8%)	4 (19%)	19 (46%)
(necessitating hemodialysis)	-	1 (5%)	8 (19.5%)
• Ischemic colitis	1 (2%)	1 (5%)	5 (12%)
(necessitating colectomy)	1 (2%)	-	2 (5%)
Operative mortality	3 (5.7%)	6 (28%)	28 (68%)
• Cardiac	1	2	5
• Multiorgan failure	2	3	10
• Respiratory	-	1	4
• Hypovolemic shock	-	-	9
5-year survival	47%	30%	20%

Table XIII. Operative outcome

Some complications influenced the mortality rate. Twenty of the 44 patients who developed respiratory failure died ($p < 0.01$). Only one of the three patients with transmural colic ischemia survived ($p < 0.05$). Nine patients necessitated reoperation for continuous bleeding. Four of these patients ultimately died ($p < 0.01$).

Compared to the period between 1984 and 1989 (Table XIV), a considerable improvement in results was observed for elective surgery (4.7% operative mortality versus 10%, $p < 0.05$). The high operative mortality for ruptured AAA was unchanged (66% and

70%). For comparison, the recent results of aneurysm repair in the younger age group (less than 80 years) are also listed in Table XIV.

	Mortality ≥ 80 years 1984-1989 (n = 36)		Mortality ≥ 80 years 1990-1996 (n = 78)		Mortality < 80 years 1994-1996 (n = 380)	
Elective repair	10%	(1/10)	4.7%	(2/42)	3.6%	(12/334)
Urgent repair	33%	(3/9)	25.0%	(3/12)	5.8%	(1/17)
Ruptured AA	7%	(12/17)	66.0%	(16/24)	44.0%	(18/41)

Table XIV. Operative mortality rate

DISCUSSION

Repair of AAA in selected octogenarians can be done with a reasonably low morbidity and mortality. At our department, elective repair in octogenarians has a mortality rate that is 1.3 times higher than that for non-octogenarians (4.7% versus 3.6% for the period 1990-1996). The same is true for urgent or emergent repair (Table XIV). Most contemporary reports on elective AAA repair in patients aged 80 years or older mention an operative mortality that is approximately two times higher than in younger patients (Dean et al., 1993, Paty et al., 1993) (Table VII). For emergent repair or ruptured AAA, the difference in results obtained in the two age groups is less evident. Outstandingly good results as obtained at the Cleveland Clinic (O Hara et al., 1995) (3.8% and 29% mortality for elective and emergent repair in octogenarians, versus 1.2% and 26% in the younger age group) are not at the reach of all centers. Despite most recent progress in perioperative care, hypovolemic shock is poorly tolerated by octogenarians, whatever their comorbid conditions. Critical hypotension involves multiorgan failure, a leading cause of postoperative death (Dean et al, 1993, Glock et al., 1990, O Hara et al., 1995, Johansen et al., 1991).

In 1992, the Joint Council of the Society for Vascular Surgery and the North American Chapter of the International Society of Cardiovascular Surgery established indications for operative repair of AAA (Hollier et al., 1992). They suggested a "wait and see" policy for high-risk patients with AAA less than 60 mm in diameter. Once the aneurysm enlarged beyond 60 mm, the risk of rupture becomes considerable. Repair of such huge AAA should be considered since the assumed benefit in terms of rupture prevention outweighs the potential operative risk. A "wait and see" policy entails some inherent risks. There is an unpredictable but likely exponential growth of the AAA over time (Nevitt et al., 1989, Brown et al., 1996, Limet et al., 1991) while patient's general condition will deteriorate by the ageing process. An intact non-operated AAA, 50 to 60 mm in diametry, exposes the patient to a significant aneurysm related mortality (Szilagyi et al., 1972, Brown et al., 1996). In our series, 24% (14/58) of the 58 medically followed AAA (mean diameter 60 ± 20 mm, 37 measuring 50 mm or more) evolued to a fatal rupture over a mean observation period of 39 months.

Controversy about management of ruptured AAA in elderly still exists (Jones et al., 1998). A decade ago, an official recommendation in England (Confidential Enquiry into Perioperative Deaths – CEPOD (Buck et al., 1987)) suggested to withhold surgery for AAA rupture in patients of 80 years old or older. In our institution, we are proponents of surgery for all ruptured AAA reaching the hospital alive, since it is the only reasonable chance of survival that can be offered to patient. In case of preoperative cardiac arrest, the chances of survival are reduced, but not inexistent. At Mayo Clinic, 20% of these patients survived to operation (Glovicszki et al., 1992).

An ethical problem and dilemma rises up when AAA rupture occurs in a patient who had already been rejected for elective repair on medical grounds or because of patient's refusal (Piotrowski et al., 1995). In such an emergency situation, there is no time enough for risk evaluation or prolonged discussion with the patient or his family. The surgeon should take his full responsibility: no resuscitation at all or an aggressive approach.

Long-term follow-up after elective AAA repair in elderly gives a 5-year survival of approximately 50%, what is lower than that of an age and sex matched general population (mean 5-year survival 63%) (O'Hara, 1995). This illustrates the fact that patients with AAA

commonly present associated cardiac disease and are thus exposed to a higher heart-related mortality (Pleumakers et al., 1995, O Hara et al., 1995, Johnston, 1994). After successful elective repair, other authors report a survival rate similar to that of the general population (Dean et al., 1993, Glock et al., 1990, O'Donnell et al., 1976). Most survivors retain a gratifying quality of life (Dean et al., 1993, O'Donnell et al., 1976, Currie et al., 1992, Magee et al., 1992).

CONCLUSIONS

We can conclude that AAA repair can be performed safely in carefully selected octogenarians, even if the results are not so excellent as in younger age group. On the basis of our data, we recommend a straight forward surgery for "otherwise healthy" octogenarians with an AAA 55 mm or more in diameter, with a rapidly expanding AAA (growth rate exceeding 0.5 cm /6 months) or with a symptomatic AAA. Although this technique was not used in the present cohort of patients, individuals with 18-FDG uptake by the aneurysm wall are also candidates. For unfit individuals with life expectancy less than two years and for mentally or physically severely disabled patients, no surgery should be performed. (see chapter 7)

CHAPTER 4

INVOLVEMENT OF MATRIXMETALLOPROTEINASES (MMPS) IN THE DEVELOPMENT OF AAA (APPENDIX 3)

INTRODUCTION

The role of inflammation in the pathogenesis of AA has only recently been investigated (Brophy, Reilly et al., 1991, Halpern et al., 1994, Koch et al., 1990). Over the past decade, it became clear that several distinct but interrelated processes contribute to the pathologic alterations observed in human AAA tissue. Some of the most important of these processes include chronic inflammation, increased production of matrix degrading proteinases and their inhibitors, and localized degradation of structurally important connective tissue proteins, especially elastin and collagen (Campa et al., 1987, Menashi et al., 1987, Rizzo et al., 1989, Sakalihasan et al., 1993).

Macrophages are known to synthesize and release over hundred identified substances with molecular weight ranging from 32 D to 440,000 D, including collagenase Types I, II, III, and IV, elastase and plasminogen activators, plasminogen activator inhibitors, collagenase inhibitors, and α_1-antitrypsin inhibitors. However, other proteases are also secreted by smooth muscle cells and fibroblasts in the wall of the aorta (Thompson & Parks, 1996).

Since the discovery of its first member by Gross & Lapière in 1962 (Gross & Lapierre, 1962), the family of matrix metalloproteinases (MMPs) (collagenase, stromelysin) (Woessner, 1991) has been shown to be involved in a number of physiological remodeling processes and in many diseases associated with excessive tissue degradation such as arthritis, tumor invasion, …. The MMPs share high level of gene homology and common features as Zn-

dependent catalytic site, requirement of Ca^{++} for activity (as shown by inhibition by EDTA). They are secreted as an inactive proenzyme requiring latent activation by proteolytic enzymes, organomercurials agents. This activation is associated to the loss of a 10 kDa propeptide. The metalloproteinases are inactivated by specific tissue inhibitors of metalloproteinases (TIMPs) (Woessner, 1991).

Recent experiments with knockout mice for Apolipoprotein E (ApoE), tissue plasminogen activator (t-PA), or urokinase-type plasminogen activator (u-PA, strongly suggest a role for plasminogen in activation of MMPs (Carmeliet et al., 1997, Longo et al., 2002). In fact, ApoE deficient mice develop microaneurysms, but their development is slowed or even abolished when the mice are also deficient for u-PA. The u-PA required for activation of plasminogen to plasmin, which in turn cleaves and activates the MMP zymogene. The pertinent MMPs are MMP-3, MMP-9, MMP-12 and MMP-13. Both u-PA and the pro-MMPs are secreted by macrophages, which colocalize to the regions of damage to the elastic lamina in the media of the microaneurysms (Carmeliet et al., 1997).

The pathogenicity of activated MMPs (MMP-9 and MMP-2) present in the human is supported by observations in animal models (Anidjar et al., 1990, Allaire et al., 1998, Silence et al., 2001, Longo et al., 2002). These metalloproteinases play an important role in the collagen turnover in the aortic wall. A considerable reduction of elastin concentration without modification of the collagen density was observed in the wall of AAAs during the early development of the lesion (Brophy et al., 1991, Campa et al., 1987, Dubick et al., 1988, Halpern et al., 1994, Koch et al., 1990, Sumner et al., 1970, Sakalihasan et al., 1993). These alterations might result from an increased proteolysis and/or decreased antiproteolytic activities (Brophy et al., 1991, Campa et al., 1987, Vine et al., 1991).

These observations led us to investigate the involvement of the metalloproteinases in the development of AAA (Appendix 3). For this purpose, we identified and quantitated MMP-2 and MMP-9, together with their degree of activation in the wall of AAAs as compared with normal aortic walls. As significant amounts of the two enzymes and their inhibitors (TIMPs) are present in the circulating blood (Vartio & Baumann, 1989, Moutsiakis et al., 1992), they

were evaluated on a comparative unit wet-weight basis in the serum and in the wall-adherent thrombus at a luminal and parietal location.

MATERIAL AND METHODS

In the first step, the involvement of MMP-2 and MMP-9 in AAA was investigated by quantifying their level of expression and extent of activation in the circulating blood, the thrombus at a luminal and parietal location and in the vessel wall of ten patients undergoing elective surgical repair compared to age-matched normal autopsy aortic samples (n = 6) and control serum samples (n = 6). Aliquots of powdered thrombus and aortic wall fragments were extracted overnight at 4°C in buffered 2M urea and the gelatinase activity was measured in the extracts and the serums by soluble assay after acetyl-phenyl mercuric acid (APMA) activation and by zymography (for more details, see Appendix 3).

The two methods of determination of the gelatinolytic activities recorded somewhat different information. In the soluble assay, the activation of the latent forms of MMPs was performed by an organomercurial reagent, which was not able to dissociate the enzymes from their complex with the TIMPs. So, this technique measured the total free gelatinase activity (72 kDa and 92 kDa). By zymography, the MMPs were separated from their inhibitors by electrophoresis and activated by sodium dodecylsulfate (SDS). This technique discriminated the 72 kDa and the 92 kDa as well as the processed activated forms as to define a distribution pattern of each form. This distribution was used to calculate the gelatinolytic activity, measured by the soluble assay, attributable to each form. The comparison between the values measured by the soluble assay (total free) and zymography (total free + complexed) provided a crude and indirect estimation of the TIMPs.

Histologic analysis

The specimens of aortic tissue and thrombus, luminal and parietal, were fixed in 3.5% saline-buffered formaldehyde and processed with standard techniques for paraffin embedding. Hematoxylin and eosin-stained sections were used to evaluate the density, localization, and

nature of the inflammatory cells. The intensity of the inflammatory reaction was scored as mild (+), moderate (++), or severe (+++).

RESULTS

The total activity was two fold higher in AAA serum than in normal serum. When compared on a unit volume basis (1 µl of serum = 1 mg wet weight tissue) to the seric gelatinolytic activity, the amount of total gelatinase was 20 and 10 fold higher in the luminal and parietal thrombus, respectively (Table XV). While the gelatinase activities in the serum of both normal and AAA were equally distributed between the MMP-2 and the MMP-9, it was mainly represented by the MMP-9 in the luminal (80%) and the parietal (70%) thrombus, suggesting a specific accumulation of this enzyme in the thrombus. The AAA wall contained an amount of gelatinase activity similar to the parietal thrombus, only slightly more elevated than the normal aortic wall. However, the preponderant form in the control wall was the MMP-2 (70%), probably arising from the medial smooth muscle cells and the adventitial fibroblasts. The AAA wall contained a significantly larger proportion of the monocytes-derived MMP, however no processed active form was observed in the luminal thrombus, while 10% of the MMP-9 and 3% of the MMP-2 were activated in the parietal thrombus. In the AAA wall, more than 20% of the MMP-9 were processed, which was never observed in the normal aortic wall and a significantly higher percentage of the MMP-2, as compared to normal aortas, was also processed. The gradient of concentration of the MMP-9 suggests that the MMP-9 could arise from the serum and diffuse towards the aortic wall, while the MMP-2 would originate from the blood vessel wall. The gradient of processing observed for both enzymes suggests that activation mostly occurs in the vessel wall.

	Serum		Tissue samples			
			Thrombus		Wall	
	Control (n = 6)	AAA (n = 10)	Luminal (n = 10)	Parietal (n = 10)	AAA (n = 10)	Control (n = 6)
Total activity						
Soluble assay*	3.0 ± 0.9	2.8 ± 0.8	41.9 ± 51.3	13.1 ± 15.8	11.1 ± 7.8	5.5 ± 4.8
Zymography†	2.7 ± 0.5	5.6 ± 2.5‡	125.4 ± 93.0	68.4 ± 56.4	71.4 ± 39.4	53.6 ± 44.6
Distribution (in %)						
MMP-2	55 ± 13	56 ± 11	19 ± 16	33 ± 17	49 ± 16	71 ± 8
MMP-9	45 ± 13	44 ± 11	81 ± 16	67 ± 17	51 ± 16‡	29 ± 8

Results are expressed in units per µl of serum or per mg of wet weight of tissue, * one unit in soluble assay corresponding to enzyme activity degrading 1 µg of gelatin in 16 hours at 37°C and † one unit in zymography assay corresponding to bleaching of 1 arbitrary volume of gelatin containing acrylamide gel.

‡Significantly different from control values, $p < 0.02$.

Table XV. Amount of gelatinolytic measured by soluble assay and by zymography and distribution between MMP-2 and MMP-9

Histological examination

Microscopic examination of the AAA wall showed medial and intimal fibrosis often associated with atherosclerosis, focal calcifications, perivascular sclerosis and thickening of the vasa vasorum. The thrombi consisted of a fibrinous material diffusely or locally infiltrated by degenerated red cells and rare leukocytes. No obvious morphologic difference was found between the luminal and the parietal thrombus. Figure 2 is a representative example of our series of specimens; the density of the inflammatory infiltrate is low in the luminal (Figure 3, A) and parietal (Figure 3, B) sides of the adherent thrombus and the inflammatory cells are preferentially localized in the adventitia and the media of the aortic wall (Figure 3,C). These cells consisted predominantly in mononuclear cells (lymphoplasmacytic cells and macrophages) beside some polymorphonuclear neutrophils. A linear regression relationship was attempted between the extent of the inflammatory cells infiltrate in the aortic wall and the

level of expression and activation of the two gelatinases (Table XVI). The only significant positive correlation (r = 0.46) was between the level of activated MMP-2 and the density the infiltrate.

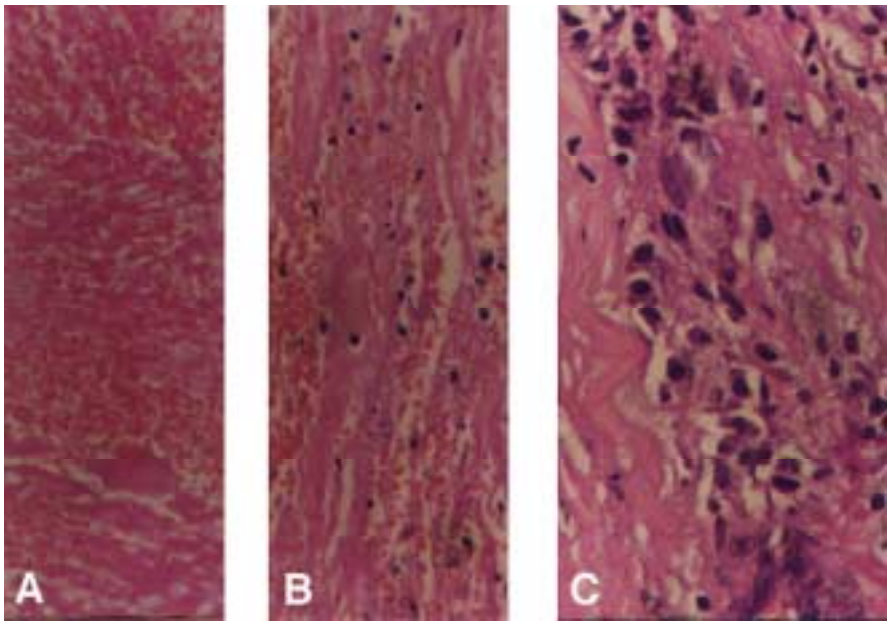


Figure3. Hematoxylin-eosin stained sections of luminal (A) and parietal (B) side of adherent thrombus, which consisted essentially of fibrinous material infiltrated by degenerated red cells and rare leukocytes. The aortic wall (C) shows medial and intimal fibrosis associated with mononuclear cell infiltrate predominating in adventitia and media.

Infiltrate	No.	MMP-9 (units/mg)		MMP-2 (units /mg)	
		Latent	Activated	Latent	Activated
+*	2	0.90 (0.78 to 1.17)	0.23 (0.18 to 0.27)	0.99 (0.24 to 1.75)	0.33 (0.17 to 0.50)
++	5	1.47 (0.30 to 2.95)	0.48 (0.03 to 1.47)	1.05 (0.64 to 2.50)	0.51 (0.22 to 0.99)
+++	2	2.86 (0.63 to 5.09)	0.25 (0.25 to 0.26)	1.62 (0.63 to 2.61)	0.83 (0.42 to 1.25)
r		NS	NS	NS	0.46

*Intensity of infiltrate was scored as mild (+), moderate (++) or severe (+++).

r, Correlation coefficient; NS, not significant.

Table XVI. Relationship between inflammatory cells infiltrate in aortic wall and latent and activated forms of MMP-2 and MMP-9

DISCUSSION

The MMP-2 was found as the predominating form in the aortic wall of the control group, whereas a significant shift toward MMP-9 was observed in the AAA specimen. MMP-2 is synthesized in the aortic wall (McMillan & Pearce, 1997). Smooth muscle cells of the intima and the media seem a significant source, as are also the adventitial fibroblasts (Herron et al., 1991). It does not differ in absolute value in the control and AAA groups. MMP-9 is synthesized and released by tissue macrophages. The higher proportion of MMP-9 in the AAA is probably related to the presence of inflammatory cells known to produce this MMP. MMP-9 was recently identified by immunohistochemical analysis (Newman et al., 1994) and in situ hybridization (Thompson et al., 1995) in macrophages infiltrating the aneurysmal aorta. The MMP-9 positive cells represented, however, a subset of only 10% to 20% of the inflammatory cells (Thompson et al., 1995). This finding might explain why we are unable to find a significant correlation between the level of expression of the MMP-9 in AAA and the score of infiltrating cells. Other types of inflammatory cells might also produce the MMP-9.

For example, neutrophils are able to secrete the monomeric 92 kDa form and a heterodimer of 125 kDa where the MMP-9 is disulfide-linked to α_2-microglobulin (Triebel et al., 1992). So, the gelatinolytic band migrating above the 92 kDa in our AAA wall samples might originate from polymorphonuclear leukocytes observed in the adventitia and the media of the AAA wall. In addition, recent reports (Newman et al., 1994 [(1)], Newman et al., 1994[(2)]) identified in AAA, besides the MMP-9, the stromelysin 1 (MMP-3), interstitial collagenase (MMP-1) in high-molecular weight forms and the activated forms of MMP-9 and MMP-3. Our results also suggest that in serums of patients bearing AAA, gelatinases are partly inhibited because of complexing with tissue inhibitor metalloproteinases (TIMP). It is however noteworthy that 5 out of 10 studied AAA patients had a detectable amount of the activated processed MMP-9, which was never detected in the control group (Appendix 5). Examining the clinical parameters of these five patients indicated that they differed by the size of the aneurysmal lesions. The mean diameter was 75.6 ± 6.2 mm in the group with detectable levels of MMP-9 versus 58.0 ± 5.7 mm in the group who showed to processed MMP-9. Our observation, that the biologic activity observed in the parietal thrombus plays important role during the evolution of the abdominal aortic aneurysm, is strongly supported by recent studies(Fontaine et al., 2002, Kazi et al., 2003). To our knowledge, our report is the first to demonstrate the presence of the activated forms of MMP-2 in significantly higher proportion in the AAA than in normal aortic wall. In our study, the increasing gradient of processing of both MMP-2 and MMP-9 observed (Figure 4) from the luminal thrombus toward the AAA wall suggests that the activation process might onset in the aortic wall.

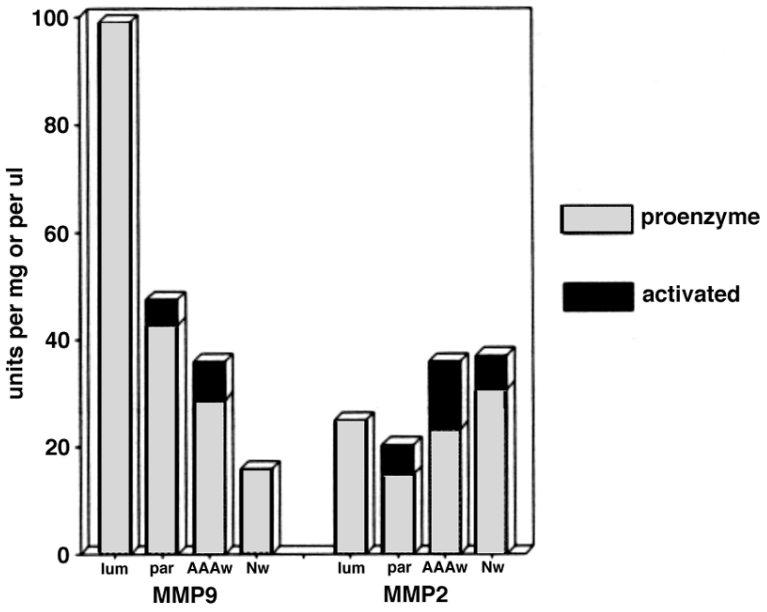


Figure 4. Illustration of gradient of decreasing level of MMP-9 activity and increasing activation of both MMP-9 and MMP-2 from luminal thrombus toward aortic aneurismal wall. * Significantly different from control values.

CONCLUSION

The main conclusions that can be drawn from our investigations are: (1) aneurysmal aortic walls contain a significantly higher amount of MMP-9 than control specimens, and (2) the activated processed forms of both MP-2 and MMP-9 are significantly increased. In fact, compared to the normal aortic wall, AAA wall contains significantly greater amount of MMP-9 (with a significant proportion of activated form) and activated MMP-2. The possibility exists that these enzymes contribute to the degradation of extracellular matrix proteins observed in AAA.

CHAPTER 5

THE ROLE OF THE MATRIX METALLOPROTEINASES (MMPS) AND THEIR INHIBITORS (TIMPS) ON THE REMODELLING OF THE ABDOMINAL AORTA IN AOTIC ATHEROSCLEROTIC AND ANEURYSMAL LESIONS. (APPENDIX 4)

INTRODUCTION

In vivo studies and experimental animal models have demonstrated that AAAs are associated with a marked angiogenic response and conversely to a moderate reaction occasionally seen in aortic occlusive disease (Allaire et al., 1998, Armstrong et al., 2002, Baxter et al., 1992, Carmeliet et al., 1997, Huffman et al., 2000, Knox et al., 1997, Longo et al.,2002, Mesh et al., 1992, Patel et al., 1996). Adventitial neovascularisation always accompanies chronic inflammation and has been seen in a number of arterial diseases, including AAA (Barger et al., 1984, Herron et al., 1991, Pearce et al., 1991, Tilson, 1992). The vascular endothelium plays an important role in the recruitment of blood cells and their migration into the media of the vessels. Vascular adhesion molecules are expressed in the endothelial cells of the vasa vasorum and are evidenced in the vessels within the plaques. In this perspective, the significantly increased expression of intercellular adhesion molecule (ICAM-1) was observed in AAA and aortic occlusive disease, primarily in the area of vasa vasorum. This enhanced expression could promote the recruitment and the entry of inflammatory cells in aortic wall through the vasa vasorum. Macrophages and lymphocytes are indeed often detected around the neo vasa vasorum. However, atherosclerosis is primarily found within the intima and media, whereas aneurysm disease typically affects the media and adventitia (Figure 5). AAA is also characterized by a medial and adventitial infiltration by inflammatory cells, while this infiltration occurs mainly in the intima of aortic occlusive disease (AOD) (Koch et al., 1990, Wolf et al., 1971). Even if previously we observed the presence of only MMP-2 and MMP-9 in the aneurysmal wall (for more details, see Appendix

4), recent publications showed that the majority of MMPs participate to degradation of all the components of the extracellular matrix, as discussed above (Wassef et al., 2001).

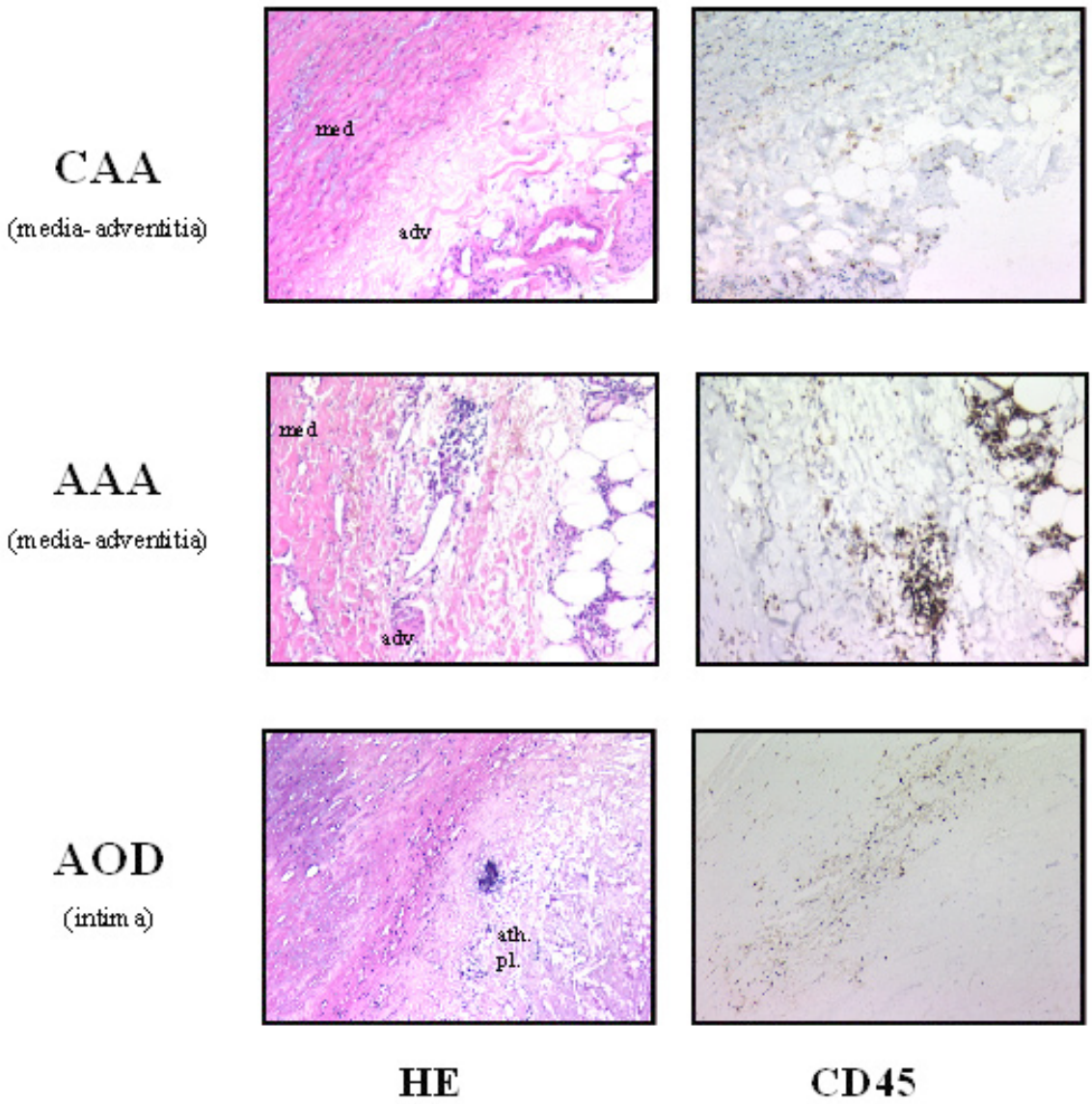


Figure 5. Histopathology and immunochemistry of normal (CAA) and diseased aortic tissues (AAA and AOD). Formalin-fixed serial sections of normal, aneurysmal and atherosclerotic abdominal aorta were stained with hematoxylin-eosin (HE) to determine the pattern (diffuse or local) of the degree of inflammation. The nature of inflammatory cells was then assessed by immunohistochemistry using standard protocols and antibodies (DAKO, Carpinteria, CA USA) against common leukocyte antigen (CD45), macrophages (CD68), lymphocytes T and B (CD3 and CD20). Only small number of mononuclear cells were diffusely observed in the adventitia of normal aorta (CAA). In AAA, there was an increased density of inflammatory cells (mainly macrophages) predominating in the adventita. In AOD, histiocytes were detected mainly around atheromatous plaques (ath. pl.) in the intima and, a to a lesser degree, in the media. In the adventitia, the weak cell infiltrate tended to be patchy with aggregates of plasma cells and lymphocytes.

Our goal is to elucidate the role of the different MMPs, of their activator (uPA) and their inhibitors (TIMP-1, TIMP-2, and PAI-1), and the interaction between the two proteinase systems in the development of atherosclerotic lesions and aneurysmal disease.Therefore we wanted to determine a large spectrum profile of MMPs, of uPAs and their inhibitors (TIMPs and PAI-1) in the wall of the aneurysmal aorta (AAA), atherosclerotic occlusive diseased aorta (AOD), normal aorta and thoracic aorta as a control.

MATERIAL AND METHODS

Patients characteristics

Full-thickness infrarenal aortic tissue was obtained from seven patients (mean age: 67 y, range 55-82y), during elective surgery for degenerative AAA. All patients were asymptomatic at the time of surgery. The mean size of the aneurysms was 58 mm (range 39-80 mm). Atherosclerotic occlusive aortas were harvested during surgery for infrarenal occlusive disease in five patients (mean age: 63y, range 50-70y). Thoracic aorta presenting weak or clinically undetectable atherosclerosis was obtained during coronary bypass surgery in seven patients (mean age: 65y, range 54-74y). Clinically normal abdominal aortas were collected from seven patients died from non-vascular disease (mean age: 60y, range 51-74 years).

RNA isolation and quantitative RT-PCR procedure

After isolation, (Chirwing et al., 1979) RNA concentration was measured by a fluorimetric assay (SpectraMax, Gemini-XS). Pairs of RT-PCR primers were selected according with the following criteria: (i) a high and similar annealing temperature; (ii) minimum complementarity between primer sequences and (iii) localization on different exons. For each investigated mRNA, a synthetic RNA (sRNA) was generated, according to previously published works, (Lambert et al., 2001, Lambert et al., 2001, Nusgens et al., 2001) in order to monitor in each tube the efficiency of both the reverse transcription and the amplification reactions. The sRNAs give rise to products of a size slightly different from that

of the endogenous mRNA. RT-PCR was performed under non-competitive conditions in an automated system (GeneAmp PCR System 9600, Perkin Elmer) using the GeneAmp Thermostable rTth Reverse Transcriptase RNA PCR kit (Perkin Elmer), specific pairs of primers (5 pmoles each), 5 ng of total cellular RNA and a known copy number of sRNA per 25 µl reaction mixture. The RT step (70°C for 15 min) using the antisense primer in presence of Mn^{2+} was followed by addition of the sense primer in presence of Mg^{2+} (i) 2-min incubation at 95°C, (ii) PCR amplification for the adequate number of cycles and (iii) a final elongation step of 2min at 72°C. The PCR conditions for the amplification of most cDNA were: 94°C for 15s; 66 °C for 20s; and 72°C for 10s. For MMP-3, -11 and -13, conditions were: 94°C for 15s, 63 °C for 30s and 72°C for 30s. The RT-PCR products were resolved on 10% polyacrylamide gels and quantified (Fluor-S-MultiImager, BioRad) after staining (GelStar dye, FMC BioProducts). Each sample was analysed in duplicate. The optical density of the endogenous RNA was normalized by the value of the sRNA and expressed in arbitrary units per unit of 28S ribosomal RNA (Figure 6).

The mRNA steady-state level of a large spectrum of matrix metalloproteinases (MMP-1, -2, -3, -8, -9, -11, -12, -13, -14 ; urokinase plasminogen activator: uPA), their physiologic inhibitors (tissue inhibitors MMPs: TIMP-1, -2, -3 ; plasminogen activator inhibitor: PAI-1) and that of structural matrix proteins (collagen type I and III, decorin, elastin, fibrillin 1 and 2) was determined by quantitative RT-PCR using a synthetic RNA as an internal standard in each reaction mixture (for more details, see Appendix 4). The mRNA levels of the selected genes were determined using total RNA purified from all aortic samples in the same run of (RT-PCR) reverse transcriptase-polymerase chain reaction.

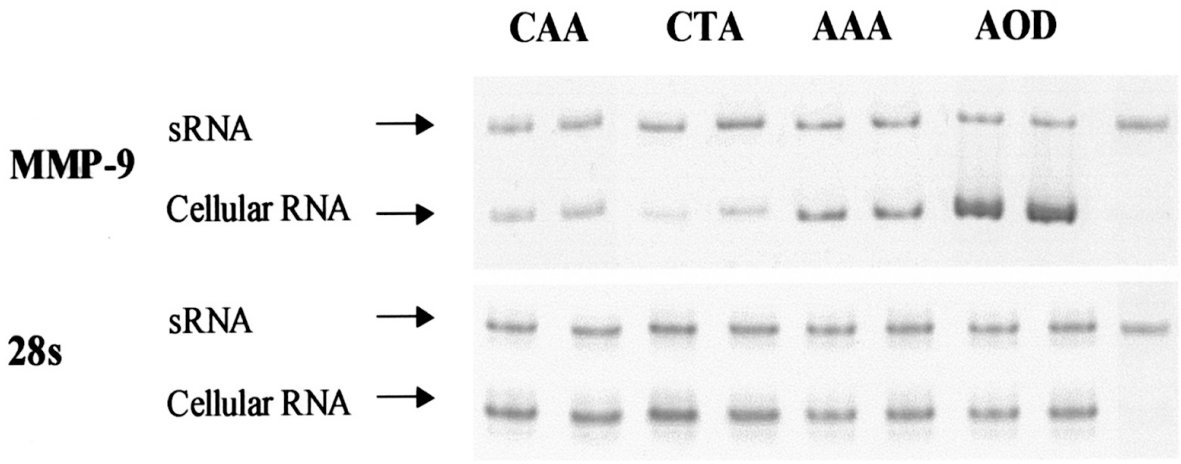


Figure 6. Representative illustration of an electrophoretic pattern of RT-PCR products amplified from MMP-9 mRNA (32 cycles), 28S ribosomal RNA (28S) (18 cycles) and their respective synthetic RNA (sRNA). The assay was performed in duplicate using RNA from normal abdominal (CAA), thoracic (CTA) or aneurysmal (AAA) and occlusive (AOD) aorta. The sample migrated in the last right lane contained all the reagents including the sRNA but no cellular RNA.

Zymographic analysis of the gelatinases MMP-2 and MMP-9

The analysis of tissue homogenates of CAA, AAA and AOD was performed following the procedure described previously (Sakalihasan et al., 1996).

Statistics

Statistical differences among groups were tested by 1-way ANOVA after normalization using neperian Log. A probability value ≤0.05 was considered as significant.

RESULTS

The MMP-3 as well as MMP-8, -9, -11, 12, 13, and U-PA usually associated with inflammatory cells were not or rarely detected in control's aortic samples. In contrast, they were largely and similarly expressed in AAA and AOD samples. However mRNAs for structural proteins and for TIMPs were more expressed than most of the MMPs (Figure 7). These observations can be considered as representative of the phenotype of the resident cells in non-diseased adult aortic wall. The mean mRNA level of all the MMPs and that of the inhibitors were always higher in AAA and in AOD than control aortas, except TIMP-2 and PAI-1 in AAA (Figure 7). Detailled results of RT-PCR assay, comparative analysis of the mRNA steady-state levels in normal thoracic aorta, comparative mRNA expression in control, aneurysmal and occlusive abdominal aorta as well as gelatinolytic activity are presented in appendix 5. In our previous study (Sakalihasan et al., 1996), we observed activated forms of MMP-9 in AAA wall while neither the pro MMP-9 nor the activated MMP-9 are detected by zymograph in AOD (Figure 8).

DISCUSSION

Since U-PA and MMP-8, -9, -12, and -13 mRNA are largely increased in AAA and AOD whereas their inhibitors (PAI-1 and TIMP-2) are lower in AAA compared to AOD, it could be explained by the alteration of the functional balance of proteolysis/antiproteolysis in AAA9).

In contrast with Carrell's observation (Carrell et al., 2002), stromelysin-1 (MMP-3) was frequently undetected except in two AAA samples. So, our results do not support the Carrell’s conclusion that MMP-3 plays a specific role during development of AAA. MMP-9 and MMP-12 were largely overexpressed in AAA and AODThe high expression of MMP-9 in AOD as observed in this study and its specific role with elastolytic activity needs further investigation.

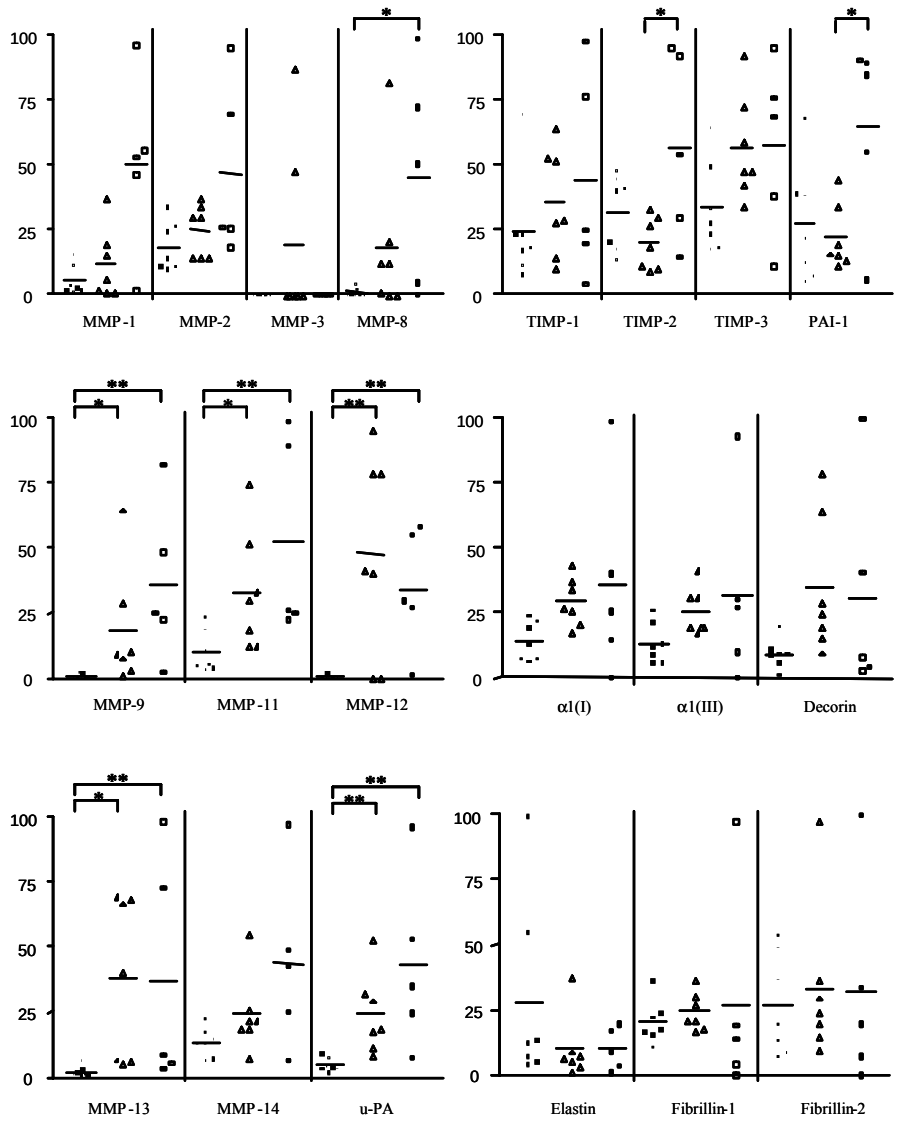


Figure 7. Expression of mRNA of MMPs, u-PA, TIMPs, PAI-1, type I and type III collagen, decorin, elastin, fibrillin 1 and 2 in samples of control abdominal aorta (CAA, solid squares), abdominal aortic aneurysms (AAA, open triangles) and aortic occlusive disease (AOD, open circles). The results are expressed in arbitrary units per unit of 28S rRNA allowing a comparative analysis between samples for each mRNA. ** $p \leq 0.01$, * $p \leq 0.05$.

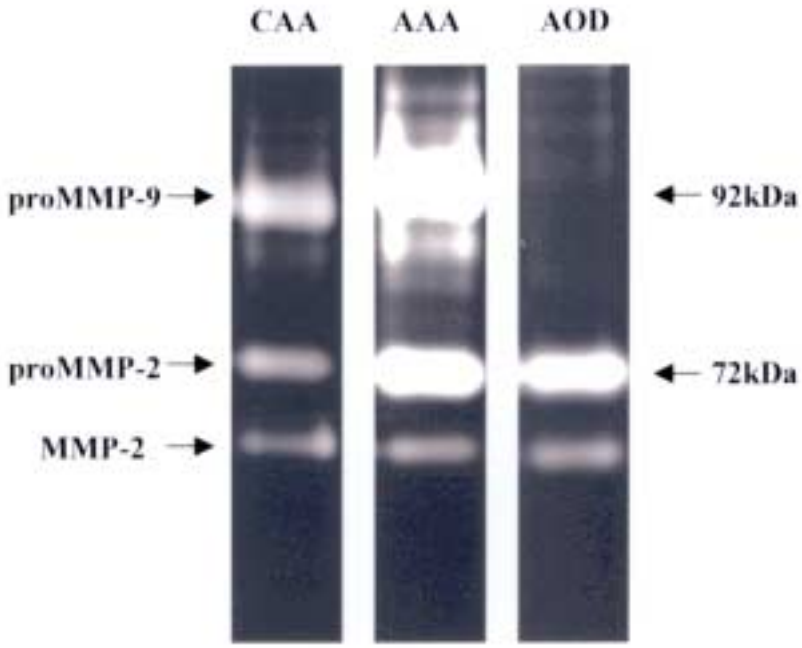


Figure 8. Representative example of MMP-2 and MMP-9 activity under latent or activated forms measured by gelatin zymography in extracts of normal (CAA) and diseased aortic wall (AAA and AOD).

MMP-8 was present in 8 out of 12 AAA and AOD patients, not detected in the study by Carrell (Carrell et al., 2002). This enzyme usually initiates type I collagen's degradation (Herman et al., 2001). MMP-13 was also overexpressed in AAA and AOD. Besides its collagenolytic activity, MMP-13 can degrade a broad spectrum of matrix protein and activate other MMPs (Knauper et al., 1997). In this study, we have also shown that a significant part of the MMP-2 was present in the AAA wall under an activated form. However, MMP-2 and its activator MMP-14 mRNA expression level were not significantly higher in AAA and AOD as compared with normal aorta.

CONCLUSIONS

Our study investigated the simultaneous implication of three biological processes, proteolysis, antiproteolysis, and repair, in the AAA and AOD. Our results show that, when compared with the basal physiologic phenotype of clinically normal age-matched aortas, both aneurysmal and atherosclerotic lesions are similarly characterized by a largely increased proteolytic repertoire, which is not compensated by a similar increased level of proteases inhibitors and structural proteins involved in the repair process. The profile of mRNA expression is quite comparable in AAA and AOD, except for TIMP-2 and PAI-1. This observation may suggest a significant role for proteinase inhibitors during the divergent evolution of AAA and AOD.

CHAPTER 6

HISTOLOGICAL AND BIOCHEMICAL HETEROGENEITY IN THE WALL OF RUPTURED AAA (APPENDIX 5)

INTRODUCTION

Increased amount of MMPs in the wall of AAA reflects an activation of inflammatory cells. An imbalance between MMPs and their inhibitors (TIMPs) may be implicated in the expansion or rupture of AAA (Baxter et al., 1992, Knauper et al., 1997, Knox et al., 1997, Thompson et al., 1995). Although several studies have analysed the wall of ruptured aneurysms, few data concerning the site of rupture are available (Hunter et al., 1996). Because of technical limitations during emergency repair of ruptured aneurysms, it is difficult to obtain circumferential samples of the aneurysmal wall to determine a profile of genes expression related to expansion and rupture. So, informations gathered from the samples collected during surgery for ruptured AAA do not reflect a local metabolic activity in the site of rupture. Therefore we studied the expression of the genes of MMPs, U-PA, their inhibitors (TIMPs and PAI-1) and major matrix proteins (elastin, collagen of type I and III) in the wall of a ruptured AAA of patient who died shortly before planned surgery in order to exactly localize the site of rupture.

CASE REPORT

A 76 year-old male was admitted in emergency for recent back pain with a history of a known AAA and hypertension. Ultrasonographs and CT-scan examination confirmed the presence of an AAA of 54 mm in its largest diameter, without any sign of rupture or leakage. Unfortunately the patient died within eight hours after admission from cardiac arrest

consecutive to hypodermic chock after rupture. At autopsy, a retroperitoneal hematoma was observed and the site of rupture was clearly identified on the right anterolateral face of the AAA.

MATERIAL AND MEDHODS

Six transmural tissue samples (0.5 cm^2) were collected from the edges of the site of rupture and every cm up to 50 mm away from this site. The samples were compared together and with 7 samples collected from the wall of non-ruptured aneurysms that we previously analysed (Appendix 4) Formalin-fixed serial sections were stained with hematoxylin-eosin and with orcein for elastin. The nature of inflammatory cells was assessed by immunohistochemistry using standard protocols and antibodies (DAKO, Carpinteria, CA) against lymphocytes (CD3, CD20 and CD45) and macrophages (CD68). Smooth muscle cells were stained using α-smooth muscle actin antibody (Sigma, St Louis, MI). RNA isolation and quantitative RT-PCR procedures performed according to the technique of Lambert (Lambert et al., 1992, 2001). All samples were analysed in duplicate in the same run. The signal of the endogenous mRNA was normalized by the value of the sRNA and expressed in arbitrary units per unit of 28S ribosomal RNA.

RESULTS

In the sample adjacent to the site of rupture, routine and immunohistological examination showed a diffuse inflammation composed of lymphocytes, macrophages and polymorphonuclear neutrophils (PMN), infiltrating predominantly the adventitia. Red blood cells were also observed, disrupting the elastic network of the residual media up to the deepest part of the adventitia (Figure 9). At distance from the rupture (10 to 50 mm) the inflammatory infiltrate was mainly composed of lymphocytes and macrophages, focally clustered in the deepest part of the adventitia. Immunohistological evaluation also revealed rarefaction of SMCs at the site of rupture while at distance from it, SMCs were present (the media was irregular in thickness up more than 30 mm away from the site of rupture (Figure 9).

As illustrated for MMP-2 in (figure 10, A), the expression of MMP-1, -2, -9, -12, -14 and uPA was high at the site of rupture, intermediate at +10 mm and lower in the more distant tissue

samples (Figure 10, A). The expression of MMP-3 and -8 was also high at the site of rupture while MMP-11 mRNA was undetectable. Expression was sporadic in the tissues sampled at distance from the rupture site (Fig. 10, B). Expression of both TIMP-1, -2, -3 and PAI-1 were also largely expressed at the site of rupture. TIMP-1 and PAI-1 decreased with increasing distance from the rupture while TIMP-2 and -3 displayed a weaker reduction (Figure 10, C). At the site of rupture, the expression of elastin was undetectable, although it was detected at different levels in the distant samples. The mRNA level for collagen type I and III was high at the site of rupture and decreased away from this site (Figure 10, D).

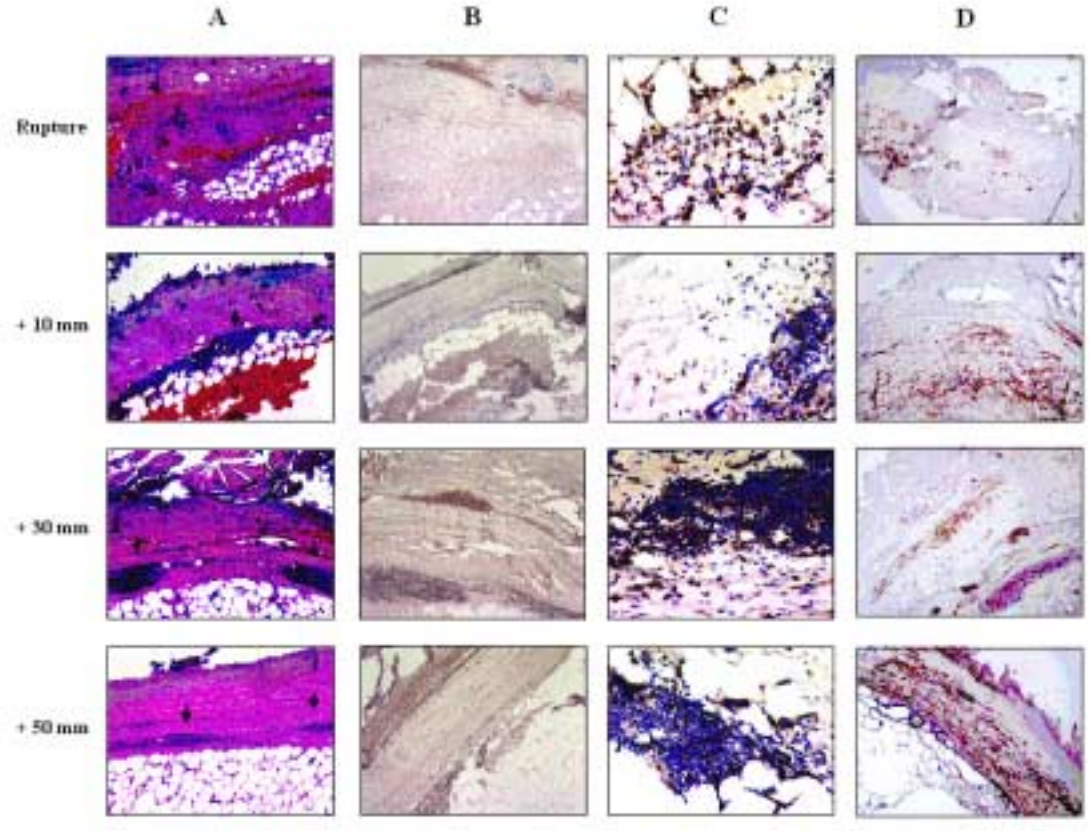


Figure 9. Histological feature of ruptured abdominal aortic aneurysm wall from the site of rupture up to 50 mm away. A. Low-power magnification shows diffuse inflammation and red blood cells in the tissue sampled near the site of rupture while the inflammation is more focally clustered (➤) at increasing distances from the rupture (hematoxylin-eosin, 100X). Intramural red blood cells (→) infiltrate the media and adventitia at the site of rupture. B. Elastic fibers staining (orcein, 100 x) shows disrupted media and adventitia at the site of rupture and a thin and irregular media up to +50 mm. C. Immunostaining (CD 68, 400 x) shows numerous macrophages diffusely infiltrating the tissue at the site of rupture and clustered with lymphocytes away from the rupture. D. Smooth muscle cells staining (a-smooth muscle actin, 100 x) shows severely disturbed distribution of smooth muscle cells at the site of rupture.

DISCUSSION

Although the size of an aneurysm, its growth rate (Limet et al., 1991) and possibly the area of endovascular thrombus may contribute to rupture, the events that immediately precede rupture are not well understood. In this study, we showed that there is an heterogeneity in the AAA wall in terms of inflammation pattern or in the expression of proteolytic enzymes involved in the aneurysmal disease. The presence of red blood cells infiltrating the media, the adventitia and the periadventitial adipous tissue indicates that an intramural haemorrhage has occurred before rupture, possibly at the time of the back pain. A diffuse infiltrate containing macrophages, lymphocytes and PMN was observed at the site of rupture up to +10 mm outwards. In the samples collected away from the rupture, focal clusters of a mixed population of lymphocytes and macrophages, but no PMNs, were observed in the deepest part of the adventitia. The presence of PMN at the location of blebs on the aortic wall (Hunter et al., 1996) and at the sites of rupture is suggestive of a local inflammatory process preceding rupture. Interestingly, the highest level of MMP-8 mRNA, the neutrophil collagenase, was also found at the site of rupture. This enzyme was recently identified in association with cleaved type I collagen in unstable atherosclerotic plaques and its expression by endothelial cells and SMCs was also described (Herman et al., 2001). The MMP-1, -2, -9, -12, -14, uPA and the inhibitors TIMPs and PAI-1 present the highest expression in the rupture area and at +10 mm (Fig. 10). This might reflect an activation of the inflammatory and resident cells by the network of cytokines and chemokines. In contrast, at further distance from the site of rupture (+20 to +50 mm), the expressions of these genes were similar to the levels observed in the wall of non-ruptured AAA (Appendix 4). The sporadic expression of MMP-3, -8 and -11 suggest an heterogeneity in the metabolic activity of infiltrating cells. Since the highest expression was observed where the haemorrhage occurred, the high expression of these genes at the site of rupture might, however, represent a consequence rather than a cause of rupture. The undetectable level of elastin mRNA at the site of rupture can be explained by the depletion and the severely disturbed distribution of smooth muscle cells (Figure 10 D), known as a major producer of elastin. It is opposed to the high level of mRNA for the two fibrillar collagens I and III at the same site, likely related to an activation of adventitial fibroblasts by inflammatory mediators and possibly by the high mechanical load. Accordingly, it has been

shown that under mechanical stimulation, fibroblasts elaborate a fibrotic tissue (Lambert et al., 1992 , Lambert et al., 2001).

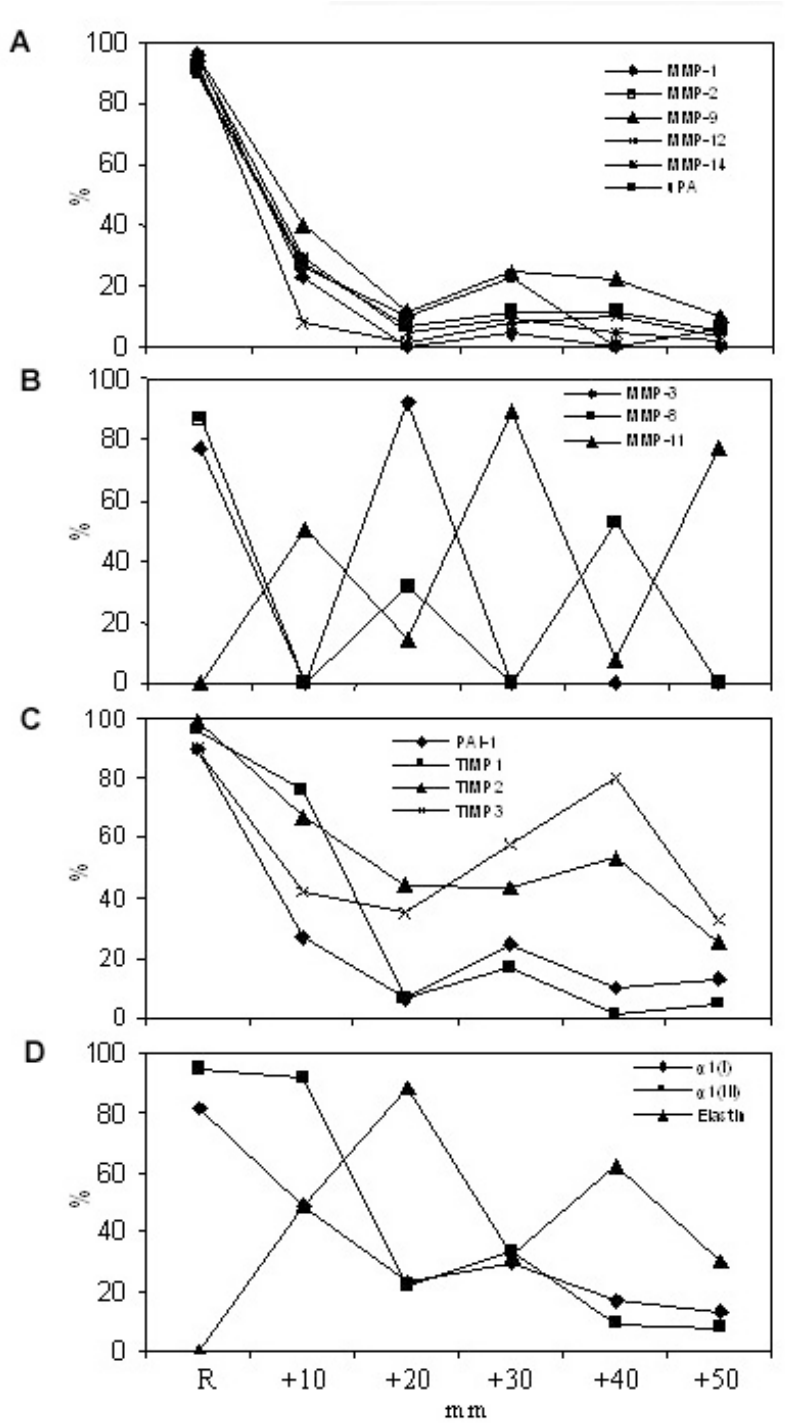


Figure 10. (a-d) Steady-sate level of mRNA for matrix metalloproteinases (a,b), their inhibitors (c) and fibrillar matrix proteins (d) in tissues sampled from the site of rupture (R) and at increasing distance. The results are expressed in arbitrary units per unit of 28S ribosomal RNA. α1(I), collagen type I; α1(III), collagen type III.

CONCLUSION

In summary, our study showed an heterogeneity in gene expression in the wall of ruptured abdominal aortic aneurysm. This may reflect a localized activation of the inflammatory and resident cells at the site of rupture. To our knowledge, this is the first time that the site of rupture of an aneurysm is compared histologically and biochemically with other part of the same aneurysm. However, more studies of the site of rupture are needed to better understand the fine cellular mechanism leading to this dramatic event.

CHAPTER 7

CONTRIBUTION OF POSITRON EMISSION TOMOGRAPHY (PET) TO THE EVALUATION OF AAA (APPENDIX 6&7)

INTRODUCTION

In the natural course of the disease, the initial rate of dilatation of the abdominal aorta is slow. The growth rate may accelerate at a later stage, possibly leading to rupture (Limet et al., 1991).

However, whatever its practical importance, the size of abdominal aortic aneurysms is rather the sole not the most accurate determinant to predict the risk of rupture. Expansion of the blood vessel precedes fissuration and rupture of a similar number of AAA, independently of their initial size (Glimaker et al., 1991, Johansson et al., 1990, Limet et al., 1991, Nevitt et al., 1989, Sterpetti et al., 1991). The process seems to depend upon the release of matrix metalloproteinases produced focally and/or activated by inflammatory cells, causing degradation of elastin and collagen in the aneurysmal wall (McMillan & Pearce, 1997, Newman et al., 1994, Sakalihasan et al., 1993, Thompson et al., 1995).

To our knowledge, no previous investigations comparing the in vivo metabolic activity in AAA wall and the increasing size of AAA have been reported.

Therefore we investigated if whole-body positron emission tomography (PET) could detect a hypermetabolic state of the aneurysm wall, eventually indicative of an accelerated wall transformation of AAA before rupture (Appendix 6).

Biochemical processes are also altered in most disease states and these alterations usually precede gross anatomical changes. Positron emission tomography (PET) is a diagnostic method that creates high resolution, three-dimensional tomographic images of the

distribution of positron emitting radionuclides in the human body. The compounds used for his purpose include metabolic substrates, ligands, drugs, antibodies, neurotransmitters and other biomolecules that are tracers for specific biological processes. The resulting PET images can be considered as “functional images” of these biochemical or physiological processes.

Historically, the initial PET studies focused on the brain and the heart metabolism. Now, PET is often used in oncological indications, where this technique revealed to be a useful method of oncological investigation for diagnosis, staging and therapy follow-up of several cancers. The radiotracers used in PET imaging have been primarily metabolic substrates that tend to accumulate to a greater extend in cancers than in normal tissues. The most widely applied substrate for tumor imaging is 18-fluorodeoxyglucose (FDG), a substrate of glycolysis. Glucose, F-18 FDG uptakes and metabolisms by malignant cells are enhanced consecutively to increase expression of the glucose permeases at the cell surface and because of an increased expression of the glycotic pathway enzymes.

MATERIAL AND METHODS

Patients

The study population consisted of a non-consecutive series of 26 patients with AAA (23 males and 3 females) documented by CT-Scan, for whom a complementary investigation by PET imaging was performed between March 1999 and August 2001. The patients presented an AAA with one or more of the following characteristics : large size (70 mm or more) (n = 11), painful AAA (n = 11), familial history of AAA (n = 1), inflammatory AAA (n = 4), rapid expansion (n = 6). PET-imaging was performed depending on the availability of the PET imaging system of the hospital.

Mean age was 71.7 years (range 56-85 years). The mean diameter of AAA was 63 mm (range 45-78 mm).

Radiopharmaceutical

After a minimum 6 h fasting, 3.7 mBq F-18-FDG per kilogram body weight was injected into a peripheral vein.

PET protocol

Static whole-body PET was performed with either an ADAC E-PET or a GE Advance tomograph. Beginning 60 min after tracer injection, emission and transmission images were recorded at each couch position (5-8) for 4-5 and 2-3 minutes, respectively. Coronal, sagittal and transaxial images were based on the use of an ordered subset expectation maximization iterative reconstruction algorithm (OSEM) including post-injection segmented attenuation correction.

Image interpretation

Two experienced investigators interpreted the PET images. The images were reviewed on hard copy and on a computer workstation (SUNSparc, SUN Microsystem, Palo Alto, CA, U.S.A.). PET imaging of the aneurysm was considered as positive when the wall of the dilated vessel could be clearly defined as illustrated in Figure 11.

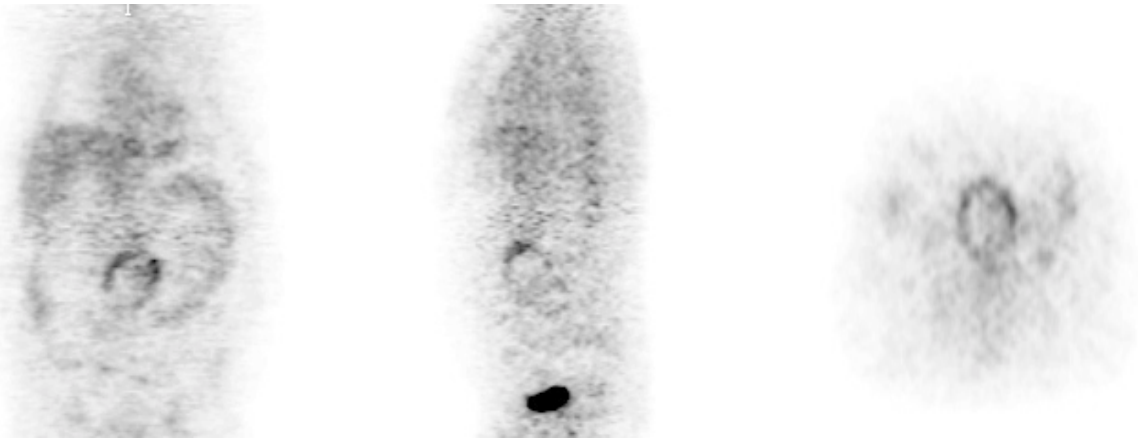


Figure 11. 18-FDG PET imaging of a patient 60-year old with rapidly expanding AAA (10 mm one month) showing a punctuated uptake of the marker in the wall of the AAA.

RESULTS

Among these 26 patients, 10 had a positive 18-FDG uptake at the level of AAA. Nine of these 10 patients required emergent (within hours) or urgent (within days after the PET Scan) aneurysmectomy for ruptured (n = 1, 70 mm), leakage (n = 1, 64 mm), rapidly expansion (n = 2, 60 and 70 mm) or unbearable pain (n = 5, 51, 70, 70, 71, 76 mm) (Table XVII). The tenth patient was not operated since he presented a huge lung carcinoma. In the 16 PET negative patients, aneurysmectomy was delayed at the convenience of the patient from one to several months. None of these patients presented a clinical evolution with the characteristics of the PET positive patients.

Patients			ø of AAA		Delay between diagnosis and surgery		
No.	Sex	Age	Initial	Last		Remarks	
1	F	64	-	71	< 1 month	Low back pain	
2	M	60	-	76	< 2 months	Painful inflammatory AAA	
3	M	70	42	51	36 months	Painful inflammatory AAA, rapidly expanding	
4	M*	79	32	70	96 months	Rapidly expanding AAA, prostatectomy	Parotid tumour†
5	M*	73	60	64	6 months	Recent nephrectomy for hypernephroma, leaking AAA	
6	M*	77	35	70	24 months	Recent bilateral carotid TEA, rapidly expanding AAA	Pulmonary CA†
7	M*	82	54	60	6 months	Recent sigmoidectomy, rapidly expanding AAA	
8	M	74	-	70	< 1 month	Low back pain	
9	M	69	-	70	2 days	Thoracic aneurysm, painful AAA	Pulmonary CA†
10	M	84	-	50	unoperated	Asymptomatic	Pulmonary CA†

* Patients who had emergency surgery.

† Lesions revealed by PET-scan.

Table XVII. Patient characteristics with positive FDG uptake

DISCUSSION

This preliminary study of PET imaging of AAA suggests an association between 18-FDG uptake by the aneurysm wall and rapid expansion of the aneurysm, recent (within 6 months) surgery or malignancy. Indeed, five of the nine operations among patients with positive PET imaging were performed on an urgent or emergent basis. No urgent surgery was required among the 15 operated patients with negative PET imaging. In this series, malignancy was also more common among patients with positive PET-imaging of the AAA.

The 18-FDG uptake in the aneurysm wall reflects the presence of a large density of inflammatory cells (macrophage, lymphocyte and polymorphonuclear cells) (Corcoran et al., 1992, Shapiro et al., 1990, Thompson et al., 1995). The lymphocytes release cytokines, known to regulate MMP expression by macrophages (Thompson et al., 1995, Corcoran et al., 1992, Shapiro et al., 1990). For example, macrophage expression of MMP-9 is positively regulated by interleukin (IL-1B), tumor necrosis factor-α (TNF-α), IL-6, down-regulated by IL-4 and interferon γ. These cytokines may contribute to matrix degradation during the progression of AAA since they down-regulate or increase the production of metalloproteinases (Thompson et al., 1995, Corcoran et al., 1992, Shapiro et al., 1990). The regulation of MMP could be altered if dense macrophages and lymphocytes infiltration is present on the wall of AAA. These AAAs could be considered as unstable. Although the activated macrophages can be detected by PET imaging (Appendix 8) and 18-FDG uptake was observed in only some patients with an huge macrophage activity. It probably correlates with increased activated proteinases and aneurysm expansion that are known to contribute to rupture. We have indeed observed an accumulation of polymorphonuclear cells accompanying a dense infiltrate of macrophages and lymphocytes only at the site of rupture (Chapter 6).

Hunter (Hunter et al., 1996) described aortic blebs as a focal saccular deformation within the walls of aneurysm, which could also be detected by CT scan. The presence of polymorphonuclear (PMN) at the location of blebs of the aortic wall and at the site of rupture is also suggestive for a local inflammatory process preceding rupture. However, the CT scan images are not representative of the metabolic state of the lesion.

It is important to stress that FDG uptake by tumor cells in vivo is dependent on numerous physiological factors, such as tissue oxygenation, regional blood flow, peritumoral inflammatory reactions (Appendix 7). On the other hand, FDG uptake is not specific for tumors (Larson et al., 1999). FDG-PET scan can also be positive in inflammatory states, especially active granuloma formation, sarcoid, abscess formation (Kubota et al., 1980). Within tumors, as well as inflammatory tissues, part of PDG is taken up by macrophages and up to 25 % of the signal reaching the scanner could be due to glycolysis from macrophages within the tumor.FDG-PET therefore must be considered as a very sensitive but less specific technique. The lack of specificity can be compensated by careful patient selection and rigorous correlation with anatomical images (including image fusion whenever possible).

Consequently, a positive PET imaging represents for us a diagnostic support to proceed without delay to aneurysm surgery, despite patients'age or operative risk (Sakalihasan & Defawe., 2005). This help in therapeutic decision is not trivial, since it is well established that the ultimate prognosis after elective surgery is much better (Rinkenbach et al., 2004).

CONCLUSION

The activity of matrix metalloproteinases that contribute to the progression of the lesion is increased in the wall of an aneurysmal aorta. These proteinases are produced and activated by inflammatory cells with an increased metabolic activity. This increased metabolic activity of the aneurysmal wall was assessed via PET imaging using 18-FDG. PET-scan was found positive in some patients bearing either large or rapidly expanding or painful aneurysms. This preliminary report shows the capacity of PET imaging to assess increased metabolic activity within the aneurysm wall. A subset of aneurysms shows an increased 18-FDG uptake, suggestive of a focally accelerated metabolism. The patients with positive PET imaging should be considered at risk for rupture and operated on without undue delay.

CHAPTER 8

CORRELATION THE FONCTIONAL IMAGING WITH THE HISTOLOGICAL FINDINGS IN THE WALL OF ANEURYSMAL ABDOMINAL AORTA (APPENDIX 8)

INTRODUCTION

Since preliminary results obtained by Positron Emission Tomography Scanning (Sakalihasan et al., 2002) suggested a possible correlation between 18-FDG uptake by aneurymal wall and the inflammatory infiltrate in the blood vessel wall, we investigated the possibility to show in vivo the presence of inflammatory cells (macrophages,PMN) by PET scanning.

MATERIAL AND METHODS

A 68-year-old male was hospitalized for unstable angina and underwent emergency coronary artery bypass surgery. During the operation, a pulsatile large abdominal aortic aneurysm (AAA) was discovered. In order to define the optimal treatment of the abdominal aneurysm, a CT-scan and Positron Emission Tomography (PET) were performed heart surgery. Surgical correction of the AAA was planned.

At the time of elective repair, full-thickness aortic wall fragments and adherent thrombus were collected. Histological analysis was performed as described in the Chapter 6 (material and methods).

RESULTS and DISCUSSION

The CT-scan confirmed the presence of the large AAA. PET imaging combined to immunohistological examination showed a region of increased 18-FDG uptake in the aortic wall. On histological analysis, an inflammatory infiltrate was noted in this area. This contrasted with the parietal thrombus (Figure 12 &13) (devoid of inflammatory cells). The luminal area showed mid level of 18-FDG uptake corresponding to circulating mediators .

DISCUSSION

PET imaging is not routinely performed in the diagnosis or treatment of AAA. This observation shows that there is a correlation between FDG uptake and the intensity of onflammatory cells infiltrations. Previously, we showed an association between increasing ^{18}F-FDG uptake and AAA expansion and rupture (Sakalihasan et al., 2002, 2004). However FDG-PET imaging is not limited to the evaluation of AAA. Indeed, enhanced uptake has been reported in various inflammatory diseases concerning the large vessels. Giant cell arteritis and Takayasu arteritis both show significantly increased glucose metabolism in the wall of the affected arteries (i.e. aorta,subclavian arteries or carotid arteries) (Belhocine et al., 2003). Furthermore, FDG uptake has been found in large arteries, in relation with the presence of active atheromatous plaques (Yun M et al., 2002).

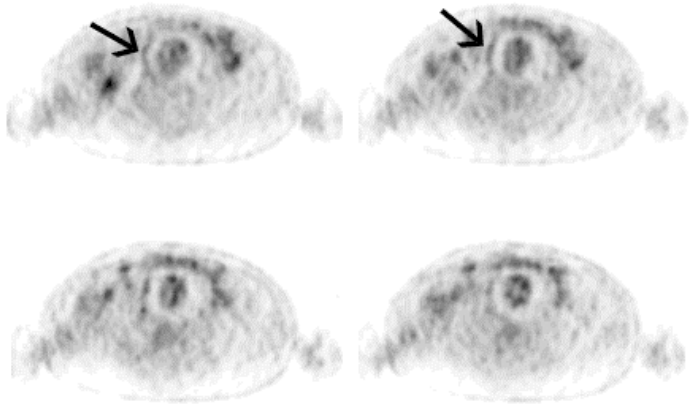


Figure 12. In this patient, a transaxial image shows a thin area of ^{18}F-FDG uptake (arrow) corresponding to aneurysmal wall. Inside, a rim without significant uptake corresponds to parietal thrombus devoid of inflammatory cells (3, 6) while the lumina

area shows mild uptake of ^{18}F-FDG possibly associated with activated macrophages primed for several days by circulating mediators released at the time of the surgery, as described previously (7, 8).

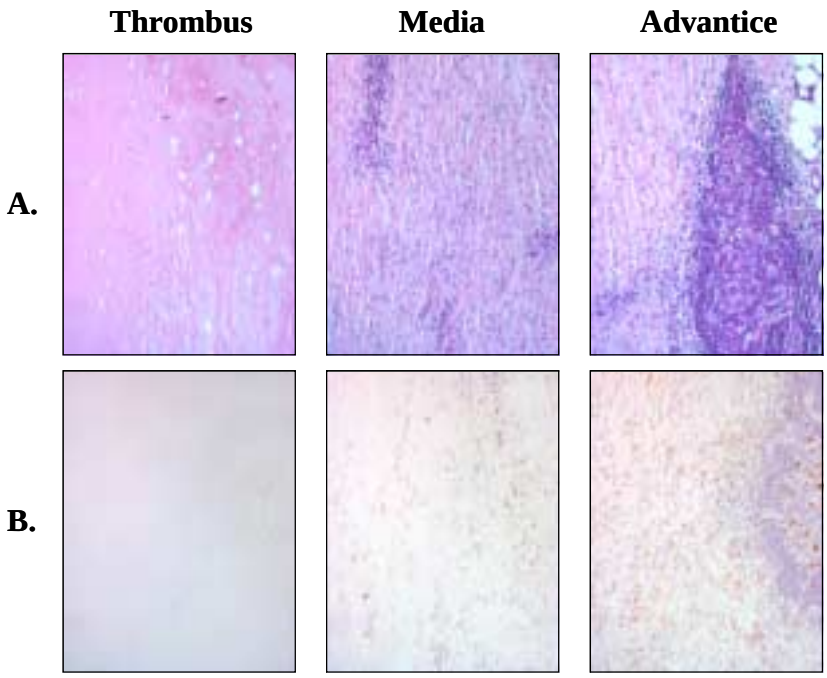


Figure 13: Microscopic features of sample collected in an abdominal aortic aneurysm wall. A. Hematoxylin-eosin (200X) staining shows a gradient of inflammation from the advantitia to the parietal thrombus with some focally clusters of inflammatory cells. B. Macrophages staining (CD68, 200X) shows a gradual distribution of macrophages from the advantitia to the media and the absence of macrophages in the thrombus.

CONCLUSION

In our observation, we observed a positive correlation between the 18-FDG uptake and the presence of inflammatory cells in the aortic wall. This observation reinforces our previous conclusions that the patients with AAA could be monitarized by PET scan before the operative decision.

CHAPTER 9

VITAMIN E (α-TOCOPHEROL) LEVEL IN PATIENTS WITH ABDOMINAL AORTIC ANEURYSM (APPENDIX 9)

INTRODUCTION

It is well established that arterial cholesterol deposit originates primarily from low-density lipoproteins (LDL). Elevated LDL concentrations are associated with an increased risk for atherosclerosis. During the initial stages of the development of atherosclerosis, an accumulation of foam cells is observed. These are macrophages that have taken up oxidized LDL. These foam cells are filled with liquid droplets of cholesterol, and are a key component of the fatty streak lesion (Esterbauer et al., 1990). LDL is an important target of free radicals in blood and oxidation of LDL is believed to be an important event in the development of atherosclerosis (Sato et al., 1990). Results of cell and animal research support the hypothesis that oxidative modification of LDL results in their enhanced uptake by macrophages leading to conversion of macrophages into foam cells (Esterbauer et al., 1987, Esterbauer et al., 1989, Steinberg et al., 1989). The mammalian cells contain endogenous defenses against free radicals. In vivo, the consumption of vitamin E (Vit E) is considered as a specific although indirect index of in vivo peroxidative processes. The significant decrease of Vit E is associated with increased free radical production (Steinberg et al., 1989).

Vit E is a major lipidic antioxidant in body tissue. It is considered as the first line of defense against lipid peroxidation, protecting cell membranes at an early stage of free radical attack through its free radical-quenching activity (Horwitt, 1986, Van Gossum et al., 1988). The consumption of plasma vitamin E is indicative of an oxidative stress. In our study

(Sakalihasan et al., 1996), we found an increased amount of metalloproteinases in the wall of AAA, reflecting an activation of inflammatory cells. An activation of inflammatory cells may determine an oxidative stress, and therefore resulting in lipid peroxidation. Thus, to test this hypothesis, we investigated Vit E levels as a marker of major antioxidant in plasma of patients with AAA.

MATERIAL AND METHODS

Three groups of male patients were screened: (1) patients undergoing elective operative repair of AAA reaching more than 4.5 cm in transverse diameter (AAA group, n = 19, mean age 72.5 ± 6.6 years), (2) atherosclerotic patients referred to our department for coronary bypass artery surgery (CAB group, n =18, mean age 61.2 ± 7.4 years), and (3) healthy volunteers (healthy volunteers group, n =13, mean age 35.2 ± 16.3 years). None of these patients were taking vitamin E supplement. Blood samples were collected the day before surgery. The plasma vitamin E levels were determined by high-performance liquid chromatography. Values were expressed μg/ml.Total lipids in plasma were measured by gravimetry, and vitamin E status was also expressed as vitamin E/total lipids ratio (mg/g). Results in the different groups were compared using Kruskal-Wallis nonparametric method because of small sample sizes and censored observations. Results were considered to be significant at the 5% critical level ($p < 0.05$).

RESULTS

The Vit E levels in AAA patients were significantly lower than the Vit E levels observed in age-matched CABG patients and in healthy volunteers. No significant difference between the total plasma lipid levels in the different groups was observed. In consequence, the Vit E/total lipid ratio was significantly lower in patients with AAA versus both control groups (coronary artery disease and volunteers) (Table XVIII).

	Control groups		
	Volunteers (n = 13)	CAB patients (n = 18)	AAA patients (n = 13)
Total lipids (mg/ml)	5.39 ± 0.81	5.09 ± 1.84	6.87 ± 2.83
Vitamin E (µg/ml)	10.90 ± 3.12	11.00 ± 4.79	1.63 ± 2.44[b]
Vitamin E/total lipids (mg/g)	2.01 ± 0.47	2.51 ± 1.60	0.26 ± 0.37[c]

CABG, coronary artery bypass graft ; AAA, abdominal aortic aneurysm.

[a] Data expressed as mean ± SD

[b] $p < 0.001$ versus CABG patients and volunteers.

[c] $p < 0.001$ versus CABG patients and volunteers.

Table XVIII. Vitamin E level, total lipids level and Vitamin E/total lipids ratio in plasmaa

DISCUSSION

Recent studies suggested an inverse relation between vitamin E status and the incidence of arterial disease (Rimm et al., 1993, Stampfer et al., 1993). Verlangieri & Bush (Verlangieri & Bush, 1992) observed that monkeys submitted to a lipid diet and supplemented with vitamin E developed less arterial stenosis than monkeys receiving the same diet with no vitamin E. Several studies have investigated the potential role of vitamin E in humans. In the prospective study of Rimm et al. (Rimm et al., 1993) and Stampfer et al. (Stampfer et al., 1993) some evidence indicated an association between a high intake of vitamin E and a lower risk of coronary artery disease in men and women.

At the present time, no other role than neutralization of free radicals is devoted to Vit E. Therefore the decrease in plasma Vit E concentration might indicate an increased oxidative stress in patients bearing AAA. As the total plasma lipid level was similar in AAA patients and in control groups, the results with Vit E/total lipid ratio confirm that the decrease of Vit E might be attributed to an excessive Vit E consumption and does not result from a decrease of the concentration of Vit E carriers. As a Vit E/total lipids ration of 0.8 mg/g or

less is usually considered as indicative of Vit E deficiency, we can conclude that all AAA patients exhibited a severe deficiency in this antioxidant. It is quite remarkable to note that Vit E was even undetectable in about 50% of these patients.

CONCLUSION

In summary, we observed evidence of an association between low plasmatic levels of vitamin E and the presence of an AAA. This decrease of the plasma vitamin E level was specifically associated to the development of AAA, since patients with coronary artery disease did not exhibit such a deficiency. Whatever the cause of this decrease, a low plasma vitamin E is associated with a diminution of endogenous defense against oxidative stress and this decrease may increase the postoperative risk. Thus, vitamin E should be regularly monitored in patients at risk for development of AAA. Perhaps vitamin E supplement should be administered to prevent deficiency and to maximize protection against injury by toxic oxygen species that can occur during the development of aneurysm. Nevertheless, further prospective studies are needed.

CHAPTER 10

FAMILIAL OCCURENCE OF AAA (LOCAL EXPERIENCES) (APPENDIX 10)

INTRODUCTION

Although AAA is frequent in the elderly, the familial clustering of cases has only recently attracted attention. Since the first case report by Martin Clifton in 1977 (Clifton, 1977), several series have confirmed AAA as one of the most frequent "familial disease". It appears probable that "not everyone is prone to aneurysm" and that a particular genetic character predisposes the ageing arterial wall to an aneurysmal transformation, perhaps in response to an accidental factor. Although some descriptive statistics are available on the familial aspects and on the natural history of the disease, the pathogeny and the genetic background still remain largely obscure, and each mode of inheritance (dominant, recessive, x-linked, multifactorial) has been advocated (Majumder et al., 1991, Powel & Greenhalgh, 1987, Tilson & Seashore, 1984).

In familial studies, persons have to be assigned a status: affected, unaffected, or unknown, whereas this is of little importance for congenital, qualitative defects. Once again the determination of the affection status is a major difficulty in the study of AAA families. In this study, we defined AAA when a dilatation was equal or larger than 30 mm.

MATERIAL AND METHODS

Between 1986 and 1991, 520 patients were surgically treated in our department for AAA. We excluded from this series patients with Marfan syndrome or Ehlers-Danlos syndrome. No systematic ultrasound screening was performed or even recommended for the siblings of the patients during the study period. We sent a written questionnaire oriented to personal and familial history to the 520 patients irrespective of any familial or surgical

particularity. The patients or their relatives who filled out the questionnaire were interviewed by phone and relatives were then contacted in the same way to obtain the most accurate and best cross-validated pedigrees.

Methods concerning descriptive statistics, relative risks, survival function, and segregation analysis are explained in details in our original report (see Appendix 10).

RESULTS

Familial aspect

Through questionnaire and phone inquiry, familial data of 324 probands allowed the establishment of 313 multigenerations pedigrees. The total number of patients with AAA in our large 313 pedigrees was 357. There were 276 sporadic cases and 81 cases belonging to multiplex pedigrees (Table XIX). Comparing familial and sporadic male cases, age at diagnosis was respectively 64.1 ± 7.9 and 66.0 ± 7.3 ($p < 0.05$), age at rupture 65.4 ± 6.6 and 75.2 ± 8.6 ($p < 0.001$), and rupture rate 32.4 % and 8.7% ($p < 0.01$)).

	n	AAA	Mean age of patients with AAA ± SD (yr)	Mean age of unaffected patients ± SD (yr)	Rupture (%)	Age at rupture ± SD (yr)
Total	1597	357	66.4 ± 7.8	68.1 ± 13.4	52 (14.6)	-
Familial subgroup						
Fathers	39	8	73.3 ± 7.6	71.6 ± 14.0	4 (50.0)	69.0 ± 8.9
Mothers	39	5	73.0 ± 7.7	71.4 ± 12.7	3 (60.0)	70.8 ± 8.0
Brothers	104	68	64.1 ± 7.9*	64.8 ± 11.5	22 (32.4)	65.4 ± 6.6‡
Sisters	44	0	-	66.4 ± 10.6	†	-
Sporadic subgroup						
Fathers	276	0	-	69.9 ± 14.2	-	-
Mothers	276	0	-	74.4 ± 12.7	-	-
Brothers	546	264	66.0 ± 7.3*	63.8 ± 11.5	23 (8.7) †	75.2 ± 8.6‡
Sisters	273	12	68.0 ± 12.5	66.4 ± 12.2	0 (0.0)	-

* $p < 0.013$
† $p < 0.001$
‡ $p < 0.001$Age statistics are given based on censoring age or age at death for the unaffected subjects and age at diagnosis for patients with AAA.

Table XIX: Descriptive statistics of 315 nuclear families with respect to their family history, sex, and position (subjects younger than 30 years excluded)

Segregation analysis

324 probands belonging to 313 pedigrees answered the questionnaire. The total number of AAA was 357. There was no familial history of AAA for 264 males and 12 females (sex ratio 22:1).In 39 pedigrees (12.5%) at least two subjects were affected, for a total 81 AAA:76 males and 5 females (sex ratio 15:1). In the familial group, all affected women were parents. Among familial cases, 23 pedigrees showed affected sibs with normal parents,10 showed an affected parent and affected child, and 6 showed more complex structures (affected cousins, uncle and nephew,…) (Appendix 10). The 582 nuclear families were analyzed with POINTER. Of those families 101 belonged to the multiplex pedigrees. They were studied separately after recoding. Eight models (sporadic, multifactorial, polygenic, dominant, recessive, codominant, mixed, and mendelian) were evaluated. The results of the analysis of the full samples are given in Appendix 10.

In our segregation analysis, testing the mixed model, the most likely explanation for occurrence of AAA in our families was a single gene showing dominant inheritance, the frequency of the morbid allele being 1/250 and its age related penetrance being not higher than 0.4. Relative risk for male sibs of a male patient was 18 (Table XX).

Age (yr)	30-49	50-59	60-69	70-79	≥ 80	Total
Relative risk	0.005/33	94.3	15.1	4.6	4.0	17.9
(95% confidence interval)	0	(0-425)	(2.9-27.3)	0-17.9	(0-86.5)	(12.8-22.9)

Table XX: Age distribution of AAA among brothers in several subgroups with calculation of relative risk

DISCUSSION

Familial Aspect

Clifton, reporting three affected brothers in 1977 (Clifton, 1977), pioneered the study of familial aggregation of AAA. Johansen and Koepsell (Johansen & koepsell, 1986)

compared the family histories of 250 patients with AAA to those of 250 controlled subjects. Among the latter, 2.4% reported a first degree relative with an aneurysm, compared with 19.2% of the patients with AAA, representing a 11.6-fold increase in risk among persons with a effected first-degree relative. Darling (Darling et al., 1989) found 15.1% of proband with an affected first-degree relative as compared to 1.8% of a control group of 500 patients of similar age and sex. Several other series were published, including our own systematic analysis of 313 pedigrees. The series are compared in Table XXI.

	Norrgard et al.	Tilson and Seashore	Johansen and Koepsell	Cole et al.	Darling et al.	Webster et al.	This study
N° of pedigrees	87 (initially 200)	50	250	305	542	91	313
Multiplex pedigrees (%)	18 (20.6)	50	48 (19.2)	37 (12.1)	82 (15.1)	14 (15.3)	39 (12.4)
Horizontal pedigrees	10	28	18	18	?	11	23
Vertical/complex pedigrees	8	22	> 19	19	?	3	16
AAA	103	127	≥307	?	669	108	357
AAA (familial subgroup)	38	127	≥ 105	91	209	31	81
Sex ratio	155:45 (3.75:1)	?	207:43 (4.81:1)	?	532:137 (3.88:1)	49/19 (4.68:1)	340/17 (20:1)
Sex ratio (familial)	30:8 (3.75:1)	11:16 (6.94:1)	?	56:35 (1.6:1)	136:73 (1.86:1)	20:10 (2:1)	76/5 (15:1)
Sex ratio (sporadic)	?	?	?	?	396:64 (6.19:1)	69:9 (7.67:1)	264:12 (22:1)
Age at diagnosis	67 (M66/F70)	?	72	?	?	M67.1/F69.2	M66.2/F69.5
Age at diagnosis (familial)	65 (n = 19)	?	?	?	M62.4/F71.2	?	M65/F73
Age at diagnosis (sporadic)	?	?	?	?	M67.8/F68.8	?	M66.6/F68
Rupture rate (%)	68/200 (initial)	?	?	?	?	?	52/357 (14.6)
Rupture rate familial (%)	14/38 (36.8)	?	?	22/52 (42)	42/209 (20.1)	?	29/81 (35.8)
Rupture rate sporadic (%)	?	?	?	?	?	?	20/276 (8.3)

M, male ; F, female

Table XXI : Comparison of different data of families observed in this study and in six preview studies

The sex ratio in favor of males varies between 4:1 and 20:1. Based on non-systematic clinical diagnosis, the proportion of AAA proband with a positive family history goes from 12 to 20%, and the relative risk of AAA for siblings was 10 to 20%. Webster (Webster et al., 1991) found 16.2% of familial history by anamnesis of 43 consecutive patients. After prospective US screening, the number of familial cases rose to 27.9%. Bengtsson (Bengtsson et al., 1989) found 29% of AAA in male and 6% in female relatives after US screening, confirming the extremely high incidence of cryptic, asymptomatic familial AAA. These discrepancies leave much room for doubt as to the accuracy of older epidemiological studies of AAA. Differences in natural history of AAA depending on the familial history were addressed in larger series. In the Darling study (Darling et al., 1989), no significant difference was found between the patients with non-familial and familial AAA in anatomic extent, or associated occlusive disease. Patients with familial AAA were more likely to be women (35% vs. 14%), and men, in their group, were affected about five years earlier than women. The risk of rupture was strongly correlated with familial disease and the presence of a female member with aneurysm (63% vs. 37%). In our own study of 313 pedigrees, we found similar differences: the age at diagnosis two years earlier ($p < 0.01$) in males of multiplex pedigrees, as well as the rupture rate were higher in the latter (32.4% vs. 8.7%). The mean rupture age was significantly different among affected brothers of the two groups ($p < 0.001$).

Ethnic differences in AAA have been considered by Johansen (Johansen & Koepsell, 1986) in a survey of autopsies and abdominal CT scans: white males have a frequency of abdominal aortic aneurysm about three times that in black mails, black females, or white females ; all three of the latter groups had comparable frequencies.

Genetic Aspect at the beginning of the nineteenths Majumder and his team (Webster et al., 1991, Majumder et al., 1991, Wabster et al., 1991) published data on first-degree relatives of 91 probands from 91 pedigrees (sex ratio 79:12). In their series, they counted as “affected” only patients with operated AAA, and used age at surgery for analysis,rejecting patients who were discovered by systematic screening. Their number of multiplex pedigrees was relatively small (13 of 91). The most likely genetic model was an autosomal diallelic locus with a recessively inherited gene for AAA. A multifactorial component did not increase significantly

the likelihood of the data set. In our own analysis of 324 probands (sex ratio 312:12) from 313 pedigrees (including 39 multiplex families), we used a less drastic definition : infrarenal/suprarenal ratio > 1.5 or absolute diameter > 30 mm. Our analysis favored a simple autosomal dominant model, with a carrier frequency of 1 of 250 and an age-related penetrance never higher than 0.4, even in the subgroup of elder men. Like Majumder et al., (Majumder et al., 1991) we noted that a multifactorial component did not lead to a significant better fit.

Both clinical considerations and experimental results allow suspect genes involved in the constitution of the normal matrix and in its turnover to play a role in the genetic background of aneurysmal predisposition. Among structural components of the conjunctive intercellular matrix are collagens, genes of the matrix metalloproteinases family (including the chromosome 11q22-23 collagenase cluster members), the tissue inhibitors of metalloproteinases, and two genes, HP and CETP, that map in the vicinity of CLG4A (although the latter two are not primarily involve in matrix metabolism) (Powell et al.,.1990). They are listed in Table XXII.

Gene	Synonyms gene symbol(s)	Chromosome location
Matrix proteins		
A1-chains of collagen type I	COL1A1	17q22
A2-chains of collage type I	COL1A2	7q22.1
A-chain of collagen type III	COL3A1	2q31
Fibrillin 1	FBN1	15q21.1
Elastin	ELN	7q11.2
Degradation enzymes		
Collagenase A type IV	MMP-2 72 kDa gelatinase CLG4A	16q13
Collagenase B type IV	MMP-9 92 kDa gelatinase	20q11-13
Interstitial collagenase	CLG4B	11q22-23
Neutrophil collagenase	CLG/MMP1	11q21-22
Matrilysin	CLG1/MMP8	11q22-24
Collagenase 3	PUMP1/MMP7	11q22-23
Stromelysin 1	CLG3/MMP13	11q22-23
Stromelysin 2	STMY/MMP3 STMY2/MMP10	11q22-23
Degradation inhibitors		
Metalloproteinase inhibitor type 1	TIMP1/HCI/E	Xp11.1
Metalloproteinase inhibitor type 2	TIMP2PA	17q25
Chromosome 16 candidates		
Cholesterol-ester transfer protein	CETP	16q21
Haptoglobin alpha chain	HP	16q21

Table XXII. Candidate mapped genes for abdominal aortic aneurysm

Loosemore et al. (Loosemore et al., 1988) suggested that a deficiency of type III collagen might be the basis for the aneurysm formation. Menashi et al. (Menashi et al., 1987) showed a low content of type III collagen in a group of patients with familial AAA. Minor normal allelic variants of COL3A1 were found in association with AAA. A less elastic aneurysm wall was found in those patients Powell et al., 1991). Thermally unstable procollagen III was found in two patients with multiple aneurysms (Deak et al., 1992). Furthermore, mutations of collagen type III are known to occur in the vascular type IV of Ehlers-Danlos (EDIV) (Superti-Furga et al., 1989). Clinical hallmarks of EDIV include facial dysmorphism (pinched nose, thin lips), fragile, easily bruisable, translucent skin with prominent venous markings, mitral valve prolapse, microangiopathy of skin capillaries with microbleedings and microaneurysms, tortuous arteries, AAA, and every commonly spontaneous arterial and bowel ruptures. In many families, EDIV is due to mutations of pro-alpha-chains of type III collagen.

In 1990, Sirpa Kontusaari showed two single base mutations in COL3A1 in two families with dominantly inherited AAA. In the first family, the mutation was shown to result in an animo acid substitution (GLY619ARG) (Kontusaari et al., 1990). In the second, presenting with AAA and easy bruisability, a single base mutation in intron 20 ($G^{+1IVS20}$) (Kontusaari, Tromp et al., 1990) was shown to induce aberrant splicing of the mRNA, hence reducing the synthesis of the α1(III) chain. This family is of peculiar clinical importance, as clinical presentations extended from the classical EDIV disease to isolated AAA. Tromp et al. (Tromp et al., 1993) carried out detailed DNA sequencing of the triple helical domain of type III procollagen on cDNA from 54 patients with aortic aneurysms, 43 of them with at least one affected relative. Only two amino acid substitutions were found (GLY136ARG, THR501PRO), the second of unclear functional significance, thus indicating that collagen III mutations are not a major cause of common AAA.

CONCLUSIONS

Systematic screening of AAA is an emerging issue. A common question is whether to apply AAA screening to a general population or to an “at risk” subgroup. We strongly recommend ultrasonographic screening of first-degree relatives aged 50 years and older, a

method that now permits simple, noninvasive, and accurate detection and follow-up of AAA. Recently in our retrospective study of the determination of the expansion rate and incidence of rupture of abdominal aortic aneurysms, we found 12% of rupture in aneurysms smaller than 44 mm and 22% when the diameter exceeded 50 mm (Limet et al.,1991). When the higher incidence of rupture in patients with positive family history and the risk of rupture even for small AAA (less than 50 mm) are considered, a more aggressive therapeutic attitude is mandatory. Rationale for a national screening program has been given by Law et al. (Law et al., 1994) who recommended one ultrasonography detection in men aged 60 years and older. As long as cost-effectiveness of those general policies has not been demonstrated, a reduced screening policy could be recommended at least for patients with other peripheral artery aneurysms and for first-degree relatives of patients with an AAA.

CHAPTER 11

MULTICENTRIC RESEARCH ON FAMILIAL AND GENETIC ASPECT OF AAA (APPENDIX 11,12 &13)

INTRODUCTION

Interviews and ultrasonographic screening studies among relatives of patients with AAA have clearly shown the increased prevalence of AAA among first-degree relatives, with up to 18% of brothers and 5% of sisters having AAA (Kuivaniemi & Tromp, 2000). Population-based ultrasonographic screening studies have also emphasized family history as an important risk factor for AAA (Lederle et al., 1997, Lederle et al., 2000). Formal segregation studies have shown that AAAs are likely to be a genetic disease with autosomal, either dominant or recessive, inheritance pattern (Majumder et al., 1991, Appendix 10).

In order to identify the relationship of the affected relatives to the proband we investigated a large number of families in which at least two individuals were diagnosed with AAA. Our goal was to identify the chromosomal regions likely to harbor the AAA susceptibility gene(s) using affected sib pair (ASP) DNA linkage analysis. We will identify affected-relative-pairs with AAA in the USA, Canada, the Netherlands, and Belgium and collect blood for DNA isolation from patients as well as those relatives that are informative for identifying alleles shared as identical by descent. First-degree family members who are 55 years or older will be offered an abdominal ultrasonography examination to detect asymptomatic AAAs. The linkage analyses will be performed as model-free affected sib-pair and pedigree analyses using two-stage design. The first stage of phase I will consist a genome scan using highly polymorphic markers located on average 10 cM apart (about 390 markers to

scan all the human chromosomes) on the 65 affected sib pairs we have collected to date (Figure 14). The second stage of phase I will include genotyping additional markers from regions that warrant further investigation based on the first stage on phase I results. In the second phase, using the additional ASPs collected, we will type markers in chromosomal regions determined in the first stage to be sufficiently interesting, i.e. exceed standard statistical threshold, to warrant further investigation, then perform model-free linkage analyses in those regions using the combined data set. Model-free methods of linkage analysis using ASPs have long been available (Campa et al., 1987). The original methods were non-parametric and compared the mean proportion of alleles that the ASP shared identical-by-descent to 0.5, the value expected if there is no linkage between the disease and the marker.

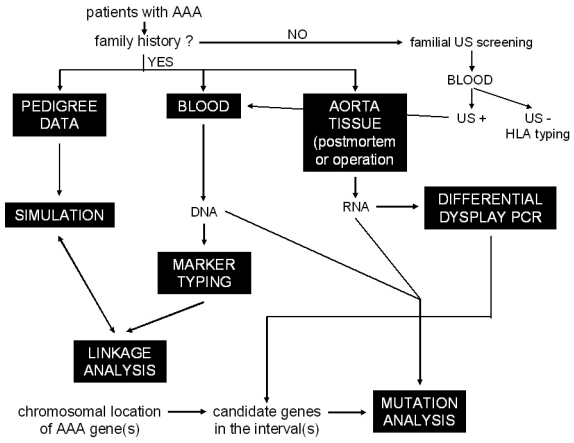


Figure 14. Integration of DNA linkage analysis and differential display PCR to identify the susceptibility gene(s) for abdominal aortic aneurysms (AAA).

MATERIAL AND METHODS

Multinational screening of familial AAA Families with at least two members with AAAs were identified for the study at the following sites: the Department of Surgery, Wayne State University School of Medicine, Detroit, Mich; the Department of Surgery, Dalhousie University, Halifax, Canada; the Department of Surgery, University of Oulu, Oulu, Finland; the Department of Cardiovascular Surgery, University of Liège, Liège, Belgium; the Department of Vascular Surgery, Vrije University Medical Center, Amsterdam, The Netherlands; the Department of Surgery, Charing Cross and Westminster Medical School, University of London, The United Kingdom; and the Department of General Surgery, University of Umea, Umea, Sweden; and through the website at http://www.genetics.wayne.edu/ags (Salkowski et al., 2001). The study was approved by the Institutional Review Board of Wayne State University School of Medicine and by the patient recruiting centers.

All patients admitted for surgical repair of AAA were interviewed by a nurse after consent was obtained. Patients who indicated that they had at least another family member diagnosed. with AAA were included into the study; a detailed family history and life-style questionnaire was sent to them, and they were subsequently interviewed by a genetic counselor. To identify individuals with heritable connective tissue disorders, such as Ehlers-Danlos syndrome type IV or Marfan's syndrome, a specific questionnaire was used assessing skin and skeletal manifestations characteristic for these disorders. Families with these disorders were excluded from the study. The family trees were drawn with Cyrillic software (Cherwell Scientific Publishing Limited, Oxford, Great Britain) (figure 15). Whenever possible, the AAA diagnosis of a deceased family member was verified by requesting the autopsy or medical records. Some family members, if 50 years old or more, had been examined with ultrasonography and were identified as affected if the infrarenal aortic diameter was 3.0 cm or greater, a cutoff point used by other investigators previously (Lederle et al., 2001). This study was approved by the Institutional Ethical Committee of Liège's University School of Medicine and by the other collaborators. To identify additional affected

relatives pairs with AAA and to collect DNA samples to identify alleles shared as identical by descent, we invited the first degree family members aged at least 50 years to give blood samples and offered them ultrasonographic examination in order to detect asymptomatic AAAs.

RESULTS

Actually, we identified 233 families in which at least two members had an AAA with an average of 2,8 AAA cases in each family. In addition there were 653 patients with AAA (Table XXIII).

Nationality	No. of families	
Canadian	80	(34%)
Belgian	59	(25%)
Dutch	47	(20%)
US American	37	(16%)
Finnish	5	(2.1%)
British	3	(1.3%)
Spanish	1	(0.4%)
Swedish	1	(0.4%)
Italian	1	(0.4%)
Total	234	

All patients with AAA were white.

Table XXIII. Nationalities of families with AAA

Most of the families were small with only two affected individuals (Table XXIV). 72% of the families show autosomal recessive inheritance pattern on the basis of the fact that

affected individuals had no affected parents, whereas in 58 families (25%), AAAs occurred in one parent of the affected individual and the inheritance mode was, therefore, consistent with autosomal dominant inheritance. In the remaining eight families (for example, families 002, and 048 in Figure 15), the familial aggregation could be explained by autosomal dominant inheritance with incomplete penetrance because some affected individuals in these families had an affected parent and others did not. Alternatively, the eight families could have autosomal recessive inheritance with a common disease allele.

No. of affected individuals	Families	(%)	Relationship to probands*			
			M	F	B	S
2	131	(56)	8	14	89	14
3	56	(24)	7	10	69	16
4	25	(11)	5	6	43	8
5	11	(4.7)	2	3	27	5
6	6	(2.6)	2	3	12	4
7	3	(1.3)	1	1	12	3
8	1	(0.4)	0	1	0	1

* Number of relatives in categories indicated.

Other relationships found were child, cousin, aunt, uncle, nephew, niece, grandparent, and great grandparent (see Table III).

M, mother ; F, father ; B, brother ; S, sister.

Table XXIV. Number of affected relatives in families with AAA

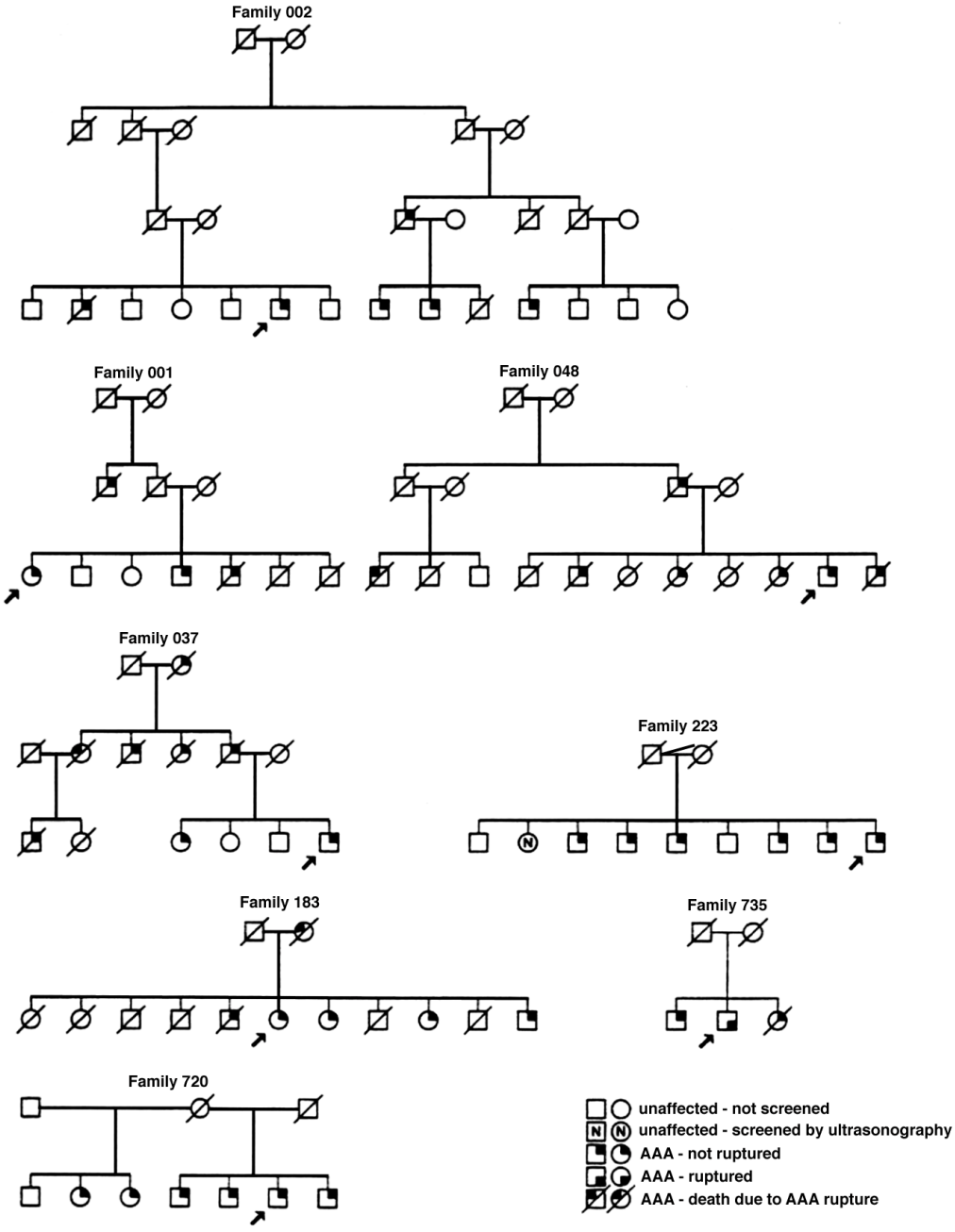


Figure 15 : Representative AAA families from our collection of 233 families. Proband in each family is indicated with arrow. Slash across symbol means death. Other symbols used are explained in insert to figure.

DISCUSSION

Our collection of 233 families with AAA had patients from nine different nationalities, but all of them were white. The underrepresentation of other ethnic groups has been noted by other investigators previously (LaMorte et al., 1995) and is in agreement with the hypothesis that genetic factors contribute to the disease.

In addition, AAAs do not consistently show any one mode of inheritance in the families, suggesting that they are a multifactorial disease with heterogeneous etiology. Most of these characteristics are shared by many other adult-onset common diseases. The approach to study them must, therefore, take into consideration these factors. First, a large collection of families is necessary for initial and subsequent follow-up studies. Second, statistical methodology must be chosen carefully to take into consideration the fact that genetic information from currently unaffected individuals might be misleading because the person's phenotype could change over the years to come.

Previous studies have suggested that although the prevalence of AAAs is lower in women than in men, AAAs might be more aggressive and perhaps more likely to be from accumulation of genetic susceptibility factors if present in women (Katz et al., 1994, Katz et al., 1997, Darling et al., 1989). We therefore investigated the transmission of AAA from one generation to another to see whether females were more likely to pass on the disease to their offspring. To our surprise, we did not find a significant difference in the transmission of the disease between the father and the mother, and both genders seemed to transmit the disease at approximately equal frequency to their offspring. It was, however, noteworthy that in 79% of the observed transmissions, AAAs were transmitted from one of the parents to a son, and in only 21% of the cases, to a daughter.

The objective of our study was to collect as many AAA families and to gather as much information about the affected family members as possible. However, some limitations in our study. With the limitation of this study as discussed in original manuscript (Appendix 11) indicate the predominance of genetic factors on multifactorial/environmental effects in the pathogenesis of AAA. Also it let us to an interpretation of the inheritance mode to be

autosomal recessive rather than dominant. The inheritance pattern in many families was also consistent with pseudodominant inheritance (i.e., apparent dominance from one parent being homozygous and the other heterozygous for a recessive allele). Pseudodominance is not probable for AAA, however, because it requires a high population frequency of mutant alleles. Such a high frequency may be possible if alleles at all loci had equal and additive effects, but that is inconsistent with the conclusion of a major gene effect from segregation studies (Majumder et al., 1991, Appendix 10). In the light of these limitations, the results presented here should be considered conservative estimates about the number of affected relatives in each family and the mode of inheritance.

CONCLUSION

Our study was not designed to compare the familial AAAs to sporadic ones or to identify differences in their risk factors. The data on the 233 multiplex families presented here emphasize the value of routinely obtaining family history from patients with AAA and considering ultrasonographic screening of unaffected siblings to detect AAAs before rupture. It is important that primary care physicians realize that AAAs do run in families even when no signs of Ehlers-Danlos syndrome or Marfan's syndrome are present, making familial AAAs a separate disease entity deserving due attention.

Analysis of coding sequences for tissue inhibitors metalloproteinases (TIMPs) genes in patients with AAA

INTRODUCTION

Tissue inhibitors of metalloproteinases (TIMPs) are major inhibitors of metalloproteinases (MMPs) (Dollery et al., 1995, Werb, 1997). A preliminary study on decreased levels of TIMPs in AAA has been reported by Brophy (Brophy et al., 1991). Furthermore, the ratio of (MMP)mRNA amount to TIMPmRNA was higher in AAA than in

normal aorta when assayed using competitive RT-PCR with gene-specific external standards (Tamarina et al., 1997). The relative deficiency observed in AAA tissues could be due to local tissue conditions inhibiting the expression or mutation in the primary structure of the TIMP genes. There at least four members in the TIMP family, namely TIMP1 (Docherty et al., 1985), TIMP2 (Stetler-Stevenson et al., 1990), TIMP3 (Apte et al., 1994) and TIMP4 (Greene et al., 1996). All four have been cloned and sequenced and the chromosomal localizations have been determined for TIMP1, 2 and 3. TIMP1 resides on the X chromosome (Spurr et al., 1987), TIMP2 was placed on chromosome 17 (DeClerck et al., 1992), and TIMP3 was localized on chromosome 22 (Stetler-Stevenson et al., 1990). In a first step with the collaboration of Professor Kuivaniemi we studied the coding sequences of TIMP1 and TIMP2 in patients with AAA and/or intracranial aneurysm (IA) to determine whether mutations in the TIMP genes are associated with these diseases (see Appendix 12).

MATERIAL AND METHODS

The materials are described previously in chapter 10 and the genomic DNA from AAA-patients was isolated either from cultured skin fibroblasts or from blood. Control DNA samples were isolated from buffy-coat fractions purchased from the American Red Cross using automated DNA isolation procedure (see Appendix 12 for details about sequencing of cDNA and confirmation and frequency of sequence variations). The methods are described in the appendix 12. The patients studied in this study included 19 individuals. The cDNA sequences of type III procollagen were previously determined for all these individuals and were found to be normal (Kuivaniemi et al., 1993, Tromp et al., 1993). Type III collagen was therefore excluded as a candidate gene for aneurysm in these individuals.

RESULTS

The sequences of the coding region of TIMP1 and TIMP2 were determined in detail (see Appendix 12). The sequence analyses carried out here provided 671 nt of TIMP1 cDNA

sequences (all 621 nt of coding sequences) from each individual. Two sequence variations were found. AAA patient JIMM429 had C/T nt 323 (proline codon at amino acid position 87 changes from CCC to CCT). All the other 18 individuals had C at nt 323. Another sequence change was found at nt 434, also reported by others (Tilson et al, 1993). Six individuals had C at this site, 11 had T and two individuals were heterozygous C/T. The sequence change converted the phenylalanine codon TTC at amino acid position 124 to TT.

For TIMP2, we analyzed 750-nt (all 660 nt of coding sequences), and two sequence variations were identified. AAA patient JIMM398 had C/T at nt 306. Another difference, G to A transition, occurred at nt 573 in three patients (JIMM332, JIMM430 and JIMM257), all of whom were heterozygous for this change. Both variations occurred at the third positions of codons and did not change the amino acids.

No significant differences in the frequencies of the nt 323 polymorphism were found between controls and AAA patients. The frequencies between the two female groups were, however, significantly different (P = 0.0019) for the nt 434 polymorphism.

DISCUSSION AND CONCLUSION

The experimental results have indicated that patients with AAA do not have any sequence variants in the TIMP1 and TIMP2 genes, and that the transitions identified in these two genes do not change the amino-acids encoded. The differences in allele frequencies of rt 573 polymorphism of TIMP2 gene between the control and AAA-groups are interesting preliminary findings that need further follow-up with larger groups and carefully selected control groups for each ethnic group. It is possible that a mutation in the gene for TIMP1 or TIMP2 contributes to the disease process in patients with aneurysms.

Genome scan for familial AAA

Linkage of familial abdominal aortic aneurysm to chromosome 19

INTRODUCTION

One of the principal goals of our multicentric study was to use affected sib pairs (ASPs) collected during the study to identify chromosomal region where certain genes harbor mutations in AAA patients. Finding a susceptibility gene for AAA could lead to a simple DNA test to identify individuals at risk for developing an AAA. Such a test could be extremely useful to pave the way of possible prophylactic drug therapy.

The aim of the present study was to find susceptibility loci for AAA with the use of linkage analysis with covariates to allow for locus heterogeneity (Goddard et al., 2001, Olson, 2002). The method of affected-relative-pair (ARP) linkage analyse was used since it was recognized as useful for identifying genes in complex genetic diseases (Weeks & Lange, 1988). The choise of 2-phase/2-stage design offers cost-effectiveness and minimizes the effort required in genotyping while maintaining statistical power to detect linkage (Guo & Elston, 2000).

METHODS

Subjects and phenotyping

Families with at least 2 members with AAA were identified; details on the family collection have been reported in appendix 11 and are summarized in Table XXV. All families were Caucasian and the number of families in each nationality were as followed: 42 Canadian, 36 Dutch, 23 Belgian, 10 American, 3 British, 3 Finnish, 1 Italian, and 1 Swedish. The study was approved by the institutional review boards of Wayne State University School of Medicine and each patient recruiting center, and the subjects gave informed consent. This investigation was conducted by Professor H. Kuivaniemi in her laboratory in Detroit (USA).

Design for DNA linkage study

An ARP design was used because the mode of inheritance of AAA is unknown and because an unaffected individual may develop an AAA subsequently or carry the susceptibility gene with incomplete penetrance (for more details see Appendix 13).

Genotyping

Before genotyping, polymerase chain reactions were performed. A whole-genome amplification was carried out to increase the amount of template DNA available for genotyping and to ensure that limited resources were used cost-effectively. Additional genotyping on chromosomes 3, 4, 5, 6, 9, 14, and 21 after the whole-genome scan was performed by deCODE Genetics Inc (for details see Appendix 13).

Category	Group 1	Group 2	Total
No. of families	36	83	119
Average No. of affected individuals per family (range)	3.4 (2-7)	3.1 (2-7)	3.2 (2-7)
Families with at least 4 affected first-degree relatives (%)	9 (25)	29 (35)	38 (32)
No. of affected individuals genotyped (male)	86 (75)	195 (155)	281 (230)
Status of AAA in			
Individuals genotyped (male)			
Elective surgery	67 (61)	131 (104)	198 (165)
Rupture	8 (7)	11 (11)	19 (18)
Detected by ultrasonography	11 (7)	53 (40)	64 (47)
Other affected individuals not genotyped (male)*			
Elective surgery	12 (11)	24 (20)	36 (31)
Rupture	10 (8)	17 (14)	27 (22)
Detected by ultrasonogrpahy	2 (1)	10 (6)	12 (7)
Unconfirmed	5 (3)	7 (4)	12 (7)
Unaffected individuals genotyped (male)	52 (20)	67 (26)	119 (46)
ASPs genotyped	62	151	213
Other ARPs genotyped	13	9	22
Total ARPs in study	75	160	235

*There were a total of 87 (29 in group 1 and 58 in group 2) affected first-degree relatives who were known to have AAA but from whom no sample was available because of death before the start of the study (n = 73) or unwillingness to provide a sample (n = 14). In 5 and 7 such cases in group 1 and 2, respectively, information about details of AAA was not available.

Table XXV. Characteristics of AAA Families

Statistical analyses

To allow for covariate-related locus heterogeneity, a covariate-based ARP LOD score method was applied (Olson, 2002). The model is a 1-parameter modification of the conditional logistic parameterization of the ASP LOD score introduced by Olson (Olson, 2002). An optimal mode of inheritance parameter (Whittemore & Tu, 1998) is specified that allows one to fit only a single additional parameter per covariate.

RESULTS

Whole Genome Scan with 36 AAA Families

A whole genome scan was performed with 36 AAA families including 62 ASPs and other 13 ARPs. Altogether 12 regions on chromosomes 3,4,5,6,9,14,19 and 21 were identified with a covariate effect significant at the $\alpha = 0.01$ level.

Follow-up Studies with 119 AAA Families

Twelve regions that were significant in the whole-genome scan were selected for a follow-up study, and additional microsatellite markers were genotyped in the 36 families and in 83 new AAA families that included 151 ASPs and 9 other ARPs (groups 1 and 2; Table XXV). Three loci (68 and 132 cM on chromosome 4, and 141 cM on chomosome 5) showed some evidence of linkage in group 2 (Table II in Appendix 13), and these regions were selected for detailed analyses (Table III in Appendix 13). Table III shows the LOD scores and parameter estimates for groups 1 and 2 as well as the total sample at the location that gave the highest LOD score for the total sample. In the combined analysis with groups 1 and 2 together, the locus on chromosome 5 did not appear significant. The region at 140 cM on chromosome 4 had a LOD score of 3.73 ($P = 0.0012$) The 70 cM region had a peak LOD score of 3.13 ($P = 0.0042$), although the parameter estimates were unstable (not shown), and

we therefore report the LOD score of 2.41, which was 4 cM away from the peak, to be able to give more accurate parameter estimates.

Chromosome 19

The chromosome 19 region was also analyzed further because (1) it had the second highest LOD score in the original genome scan (Table II, Appendix 13); (2) Olson et al. have recently identified a putative locus for intracranial aneurysms on chromosome 19 (Olson et al., 2002), and (3) it contains a large number of biologically plausible candidate genes (National center for Biotechnology Information. Available at : http://www.ncbi.nlm.nih.gov, 2003). The highest LOD score on chromosome 19 for group 2 was 4.12 (P = 0.00054) near D19S416 and 58.69 cM from the p-terminus when sex, N_{aff}, and their interaction were used as covariates. In the combined analysis with groups 1 and 2, including 213 ASPs and 22 other ARPs, the maximum LOD score was 4.75 (P = 0.00014) at 58 cM, just proximal to D19S416, with sex, N_{aff}, and their interaction as covariates. The interaction term (sex*N_{aff}) was significant in the total sample (P = 0.00317) as well as in the 2 subsamples. These results suggested that female-female pairs from families with larger numbers of affected persons are most at risk from this locus, although this locus also gives substantial risk to male-male pairs from families with fewer affected persons. Both groups 1 and 2 had the peak LOD score at same location on chromosome 19. The best, most parsimonious model was the one with N_{aff} as a covariate in group 1 and a model using sex, N_{aff}, and their interaction as covariates in group 2 (for more detail, see Appendix 13).

DISCUSSION

No evidence of linkage was found no evidence of linkage unless sex and number of affected persons were included as covariates in the linkage model. How then should our results be interpreted ? As Dizier and coworkers (Dizier et al., 2000) have shown, absence of a linkage signal can be due to a factor on which the siblings differ, such as a characteristic of the disease (eg, severity), or an environmental factor. For common diseases that are genetically complex, such situations may be the rule rather than the exception (Goddard et al., 2001, Olson, 1999, Olson, 2002). By allowing for heterogeneity in the analysis by including

covariates chosen a priori, we avoid these concerns and are able to detect linkage signals obscured by the presence of heterogeneity.

There has been no previous DNA linkage studies with AAA. Three studies, however investigated familial thoracic aortic aneurysms and dissections (TAAD) and identified linked loci on 5q (Guo et al., 2001) 11q (Vaughan et al., 2001) and 3p24-25 (Hasham et al., 2003). It is noteworthy that no patient with thoracic aortic aneurysms or dissections was included in our collection of AAA families.

There are several plausible candidate genes in the 2 regions with the highest LOD scores, such as IL15 (interleukin 15; a plausible candidate gene with respect to inflammation in AAA), GABI (GRB2-associated biding protein 1; an important mediator of branching tubulogenesis and a central protein in cellular growth response, transformation, and apoptosis), and EDNRA (endothelin repeptor type I; an endothelin-1 receptor expressed in many human tissues with the highest level in the aorta) around 140 cM on chromosome 4, as well as LRP3 (LDL receptor-related protein 3), HPN (transmembrane protease, serine 1; a serine-type peptidase involved in cell growth and maintenance), PDCD5 (programmed cell death 5; a protein expressed in tumor cells during apoptosis independent of the apoptosis-including stimuli), and PEPD (peptidase D; an Xa-Pro dipeptidase important in collagen catabolism) on chromosome 19. (National Center for Biotechnology Information. 2003, HUGO Gene Nomenclature Committee. 2003) LRP3 is particularly interesting because conditional knockout mice for LRP1, another member of the gene family, developed arterial aneurysms and atherosclerosis (Boucher et al., 2003).

It is likely that additional AAA loci will be identified by testing other possible covariates, such as smoking, hypertension, and coronary artery disease, which was not possible in this study because these risk factors are so common both in the general population and in patients with AAA that the relatively small number of families in this study did not provide enough power to study them.

CONCLUSION

Our genetician group have identified susceptibility loci for AAA on chromosomes 4q12, 4q31 and 19q13 by a whole genome scan using ARP linkage analysis with covariates to allow for heterogeneity. By analyzing two independent cohorts, the presence of these region was confirmed. These loci will be the focus of future studies to identify genes contributing to the development, growth and rupture of AAA.

GENERAL CONCLUSIONS AND PERSPECTIVES

Abdominal aortic aneurysm being a serious medical problem, we conducted a one city-based epidemiologic study to assess the prevalence of AAA in our population; 4% of the 65- and 75-year-old males in Liège were found to be affected by the disease. The presence of hypertension and smoking habits was significantly higher in AAA group as compared with non-AAA group. Our results demonstrate that ultrasound is a cost-effective method for routine screening in men older than 65 years. In addition, recent reports demonstrated that screening 65-80 year-old males for AAA could reduce the incidence rate of ruptured AAA by 49%.

Surgical repair of AAA in selected octogenarians can be done with a reasonably low morbidity and mortality. At our department, elective repair in octogenarians is performed with a mortality rate that is 1.3 times higher than that for non-octogenarians (4.7% versus 3.6% for the period 1990-1996). The same is true for urgent or emergent repair. This is in agreement with the literature data since most contemporary reports on elective AAA repair in patients aged 80 years or older mention an operative mortality that is approximately two times higher than in younger patients (Dean et al., 1993, Glock et al., 1990, O Donnell et al., 1976, Treiman et al., 1982, Robson et al., 1989, Paty et al., 1993). For emergent repair or ruptured AAA, the difference in results, observed between young and aged patients, is less evident. As discussed in chapter II, outstandingly good results as obtained at the Cleveland Clinic (O Hara et al., 1995) are not achieved by all centers. Elderly patients often present concomitant systemic diseases with resultant increase in the relative operative risk. For “otherwise healthy” octogenarians free from vital organ diseases, elective AAA repair can be done with no mortality. For intermediate-risk and high-risk patients, mortality rates increase to 6.25% and 10%. In case of AAA, it is worthy noted that, once again, the difference in mortality between the different risk categories is no longer evident. This can be explained by the intrinsic fatality of ruptured AAA, with the spectrum of hypovolemic shock and multiorgan failure, a leading

cause of postoperative death whatever the presence of co-morbid conditions (Dean et al., 1993, Glock et al., 1990, O Hara et al., 1995, Johansen et al., 1991).

Based on the above data, controversy still exists about the management of ruptured AAA in elderly (Dean et al., 1993). A decade ago, it was recommended to withhold surgery for ruptured AAA in patients 80 years old or older (Buck et al., 1987). Also argued was the tremendous cost for the health insurance. At our institution, the cost to save one patient with ruptured AAA was evaluated at 30,000 euros, versus 9.000 euros for successful elective AAA repair (Goffard, 1993). Dean (Dean et al., 1993) and Johansen (Johansen et al., 1991) calculated similar costs for both groups. In a more recent report from the Mayo Clinic, no single preoperative criterion could be identified that justifies to deny surgery for patients with a ruptured AAA (Gloviczki et al., 1992). Forty-four percent of their octogenarians, operated on for rupture, survived. Thus we favor surgery for all ruptured AAA reaching the hospital alive, since it is the only reasonable chance of survival that can be offered to the patient (Budd et al., 1989, Gloviczki et al., 1992).

In conclusion, according to our local experiences, AAA repair can be performed safely in carefully selected octogenarians, even if the results are not as excellent as in the younger age group. Thus, advanced age, on itself, should not be an exclusion criterion for AAA repair. Management of octogenarians with a non ruptured AAA of 50 mm or more requires sound clinical judgment in each single case, and a complete information of the patient and of his family. On the basis of our data, we recommend a straightforward surgery for “otherwise healthy” octogenarians with a 50 mm or more AAA, with a rapidly expanding AAA (growth rate exceeding 0.5 cm / 6 months) or with a symptomatic AAA. For “at risk” patients, we adopt a watchful waiting and AAA surveillance up to 60 mm. For unfit individuals with life expectancy less than 2 years and for mentally or physically severely disabled patients, no surgery should be performed.

Considering all the above elements, it was logical to attempt to discriminate patients at higher risk of rupture. This was one of the basis of our study about PET scan and metalloproteinase activities. Our preliminary study on PET evaluation of AAA suggests a possible correlation between 18-FDG uptake by the aneurysm wall and the triggering of MMP

activity leading to rupture. Our recent observation supports a relation between positive PET imaging and clinical status of AAA. Therefore a positive PET imaging could be an additional factor for recommending surgery.

Elastic fibers and fibrillar collagens are the main determinants of the mechanical properties of the aorta and in our doctoral thesis, we observed abnormalities of elastine and collagen in the AAA wall. Elastin and associated proteins form a network responsible for the visco-elastic properties of the arterial wall. Elastin is stabilized by molecular cross-links. Elastic fibers associated with smooth muscle cells (SMCs) are most abundant in the media of the aortic wall. Collagen, in polymeric form, is also a significant component of the media and the surrounding fibrous adventitia. Two specific types of fibrillar collagen (types I and III) provide tensile strength and help to maintain the structural integrity of the vascular wall. Beside elastic and collagen fibers, proteoglycans are also involved in the organization of the aortic wall (Melrose et al., 1998). In the aortic wall, elastin and collagen can be degraded by specific proteases displauing elastase and collagenase activity. These proteases are produced by resident cells of the vascular wall (medial SMCs and adventitial fibroblasts) and by the cells of the lympho-monocytic infiltrate. These inflammatory cells in the media and adventitia arise from the aortic blood but also from the medial neovascularisation which is characteristic of the AAA (Herron et al., 1991, Holmes et al., 1995, Shah, 1997). Leukocyte recruitment into the aortic wall is promoted by elastin degradation fragments as well as pro-inflammatory cytokines, chemokines, and prostaglandin derivates released by both resident mesenchymal cells and the inflammatory cells themselves (Koch et al., 1993, Newman et al., 1994, Walton et al., 1999, Hance et al., 2002). The proteolytic enzymes degrading elastin and collagen are defined as Matrix MetalloProteinases (MMPs) locally activated by either other MMPs or by plasmin generated by plasminogen activators (Carrell et al., 2002, Defawe et al., 2003, Rao et al., 1996, Reilly, 1996, Sakalihasan et al., 1996, Thompson et al., 1996, Carmeliet et al., 1997, Curci et al., 1998, Davis et al., 1998, Mao et al., 1999, Shi et al., 1999, Nollendorfs et al., 2001, Silence et al., 2001, Longo et al., 2002, Tromp et al., 2004, Eriksson et al., 2004). The role of MMPs and plasmin in AAA development has been confirmed in animal models (Carmeliet et al., 1997, Silence et al., 2001, Longo et al., 2002, Anidjar et al., 1990, Pyo et al., 2000, Dobrin, 1999, Ailawadi et al., 2004, Deng et al., 2003). As presented in (chapter 4), we

therefore investigated the potential mechanisms that could be determinant in elastin and collagen degradations. The activities of two metalloproteinases - MMP-9 and MMP-2 respectively released by inflammatory cells and SMC- were measured. The wall of aneurysmal aorta displays a significant level of metalloproteinase activity. A gradient of concentration of MMP-9 still exists between the aortic wall and serum. This suggests that the MMP-9 could arise from the serum and diffuse through the parietal thrombus towards the aortic wall. The development of AAA is also associated with a mural thrombus in the majority of the cases. In contrast to arterial occlusive diseases (AOD), blood flow is maintained in AAA resulting in a continuous remodeling activity of the thrombus components. The evolution of the aneurysmal diameter has been reported to correlate with the plasma concentration of fibrin formation and degradation by products (Yamazumi et al., 1998) as well with concentration of the circulating complex plasmin-$_2$-anti-plasmin (Lindholt et al., 2001) potentially related to thrombus turnover. The role of an adherent thrombus in aneurysmal degeneration has also been investigated. While the thrombus may significantly reduce aneurysmal wall stress, its increasing thickness leads to local hypoxia at the inner layer of the media; this may induce increased medial neovascularisation and inflammation (Vorp et al., 2001). The implication of the thrombus as a source of proteases contributing to aneurysmal evolution has also been suggested after the initial report of a high MMP-9 activity in the thrombus (Sakalihasan et al 1996). In addition, Fontaine et al.(Fontaine et al., 2002) provided evidence of polymorphonuclear neutrophils (PMN) trapping and MMP-9 storing within the aneurysmal thrombus. They also demonstrated the presence of plasminogen in the thrombus and its activator (u-PA) in the aneurysmal wall. This might result in local generation of plasmin, an activator of MMPs, in the aneurysmal wall. In contrast, the MMP-2 would originate from blood vessel wall. Histological examination of samples showed an infiltrate of inflammatory cells in the AAA wall, which might also explain the higher proportion of MMPs in the AAA walls and the activation of MMP-2 and MMP-9. Metalloproteinase are released by activated inflammatory cells. Activation of inflammatory cells may induce an oxidative stress, resulting in lipid peroxidation. In the study presented in chapter 9, we observed significantly lower plasmatic Vit E levels (a lipophilic antioxidant) in AAA patients compared to age-matched control patients presenting with coronary heart disease and in healthy

volunteers. These observations suggest that plasmatic Vit E concentration might be indicative of an increased oxidative stress in patients bearing AAA.

In aneurysmal and other sclerotic aortic diseases, an inflammatory infiltrate is present. In AAA, the inflammatory cells are present in the media and the adventitia, while this infiltration occurs mainly in the intima during aortic occlusive disease. As reported in chapter VI, we investigated, therefore, the role of MMPs, their activator (uPA) and their inhibitors on the remodeling of the abdominal aorta both in aortic atherosclerotic and aneurysmal lesions. Our results demonstrate that the aneurysmal and the atherosclerotic lesions are characterized by a similar largely increased proteolytic process. In contrast, the counterbalance by increased level of protease inhibitors and structural proteins involved in the repair process differs between the two pathologies. In fact, the profile of mRNA gene expression of TIMP-2 and PAI-1 is significantly different between AAA and aortic atherosclerosis. Although, in the aneurysmal wall, the amount of Tissue Inhibitors of Matrix metalloProteinases (TIMPs) is also increased (Thompson et al., 1996), the balance proteases/antiproteases seems to be in favor of proteolysis (Defawe et al., 2003, Knox et al., 1997, Tamarina et al., 1997). The significance of this imbalance during AAA development is reinforced by experimental studies in which the antiproteases are overexpressed or genetically inactivated (Allaire et al., 1998, Allaire, Hasenstab et al., 1998, Rouis et al., 1999). In one report (chapter 6), we had the opportunity to demonstrate an heterogeneity in the activity of the MMP and in the degree of infiltration inside the aortic wall of a ruptured aneurysm. Cell infiltration and metalloproteinases expression were increased at the site of rupture.

Genetic aspects of AAA have been the subjects of very few studies. Because of the irregular clustering of cases, some authors inferred that these aspects were miscellaneous and ethnical dependent. In an non-prospective and non-systematic study, Tilson & Seashore (Tilson & Seashore, 1984) presented 50 families, including three pairs of identical twins, collected by various teams. No data were given either on the mode of ascertainment or on the number of isolated AAA in the same population. Based on an empirical approach to the results, the authors favored a frequent X-linked dominant form and a less common autosomal dominant, or a multifactorial model.

In the Blotchy mouse, deficiency in crosslinking of collagen and elastin is the consequence of a decrease in the conversion of lysine to lysine-aldehyde residues resulting from a mutation on chromosome X (Andrews et al., 1975). This condition is associated to decreased skin resistance and to AAA development propensity. This congenital biochemical defect is close to the pathology resulting from administration of lathyrogens in Turkey (Gresham & Howard, 1961) and mouse (Brophy et al., 1988), which prevents the formation of aldehydes by inactivating lysyl-oxidase. Experimental deficiency in copper (a cofactor of lysyl-oxidase) may also induce aneurysms in the pigs and in the chicken. Although those experiments represent adequate experimental models for inborn defects associated to AAA formation, caution is advisable before extrapolation to the human condition for two reasons. Firstly, the aneurysmal transformation takes several decades in man whereas in animal models it requires a relatively short time ; secondly, the composition of the aorta in term of fibrous protein is not strictly similar in man and animals. This requires a more complex and fine analysis of genetic transmission of AAA in humans.

Most studies of common diseases assume that genetically determined factors are numerous and give an equal and individually small contribution to the phenotype. In polygenic models, a quantitative trait (with threshold) or a qualitative trait results from addictive effects of several biallelic (A,a) loci, each locus having a same weighted effect. Loci are unlinked (independent transmission), and heterozygotes (Aa) contribute to an effect, which is intermediate between the effects of both types of monozygotes (AA, aa). Each locus accounts for a part in the genetic variance of the population. The parent-child and child-child correlations are 50% for each locus. In those models, formal genetic analysis is limited to the computation of heritability, which is the proportion of total phenotypic variance due to polygenic effects. Assuming a multifactorial model, in a series of 60 patients with 25 of them having a positive familial history, a heritability of 70% was calculated using Falconer's method (Powell & Greenhalgh, 1987).

A segregation analysis is basically the comparison of the observed proportion of affected offspring with the expected proportion according to a particular genetic hypothesis. To assess evidence of major gene in the presence of other familial sources of correlation

(polygene, sociocultural factors, etc…), pedigrees may be analyzed using the pointer strategy (Morton & Lalouel, 1983). This methodology was developed as a tool for analysis of multigenerational pedigrees and is based on the mixed model, a mathematical model combining genetic and multifactorial components (Elston & Stewart, 1974, Lalouel & Morton, 1981, Morton & McLean, 1974). It includes modifiable parameters, which allow the definition of several submodels (purely dominant, multifactorial, etc…). The likelihood of the observed sibships conditional on the phenotype of the parents is computed and maximized through fine-tuning of those transmission parameters. The likelihood of the general model (mixed) is compared with appropriate statistical tests to restricted submodels, the best model being the more likely or, for non-significant differing submodels, the most parsimonious in terms of parameters. Cumulative incidence of the trait in the population (stratified, if necessary, for age and sex in several subgroups) and ascertainment probability of the samples must be known. It implies that reliable epidemiological data are available for the studied population. Genetic heterogeneity cannot be directly addressed by this mode of analysis.

The analysis of AAA by the segregation method was attempted by only our team and by Majumder's team. In Majumder's work collecting 91 probands,an autosomal diallelic locus with a recessively inherited gene for AAA was considered as the most likely genetic model (Majumder et al., 1991, Webster et al., 1991, Webster, St Jean et al., 1991). A multifactorial component did not increase significantly the likelihood of the data set. In contrast our own analysis of 324 probands (chapter 10), favored a simple autosomal dominant model, with a carrier frequency of 1 of 250 and an age-related penetrance never higher than 0.4, even in the subgroup of elder men. Like Majumder et al, we did not oberveved a significant better fit with a multifactorial component. Whether differences in the inheritance mode between the two studies come from differing ascertainment, variable definitions of the affected status, or differing local epidemiology (suspected on the basis of our unusually high sex ratio) remains unknown. For example the mode of selection of Majumbder's cases does not appear clearly. Their sample of familial cases was very small.A simple explanation would be that their sample, by chance, contained less pedigrees with subjects affected in two generations. Methodology was different. They considered AAA when aortic diameter was > 50 mm; we used a more common definition as discussed above. Nevertheless, the importance

of a single genetic factor if the appearance of AAA remains the most important lesson resulting from those formal genetic works. The difference between sexes may nevertheless indicate that an adjuvant factor (hypertension, atheromatosis) may act as a modifier on penetrance and/or expressivity of the disease (for more details, see Appendix 10 and 11).

In our investigations about the familial aspects of AAA, we identified a group of patients where rupture occurred more often and earlier than in sporadic groups. As ultrasound allows simple, non-invasive, accurate detection and follow-up of AAA, we therefore recommend ultrasound screening of first-degree relatives aged 50 years and older. Considering the higher incidence of rupture in patients with positive family history and the risk of rupture even for small AAA (a maximum diameter between 40 and 55 mm), a more aggressive therapeutic attitude is mandatory.

Professor Kuivaniemi and her team studied different possible biochemical and genetic factors involved in the development of aneurysm. In a study of DNA sequences for type III procollagen, they concluded that mutations in type III procollagen are an infrequent cause of AAA disease. In view of our largest familial AAA samples, they contacted us to perform a multicentric AAA study. The screening study increased the number of those family members known to have AAA and available for the genetic study. The screening of asymptomatic family members of AAA-patients with ultrasonography, should detect those individuals who have dilatations of AAA, but have not suffered a rupture nor had any symptoms caused by the AAA.

During this multicentric familial screening program, 233 families were identified, in which at least two members had an AAA with an average of 2.8 AAA cases/family. In contrast to our own single institution study, the inheritance mode was autosomal recessive in 72% of the families and autosomal dominant in 25% of the families. The origins of such differences in the results of the two studies must be questioned. There was some limitations in the multicentric study. Firstly, there was a difficulty in handling the subjects who were lost from family contact or who were not first-degree relatives of the immediate family. As pointed out in the original discussion (see Appendix 11), this would lead to underestimating the magnitude of a genetic component of AAA. Secondly, ultrasound screening was

performed with a cut-off for 50 years of age. It is possible that the siblings had not reached a minimum age for AAA development. This is the reason why we cannot prove the absence of AAA and, therefore, only the presence of AAA is considered. For example, a subject "truly" unaffected by AAA ("absence of AAA") is difficult to distinguish from a subject free from the disease a the time of screening but in whom AAA will onset later. Thirdly, dead parents with AAA were sometimes included on basis of autopsy record revealing ruptured AAA. Subjects could be missed or not included when the exact cause of death was unknown or when autopsy records were unavailable despite sudden death. This could be especially true with the older generations in the pedigrees and could lead to an interpretation of the inheritance mode to be autosomal recessive rather than dominant. The inheritance pattern in many families was also consistent with pseudodominant inheritance (i.e., apparent dominance from one parent being homozygous and the other heterozygous for a recessive allele). Pseudodominance is not probable for AAA, however, because it requires a high population frequency of mutant alleles. Such a high frequency may be possible if alleles at all loci had equal and additive effects, but that is inconsistent with the conclusion of a major gene effect from segregation studies (Majumder et al., 1991). In the light of these limitations, the results presented of this multifactorial srudy should be considered as conservative estimates about the number of affected relatives in each family and the mode of inheritance.

Parallely, blood samples were collected and analyzed during the multinational study. In the first step, this cooperative international research permitted to determine the coding sequences of TIMP1-2 in patients with AAA. The aim was to investigate the possibility that aneurysms were caused in these individuals by defects in the genes for these inhibitors of metalloproteinases. No mutations were found in the TIMP1 and TIMP2 genes in aneurysm patients. In constrast, the differences in allele frequencies of nt 573 TIMP2 polymorphism between the control and AAA groups are interesting preliminary findings. They however need further investigations in larger groups and in carefully selected control groups for each ethnic group. It is also possible that mutations in the promoter sequence or large scale rearrangement in the genome (not easily detected by the RT-PCR used in the study) are implicated.

In a second step, affected sib pairs collected during the study were used to identify chromosomal region that certain genes harbor mutation in AAA patients. The eventual susceptibility locus for AAA was identified on chromosome 19 in a region containing candidate genes as LRP, HPN and PEDP3 that are potentially relevant to the pathogenesis of AAA.

In summary, from clinical observations has emerged the concept that AAA has a familial incidence, linked with specific genes, the locus of which is going to be identified; the gene expression leads to the activation of metalloproteinases that results in aortic wall protein alteration. This unifying concept will, undoubtedly, help to better management of the treatment and prevention of AAA.

At the end of the present work , what are the perspectives of future development?

In a first step, we would like to create a bank of biological material containing a collection of samples of tissue and blood samples from well-characterized patients. This material would be used for biochemical and genetic studies (Figure 16).

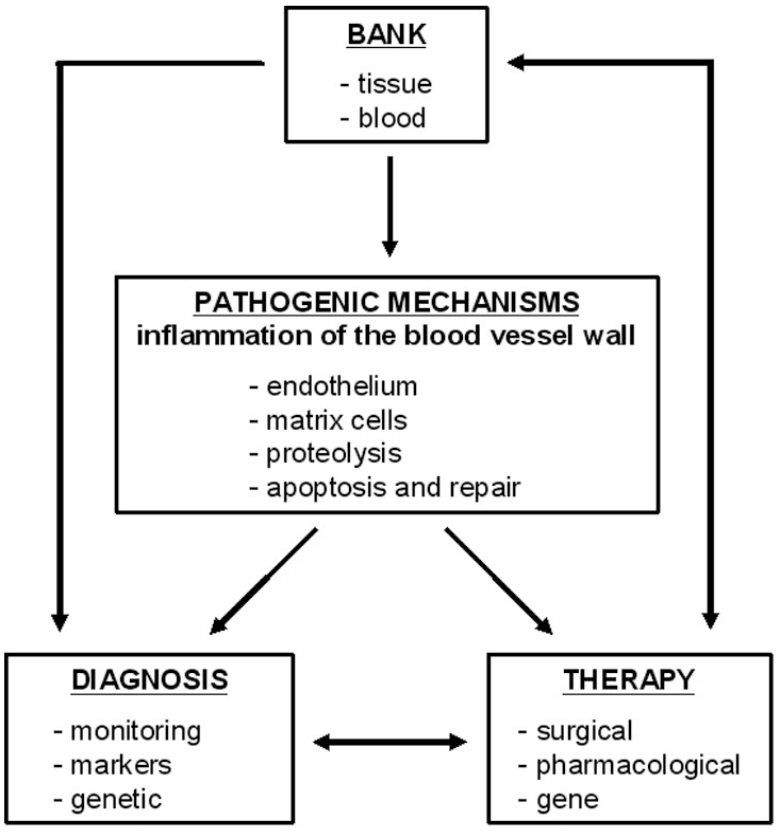
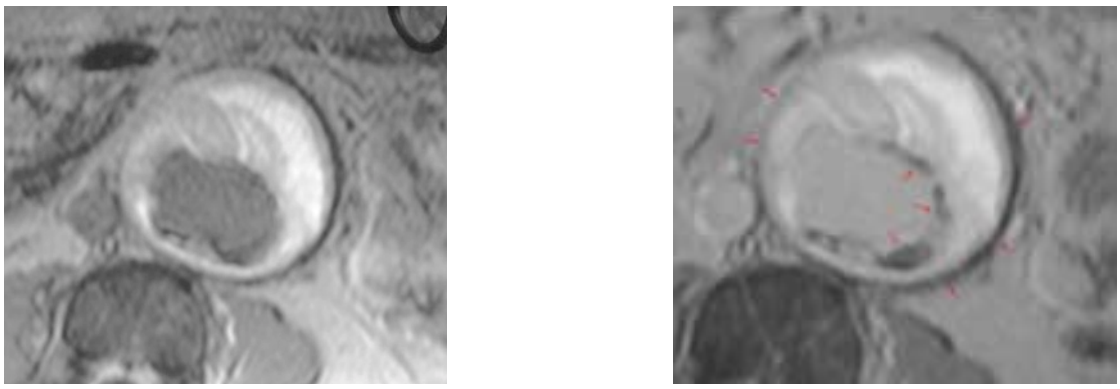


Figure 16. The Abdominal Aortic Aneurysm (AAA) Project

For the present, the knowledge that we seek to accumulate will allow the elaboration of a better therapeutic classification for surgery. Preliminary data obtained by Positron

Emission Tomography scanning (PET scanning) imaging of the inflammatory infiltrate in the blood vessel wall using non-specific markers (18-FDG) have been obtained. We will compare the PET imaging with morphological and biochemical analyses of specimen of the excised aneurysm wall. These observations should provide more insight into the pathogenesis of aneurismal disease, and may help us to monitor progression of AAA.

Since we hypothesized that the clinical status of AAA with 18-FDG uptake by the aneurysmal wall is linked to the presence of an inflammatoryinfiltrate, we recently investigated the possibility to in vivo demonstrate the presence of macrophages. In a study,with the collaboration of the departement of the radiology and pathology of the St Joseph hospital in Liège, we used magnetic resonance imaging (MRI) magnetic resonance to detect an inflammatory activity of the aneurysmal wall using blood-pool MRI contrast agents like supra paramagnetic iron oxide (SPIO) particles. The SPIO is known for his affinity for the inflammatory cells, especially monocytes and macrophages (Weissleder et al. 1990). It has been hypothesized that macromolecular SPIO particles of a diameter similar to that of LDL (15-25 nm) enter in atherosclerotic plaques with increased endothelial permeability and that they accumulate in plaques displaying a high macrophage tissue content (Schmitz et al., 2000). The MR images of AAA wall with labeled macrophages MR (Figure 17) are correlated with histological examinations of the aneurysmal wall and thrombus (Figure 18).



A B

Figure 17. Axial 2D gradient-echo MRI at the level of the infrarenal aneurysmal aorta prior (a) and one hour after (b) SPIO administration. The aortic wall and parietal calcifications are clearly visible (a, arrow). On the postcontrast image, a heterogeneous pronounced signal loss related to SPIO uptake at the level aortic wall is seen (b,arrow).

In the near future PET and MR examinations will be performed in patients recruited for open AAA surgery.In a first step, the images obtained by two differents techniques will be confronted and correlated. In a second step, after localisation of the region of 18-FDG and SPIO uptakes with CT-PET and MR angiography respectively, tissues, luminal and parietal thrombus specimens will be resected during surgery. Blood samples will also be collected before surgery. Then biochemical andhistological results will be correlated with anatomical functional imaging.

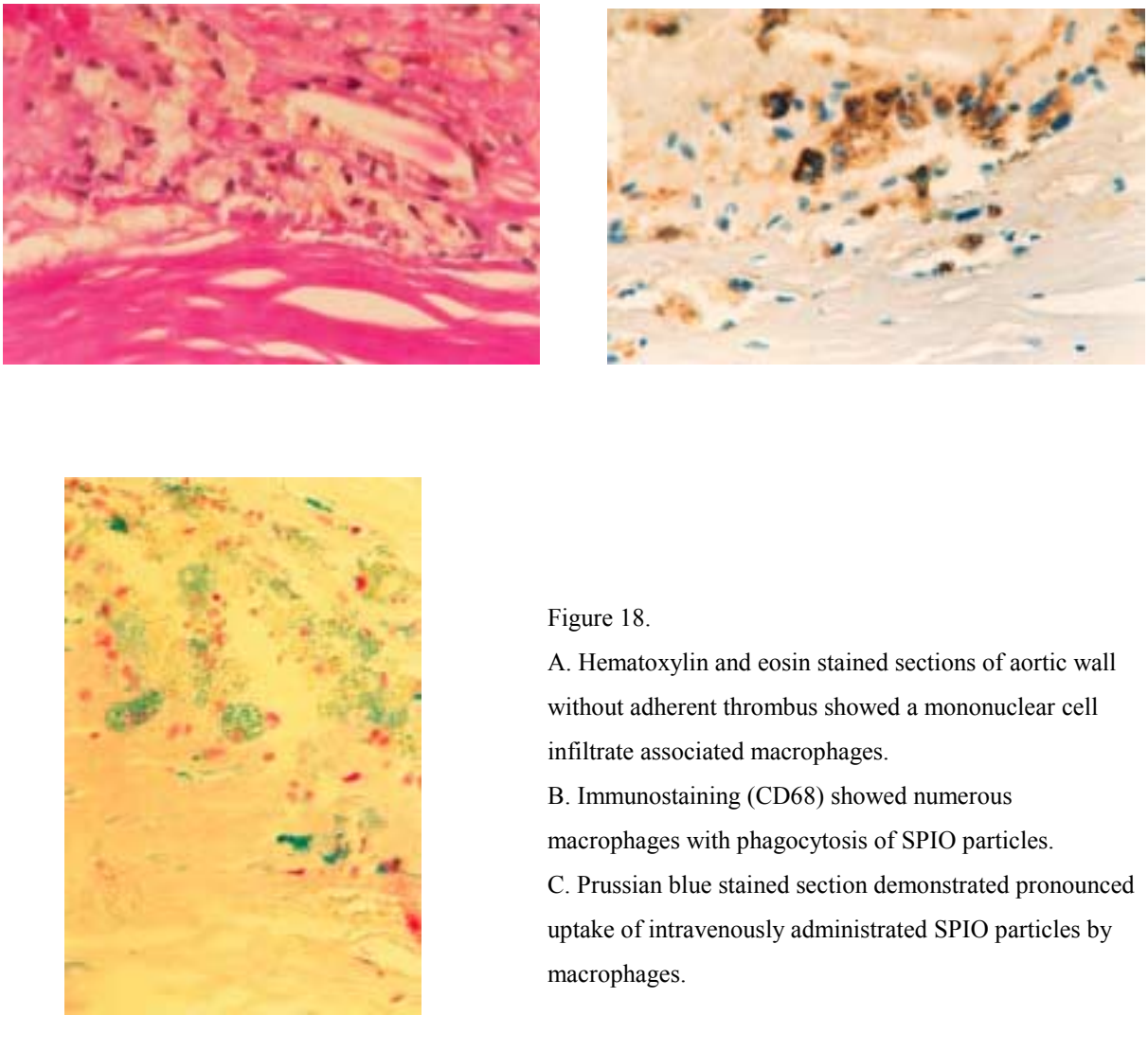


Figure 18.
A. Hematoxylin and eosin stained sections of aortic wall without adherent thrombus showed a mononuclear cell infiltrate associated macrophages.
B. Immunostaining (CD68) showed numerous macrophages with phagocytosis of SPIO particles.
C. Prussian blue stained section demonstrated pronounced uptake of intravenously administrated SPIO particles by macrophages.

One interesting future perspective is to stop or to slow the expansion of AAA. Complete arrest of tobacco smoking is one prerequired prophylactic measure for AAA since it allows a reduction of its growth rate (Brady et al., 2004). The central role of the matrix metalloproteinases in AAA development and rupture supports the interest of pharmacologic inhibitors of these proteases. Tetracyclines provide a potentially effective therapy. As shown in animal models (Curci et al., 1998), doxycycline, a synthetic tetracycline derivative, is able to prevent MMP-mediated aneurysmal growth. Moreover, one clinical study suggested that doxycycline treatment prevents aneurysm growth in man (Baxter et al., 2002). The use of synthetic MMP activity-inhibitors, such as batimastat (BB-94), has also been shown to suppress the expansion of experimental AAA (Bigatel et al., 1999, Moore et al., 1999). However, Defawe et al. (Defawe et al., in press) recently showed that MMPs may alter matrix remodeling independently of their proteolytic function suggesting that the role of MMPs may be more complex than mediating a degradation process. Another attractive option of aneurysmal pharmacotherapy is to target the inflammatory response and interfere with the MMPs pathway. Non-steroidal anti-inflammatory drugs, such as indomethacin, are known to prevent AAA development in animal models (Holmes et al., 1996). In the past decade, a number of substances have been proposed for the treatment of asymptomatic AAAs. The use of β-blocking agents (e.g. propranolol) seems to attenuate the growth rate of large (>5 cm) AAAs and even reduce the size of experimental AAAs (Gadowski et al., 1994, Slaiby et al., 1994). Nevertheless, a recent Canadian randomized trial reported that propranolol does not have a significant effect on the growth rate of small AAAs (Propranolol for small abdominal aortic aneurysms : results of a randomized trial, 2002). Besides their cholesterol lowering effects, statins reduce the expression of various inflammatory molecules including MMPs (Bellosta et al., 1998). The addition of cerivastatin in AAA tissue organ cultures has been shown to down-regulate the production of MMP-9 suggesting that members of the statins family may prevent elastolysis in patients with AAA164. Long-term statin use in patients who underwent successful AAA surgery was also associated with reduced mortality (Kertai et al., 2004). Several innovative experimental studies demonstrated the potentials of cell therapies (e.g. seeding of cells overexpressing antiproteases in aneurysmal walls) to prevent aneurysmal progression and rupture in animal models (Allaire et al., 2002, Gomes et al., 2001, Allaire et

al., 2004). We plan to determine the role and effect of statins in in vitro models well established model of SMC culture in the "Laboratoire de Biologie des Tissus Conjonctifs". These in vitro results will constitute a basis for determining the opportunity and the modalities of an in vivo clinical study designed for investigation of the effect of statins on the progression of AAA.

Our work on genetic risk factors for AAA will contribute to the scientific community of AAA research by identifying genes and pathways that are important for the pathogenesis of AAA. This will perhaps open new avenues of research for other groups investigating the function of candidate genes in transgenic animals and possible pharmacological intervention in the identified pathways. Our work will also advance related fields of research such as statistical genetics by providing real data sets used for testing new statistical approaches for data analysis of complex diseases.

Future studies will be centered on the collection of another set of ASPs. They will be analyzed in order to identify candidate loci, using markers located more closely to each other (2 cM) than the ones used in the first screen. Then, candidate genes located in the support interval, will be analyzed for the presence of mutations (genetic alterations). If such mutations are detected, simple DNA tests can be designed to test individuals at risk to develop in each family those harboring mutations and being, therefore, at higher risk to develop an aneurysm.

The long-term goal of the AAA project is to identify the gene or genes that harbor mutations in patients with AAA. The results obtained in this studies are likely to yield important information regarding the genetic factors contributing to the development of AAA, and could provide the basis for genetic testing to identify those individuals at risk for developing AAA. Such individuals could then be monitored by ultrasonography to detect the dilatation of abdominal aorta and surgically repair the aneurysms before they rupture and lead to massive bleeding and often sudden death.

The past five decades have been marked by continuing progress in diagnosis, management, timing of interventional therapy and assessment of endovascular repair versus conventional surgery. Hopefully, the upcoming decades will provide preventive treatments

that could be applied to selected groups of individuals identified as high risk for AAA by genomic and/or imaging technology. The first goal in AAA history was the prevention of rupture; the next aim will be the prevention of AAA growth.

Tomorrow, a better understanding of the biological processes responsible for the mechanical failure of the blood vessel will generate the prospect for a pharmaceutical prevention of the simple occurrence of AAA.

Can we hope that the additional knowledge, however modest, issued from our research, will contribute to its development ?

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PERTINENT PUBLICATIONS POSTERIOR TO THE “THESE DE DOCTORAT EN SCIENCES CLINIQUES A L’UNIVERSITE DE LIEGE” 1994

APPENDIX 1

Routine ultrasound screening for abdominal aortic aneurysm among 65- and 75-year-old men in a city of 200,000 inhabitants. C. Vazquez, N. Sakalihasan, J.B. D'Harcour, R. Limet. Ann Vasc Surg 1998;12:544-549

Routine Ultrasound Screening for Abdominal Aortic Aneurysm among 65- and 75-Year-Old Men in a City of 200,000 Inhabitants

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Unruptured abdominal aortic aneurysm (AAA) is seldom recognized. Thus it is difficult to know whether the incidence of AAA in the general population is high enough to warrant routine screening at least in men after a certain age. Ultrasound screening studies to evaluate the incidence of AAA have been carried out in several English-speaking and Scandinavian countries. The purpose of this report is to describe the results of a study carried out in Belgium. All 65- and 75-year-old men living in the city of Liege, Belgium, were given the opportunity to undergo a free ultrasound examination. Only 41% of the target population was examined. AAA defined as abdominal aortic diameter of >30 mm was observed in 28 subjects (incidence: 3.8%). Mean abdominal aortic diameter was 34.7 mm. A diameter >29 mm was observed in 33 subjects (incidence 4.5%). Mean abdominal aortic diameter was 30.4 mm. On the basis of epidemiological data collected, a high-risk population for AAA was identified. Arterial hypertension ($p < 0.05$), previous coronary artery surgery ($p < 0.05$), and smoking ($p < 0.06$) were more common in subjects with than without AAA. The overall cost of screening was $18.175. The cost per AAA diagnosed was $551.00. (Ann Vasc Surg 1998;12:544-549.)

INTRODUCTION

In the United States, ruptured abdominal aortic aneurysm (AAA) accounts for 1.2% of mortality among men over 65 years of age and 0.6% among women in the same age group.[1,2] In England and Wales in 1983, ruptured AAA was the cause of 1.9% of deaths among males and 0.7% among females over 65 years.[3] In The Netherlands in 1990, the incidence of fatal ruptured AAA was 1.4% for men and 0.5% for women over the age of 55 years.[4]

Nearly 40% of patients presenting ruptured AAA die before admission to the hospital.[5] Operative mortality ranges from 40 to 50%.[1,5,7] According to Ingoldby et al.,[7] overall mortality due to ruptured AAA is 80.2%. This contrasts starkly with the operative mortality of elective surgery for unruptured AAA which, thanks to progress in surgical techniques, is <3%.[1,8]

Although a significant number of patients are still treated after rupture, the number of elective procedures for AAA has risen over the last 30 years. This is probably due not only to longer life expectancy and improvement in diagnostic techniques but also to an increasing incidence of AAA in both men and women as reported in several studies.[4,9-11]

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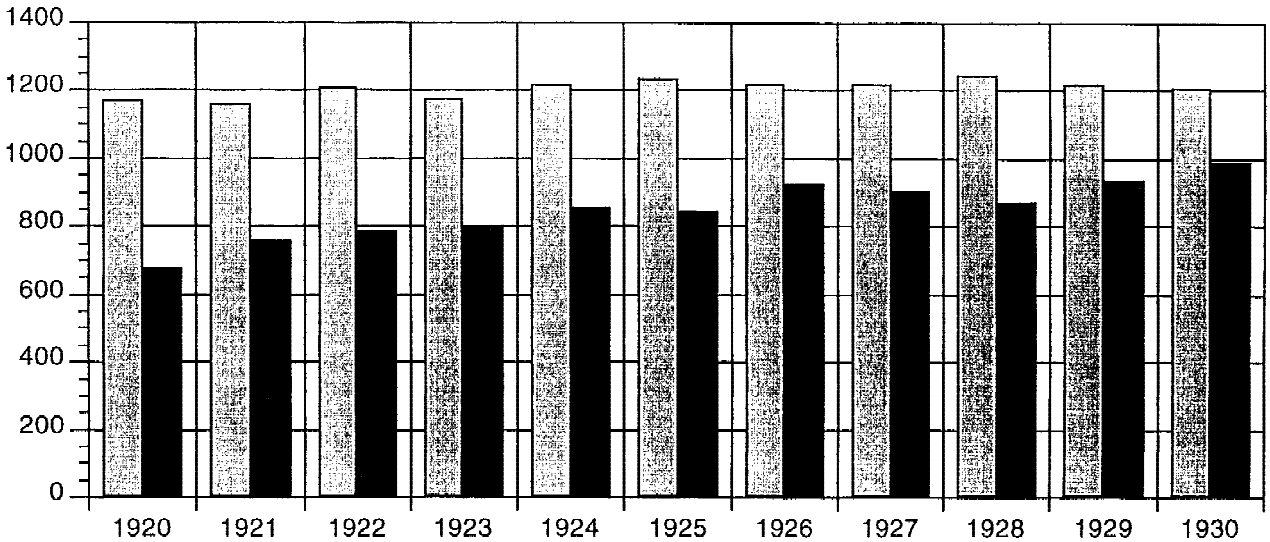

Fig. 1. Birth statistics for the city of Liege between 1920 and 1930. A total of 9344 men and 13,270 women were born during that period, with 729 men born in 1920 and 1035 men born in 1930. Black bars, men; shaded bars, women.

Care costs are two- to three-fold higher for patients treated after rupture than for patients treated electively.[2,12]

The purpose of this study was to evaluate the incidence of AAA in a city with an estimated population over 200,000 and identify a population at risk. For this purpose, we carried out routine ultrasound screening and collected epidemiological data in 65- and 75-year-old males living in Liege, Belgium. This study was funded by the French-speaking Belgian community.

MATERIAL AND METHODS

This study was undertaken with the approval by the Board of the Physicians' Association and the Ethics Committee of the University Hospital of Liege. Between December 14, 1995 and November 24, 1996, a personalized letter offering the opportunity to undergo free ultrasound screening for AAA was sent to 1764 men born in 1920 (n = 729) and 1930 (n = 1035). Selection was based on birth records for the city of Liege, Belgium (Fig. 1).

Before ultrasound procedure, each subject responded to a physician-administered epidemiological survey concerning risk factors for cardiovascular disease (hypertension, diabetes, hypercholesterolemia, smoking), previous cardiovascular events (angina, myocardial infarction, stroke, transient ischemic attacks), ongoing medical treatment, previous cardiovascular surgery (aortic, cardiac, or peripheral vascular surgery), and family history of AAA (documented AAA treated surgically, documented AAA not treated surgically, sudden death). Sketchy, unreliable responses were not taken into account.

Ultrasound examinations were performed by a radiologist and surgeon from the Medical Imaging and Cardiovascular Departments of the University Hospital Center of Sart-Tilman in Liege, Belgium. The devices used were a Toshiba Sonolayer SSH/140A (3.75 mHz transducer) between December 14, 1995 and June 30, 1996, and a Siemens Sonoline Elegra (3.5 mHz transducer) between September 14, 1996 and November 24, 1996. Both anteroposterior diameter (external edge of the aortic wall) and transverse diameter of the infrarenal aorta and the iliac bifurcation were measured. Infrarenal abdominal aortic diameter was considered normal up to 29 mm.[9,13] Corollarily AAA was defined as an anteroposterior abdominal aortic diameter >29 mm. Statistical analysis was performed using an SAS system.

RESULTS

Of the 1764 subjects to whom letters offering the opportunity of undergoing AAA screening were sent, a total of 727 (41%) consented. Of these 727 subjects, 465 were born in 1930 (64%) and 262 in 1920 (36%). Ultrasound was feasible in 98.5% of cases. Ultrasound failed in 11 cases because of obe-

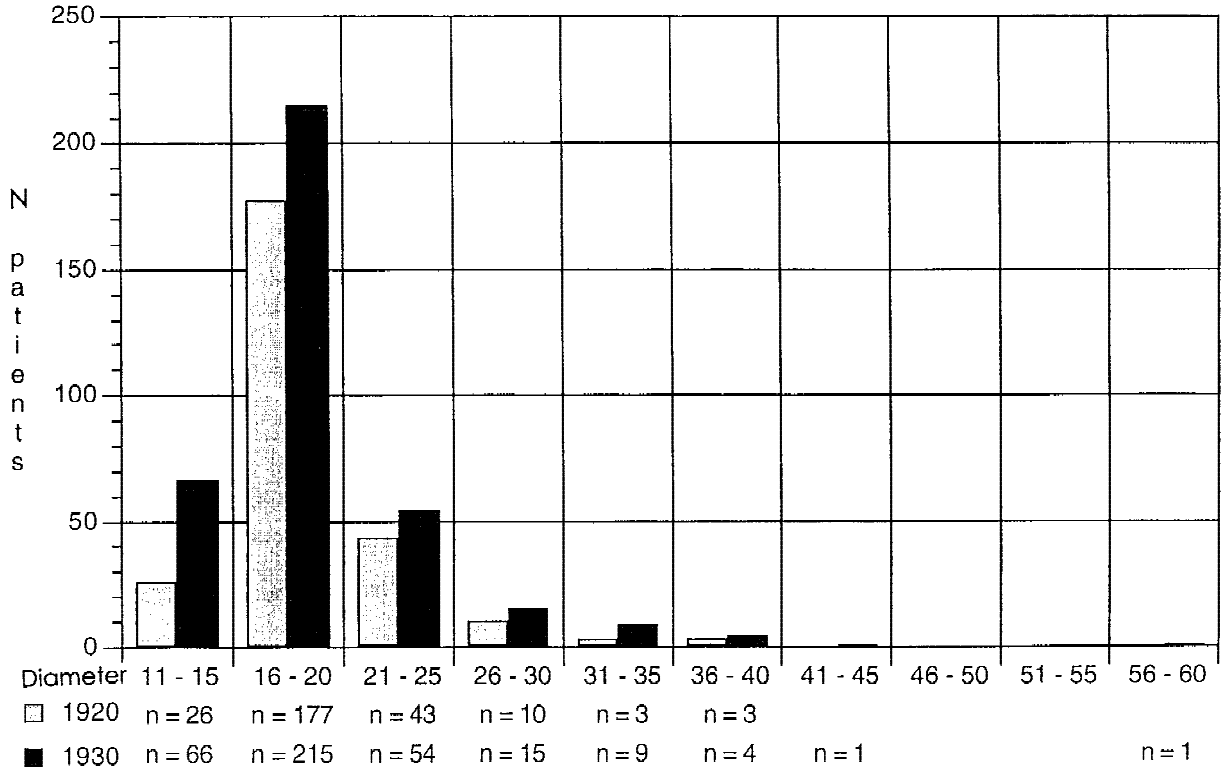

Fig. 2. Infrarenal abdominal aortic diameter in function of age.

sity (n = 5), sequellae of previous surgery (n = 2), and air artifacts (n = 4). Findings in failed cases were classified normal.

The mean anteroposterior diameter of the infrarenal abdominal aorta for the 727 subjects examined was 18.9 mm (range: 11.5-60 mm). Mean anteroposterior diameter was 18.9 mm for the 465 subjects born in 1930 vs. 19.2 for the 262 subjects born in 1920 (Fig. 2). Overall, the mode was 17 mm and the median was 18 mm.

Anteroposterior diameter was >29 mm in 33 subjects including 21 born in 1930 (4.5%) and 12 born in 1920 (4.6%). In these 33 patients, the mean anteroposterior of the infrarenal abdominal aorta was 30.9 mm, i.e., 31.2 mm for subjects born in 1930 and 30.3 for subjects born in 1920. The incidence of AAA defined as anteroposterior diameter >29 mm was 4.5%.

Twenty-eight subjects presented an abdominal aortic diameter >30 mm, including 18 (3.9%) born in 1930 and 10 (3.8%) born in 1920. The mean anteroposterior diameter of the infrarenal aorta in these 28 patients was 34.7 mm, i.e., 35.6 mm for subjects born in 1930 vs. 33.9 mm for subjects born in 1920. The incidence of AAA defined as anteroposterior diameter >30 mm was 3.8%.

Risk factors for cardiovascular disease are listed in Table I. Hypertension was noted in 204 subjects (28.1%), dyslipidemia in 185 subjects (25.4%), smoking in 163 subjects (22.4%), and diabetes in 85 subjects (11.6%). Table I also indicates risk factors for cardiovascular disease in subjects without (n = 694) and with (n = 38) AAA. The only statistically significant difference between the two populations was for hypertension ($p < 0.05$). The difference for smoking was nearly significant ($p < 0.06$).

Table II shows data regarding previous cardiovascular surgery. A total of 72 subjects had undergone previous cardiovascular surgery. The procedure was coronary artery bypass grafting (CABG) in 44 subjects (6.2%), aortic surgery in 13 (1.2%) (AAA repair in 5 cases and aortobifemoral bypass grafting in 8 cases), and peripheral vascular surgery (PVS) in 15 (2.1%). Table II also indicates previous surgery for cardiovascular disease in subjects without (n = 694) and with (n = 38) AAA. The only statistically significant difference between the two populations was for CABG ($p < 0.01$).

Data collected regarding medical treatment and family history of AAA were considered too sketchy and unreliable for meaningful analysis.

The cost of each screening examination for AAA

Table I. Risk factors for cardiovascular disease in the study population (n = 727) without (n = 694) or with (n = 33) AAA

	Without AAA		With AAA		
	n	%	*n*	%	*p*
Dyslipidemia	172	24.92	13	35.13	NS
Diabetes	78	11.30	7	18.91	NS
Hypertension	188	27.24	17	45.94	<0.05
Smoking	156	21.73	13	35.13	<0.06

NS, nonsignificant.

Table II. Incidence of previous cardiovascular surgery in the overall population, subjects without AAA, and subjects with AAA

	Aortic surgery	Coronary artery bypass	Peripheral vascular surgery
Overall population (n = 727)	13(1.78%)	44(6.05%)	16(2.18%)
Subjects with AAA (n = 33)	1(3.03%)	6(18.1%)	2(6.06%)
Subjects without AAA (n = 694)	12(1.77%)	38(5.48%)	14(1.87%)

The difference between the two subgroups was not significant for aortic surgery or peripheral vascular surgery. The difference was significant for coronary artery bypass ($p < 0.01$).

was calculated on the basis the officially established rate for ultrasound study: $25.00/procedure. Thus the overall cost of screening was $18.175 (875 × 727). The cost of diagnosis of each AAA was $551.00, calculated by dividing the overall cost by the number of AAA discovered ($551.00/33 AAA).

DISCUSSION

The reliability of population-based studies depends on compliance. The percent of compliance in previous AAA screening studies in English-speaking and Scandinavian countries has ranged from 51 to 81%.[9,14] At only 41.1%, compliance in our study can be considered too low to allow comparison. Low compliance was probably due to differences in attitude not only of the general public but also of physicians (suspicion of private practitioners screening efforts outside their control, lack of motivation, and more undisciplined Latin mentality). Lower compliance by 75-year-olds than 65-year-olds was probably due to more limited mobility of the older group. In comparison, compliance rates observed in different sections of Liege, Belgium during a breast cancer screening program carried out between 1992 and 1995 ranged from 18 to 43.6% but it should be noted that the target population was younger and that a mobile unit was used, thus making participation more convenient.[15]

Ultrasound is considered the method of choice for AAA screening.[9,14] In addition to being feasible in 98.5% of cases, the sensitivity of ultrasound is over 95% and its specificity is 100%. Other advantages of ultrasound include noninvasiveness, good reproducibility, and cost-effectiveness (seven-fold less expensive than an abdominal CT scan). However, Ellis et al.[12] reported that ultrasound underestimates aortic aneurysm diameter in comparison with the CT scan, particularly if transverse diameter rather than anteroposterior diameter and/or the suprarenal rather than the infrarenal aorta are studied. Yucel et al.[16] stated that differences between anteroposterior measurements made by ultrasound and CT scan must be >4 to 5 mm to be significant. Grimshaw and Docker[17] emphasized that standard deviation between measurements obtained in two ultrasound examinations by the same operator should not exceed ±1.3 mm. In an experimental study carried out by Comstock et al.,[18] the mean difference in measurements obtained by two operators was 2.1 mm for anteroposterior diameter and 3.1 mm for transverse diameter. Similarly, Akkersdijk et al.[14] reported that the standard deviation between measurements obtained by different operators was 2.2 mm for anteroposterior diameter and 5.3 mm for transverse diameter. In the present study, we considered only anteroposterior diameter.

Setting strict criteria for defining AAA is difficult since the diameter of the aorta varies according to several factors such as sex, age, and body surface. Various diameters have been used for diagnosis of AAA in previous ultrasound studies. McGregor et al.[19] proposed an infrarenal aorta diameter >30 mm. In a pilot study including 550 men between the ages of 65 and 75 years, Smith et al.[13] proposed an infrarenal diameter of 29 mm because it was three standard deviations greater than the mean diameter of 21 mm. This definition has the advantage of being based on statistical data obtained in a relatively homogeneous, controlled population. The Ad Hoc Committee of the ISCS/SVS[20] defines AAA as an infrarenal diameter >150% of ''normal.'' However, this definition is difficult to use in screening studies as mean diameters must be calculated in function of age, sex, and body surface. Had we used the Ad Hoc Committee definition for our study in

Table III. Data from previous screening studies for AAA

Reference	Compliance (%)	Number of subjects	Age (years)	Incidence of AAA[a] (%)
15	58.8	4.237	65-80	4.3
13	76.3	2.664	65-75	8.4
3	51.7	497	65-74	4.2
9	75.0	364	74	8.5
This study	41.0	727	65 and 75	4.53

[a]Defined as maximum transverse diameter >29 mm.

which mean diameter was 17 mm, the cutoff point between normal and aneurysmal would have been 25.5 mm and the incidence of AAA would have been 5.22% (38 cases).

In the present study, we targeted 65- and 75-year-old men for routine ultrasound screening for AAA. The highest incidence of AAA is in subjects over 80 years, the optimal effect on mortality in function of age at diagnosis is observed between 70 and 75 years, and the lowest operative mortality is observed between the ages of 55 and 65 years. Thus, since the detection rate is lower and the cost of screening per case diagnosed is higher[13,21] in these cases, the best age group for screening in terms of cost effectiveness is that between 65 and 75 years.[9,13,21] In the city of Liege, there are 9344 men between the ages of 65 and 75 years. We chose to study 65-year-olds (born in 1930) and 75-year-olds (born in 1920) to obtain an easily useable sample for statistical analysis.

According to the Ad Hoc Committee of the ISCS/SVS,[20] mean infrarenal abdominal aortic diameter measured by ultrasound ranges from 14.1 to 20.5 mm (margin of error 0.04 to 0.37). Sonesson et al.[22] studied infrarenal aortic diameter in function of age, sex, and body surface in healthy subjects and reported a diameter of 15.7 ± 1.5 mm for the 61.4 ± 3.6 year age group and 17.3 ± 2 mm for the 68.6 ± 2.7 year age group. In our study, mean aortic diameter was 18.9 (range: 11.5 to 60 mm) and the mode was 17 mm. Anteroposterior aortic diameter >30 mm was observed in 28 male subjects (mean diameter: 34.7 mm) and the incidence of AAA was 3.8%. Anteroposterior aortic diameter >29 mm was observed in 33 cases (mean diameter: 30.9 mm) and the incidence of AAA was 4.5%. This incidence of AAA is comparable with previous reports[3,9,13,14] (Table III).

On the basis of epidemiological data obtained, we attempted to define a high-risk population for AAA. The incidence of hypertension was 45.9% in the AAA Group as compared with 27.2% in the general population ($p < 0.05$) and the incidence of smoking was 35.1% in the AAA group as compared with 21.7% in the general population ($p < 0.06$). The higher incidence of CABG [18.1% in the AAA group versus 5.5% in the general population ($p < 0.01$)] could be the result of a bias as patients who have undergone previous surgery may be more aware of the risks of cardiovascular disease and thus more likely to take part in screening.

The effectiveness of screening studies could be enhanced by specific targeting of high-risk groups. The incidence of AAA in subjects over the age of 65 with hypertension was 12% in the study of Allen et al.[23] and 9% in the study of Lederle et al.[24] In our department, screening for AAA was carried out on 110 patients scheduled to undergo surgery for either coronary artery disease ($n = 72$) or peripheral vascular disease ($n = 38$). The incidence of AAA was 9.7% in patients with coronary artery disease and 2.6% in patients with peripheral vascular disease.[25,26] In a group of patients who underwent coronary artery surgery between the ages 34 and 77 years, Nevelsteen et al.[27] reported that the incidence of AAA was 8.8%. In a study of patients with peripheral vascular disease between the ages of 31 and 83 years, Shapira et al.[28] observed that the incidence of AAA was 4.6%. In a group of male patients with peripheral arteriopathy, Allardice et al.[29] reported that the incidence of AAA was 17%.

CONCLUSION

Estimates of the incidence of AAA in the general population vary. In our series of 65- and 75-year-old men, the incidence of AAA was 4.5%. Although surgical treatment of all these AAA may not be required, surveillance is necessary. The only way to reduce the 80% mortality rate observed after ruptured AAA (80%) is to perform elective surgical repair before rupture. To increase the number of elective procedures, routine screening is necessary using a more reliable technique than simple palpation of the abdomen. The results of this study demonstrate that ultrasound is a cost-effective method for routine ultrasound screening in 65- and 75-year-old men. The efficacy of routine screening could be further improved by targeting a subgroup at risk. In this regard, the present study showed that subjects with hypertension, a history of coronary artery bypass, and smoking constitute a high-risk population for AAA.

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APPENDIX 2

Abdominal aortic aneurysms in octogenarians. **H. Van Damme, N. Sakalihasan, C. Vazquez, Q. Desiron, R. Limet.** *Acta Chir Belg 1998, 98 : 76-84*

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Abdominal Aortic Aneurysms in Octogenarians

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Key words. : Aortic aneurysm ; aorta, abdominal ; aged 80 and over ; ruptured aneurysm

Abstract. The decision on whether to operate or not abdominal aortic aneurysms (AAA) in elderly depends on the relative risk of the operation versus the natural course of the unoperated AAA.
From January 1984 to December 996, 138 patients, aged 80 years and older, were referred to our department for an aneurysm of 40 mm or more (transverse diameter) of the infrarenal abdominal aorta (95 asymptomatic, 15 painful, and 28 ruptured AAA). For 58 patients with asymptomatic AAA, operation was denied at referral because of transverse diameter less than 50 mm (n = 21), patient refusal (n = 10) or unacceptable operative risk or poor general condition (n = 27). Thirty-four of these observed AAA were ultimately operated after a mean delay of 41 months because of aneurysm enlargement (n = 15), aneurysm tenderness (n = 6) or rupture (n = 13). Overall, 52 patients had immediate (n = 37) or delayed (n = 15) elective repair of their AAA, with an in-hospital mortality of 5.7%. Urgent operation was done for 21 patients with a painful AAA. Six patients died at hospital (28% mortality rate). Emergent surgery was applied to 41 patients with ruptured AAA (including 13 AAA who ruptured during surveillance). The operative mortality in this subgroup attained 68%.
Follow-up for the 77 survivors and the 24 non-operated patients averaged 43 months. The 5-year survival (operative mortality included) is 47% for electively operated patients, 30% for urgently and 20% for emergently operated patients. For comparison, the 5-year survival of an age and sex matched Belgian population is 63%. For the 24 medically followed AAA, the 5-year survival was 33%. In six cases, the cause of death was rupture of the AAA. Of the 58 patients for whom operation was initially not considered, 19 (33%) presented AAA rupture (13 operated in emergency and 6 who never came to surgery).
The operative outcome of AAA repair in octogenarians is less favourable than in the younger age group (3.6% mortality after elective repair, 44% after operation for AAA rupture, according to our institution data).
The authors conclude that AAA surgery should not be denied to octogenarians on the basis of advanced age alone. They recommend a straightforward surgery for otherwise healthy octogenarians with AAA of 50 mm diameter, surveillance up to 60 mm for high-risk patients and no surgery for unfit, bedridden or demented patients.

Introduction

Abdominal aortic aneurysm is a disease primarily affecting elderly men. From population-based screening studies, one can estimate the prevalence of AAA (40 mm or more in transverse diameter) as 4% for octogenarians, with a male/female ratio of 5 over 1 [1, 2]. At 80 years, life expectancy attains 7.52 years [3]. Currently, 3.5% of the Belgians are 80 years or older, what means that about 14.000 Belgian octogenarians could be expected to have an AAA of 40 mm or more. Ageing of the population is a health concerning problem. It is expected that the cohort of octogenarians will double in the coming 30 years. In the official Belgian mortality statistics of 1991, aortic aneurysm is recorded as main cause in 0.55% of all deaths among octogenarians, and ranks fifteenth among all causes of death [3]. Bengtsson [4] found an analogous incidence of ruptured AAA in the Malmö autopsy study.

The medical decision making of surveillance or surgical repair should balance the relative operative risk against the natural course of the unoperated AAA. In other terms, it is essential to determine if both the risk of rupture and the safety of surgery justify operation.

The natural history of AAA is one toward rupture [5]. Aneurysms greater than 50 mm (transverse diameter) should be considered as a life-threatening but curable condition [6]. The annual rupture rate for a 50 mm AAA is estimated at 4% [5, 7, 8]. Risk factors other than the aneurysm size, such as concurrent chronic obstructive lung disease, uncontrolled diastolic hypertension [7] and positive family history [9], increase the probability of aneurysm rupture.

Widespread agreement exists that AAA of 50 mm or greater should be repaired, except in individuals with major coexisting disease, resulting in excessive operative risk and reduced life expectancy [6]. Octogenarians often suffer concurrent systemic disease that adversely

influence the operative risk. They are especially vulnerable from a pulmonary standpoint. A prophylactic operation with a mortality risk exceeding 7% is not easily suggested to an octogenarian for an asymptomatic AAA. On the other hand, the cumultive risk of death from AAA rupture attains 30% for a 80-year old patient with a life expectancy of 7.52 years. Large AAA evolve, soon or late, to rupture that is always fatal if untreated. Most patients with ruptured AAA die before reaching the hospital, and for those who benefit surgery, operative mortality is as high as 70% [10].

Once an AAA is documented, aortic replacement before rupture should be invariably considered. This recommendation is based on the premise that aortic replacement will avoid death by rupture and extend useful life expectancy.

One should analyse which octogenarian could derive a clear advantage from surgery. Is the patient able to withstand major surgery? To answer these questions and to define an appropriate strategy, we reviewed our experience with AAA in octogenarians during the last 12 years.

Material and methods

A retrospective study was done for all octogenarians referred to our department for infrarenal AAA of 40 mm or more in diameter, from January 1984 to December 1996. We used the computerized vascular registry of our department to obtain demographic data and to analyse basic information on the diameter and aspect of the AAA, on comorbid conditions, on relevant medical history, and on the adopted management of the AAA (surveillance versus repair).

Aneurysms clinically presented as asymptomatic, painful or ruptured. Hospital records were analysed in detail, with special attention to the risk evaluation and the decision making on whether to operate or not. The only operation applied was standard aortic replacement. No exclusion techniques with extra-anatomic bypass or endoluminal stent graft were used. The operative outcome is analysed in function of the type of surgery (elective, urgent or emergent). Patients were subdivided in risk categories in function of age and cardiac, pulmonary and renal functional reserve, in accordance to the risk evaluation scale of the Joint Council of the Society of Vascular Surgery and the North American Chapter of the International Society of Cardiovascular Surgery [6]. Low risk patients are younger than 85 years, have a preserved cardiac function, mild or no obstructive lung disease and serum creatinine less than 20 mg/l. Intermediate risk patients are aged 85 to 90 years, or have decreased cardiac output (ejection fraction between 20 and 50%) or suffer angina at minimal exertion, or have incapacitating lung disease (forced expiratory volume (FEV1) between 800 and 1200 ml/s), or impaired renal function (serum creatinine between 20 and 30 mg/l). High risk patients are 90 years or older, or suffer congestive heart failure, or have ejection fraction lower than 20%, or have severe respiratory insufficiency (FEV1 less than 800 ml/s), or need home oxygen, or have significant renal failure (serum creatinine $>$ 30 mg/l or haemodialysis). The operative outcome is further analysed in function of the risk category.

For comparison, the results of AAA repair in the younger than 80 years age group are given.

Operative mortality is defined as in-hospital mortality (only patients discharged from hospital are considered as operative survivors), rather than 30-day mortality.

An estimate of cost for elective and emergent surgery was made [11] A detailed analysis of the hospital charges was done for the most recent 40 patients operated electively (n = 20) (1993-1996) or emergently (n = 20) (1990-1996). Cost analysis concerns hospitalization, including stay in intensive care, pharmaceutics, transfusion products and medical fees. Cost effectiveness is calculated by dividing the overall cost of each group by the number of survivors. By this way, the bias of early deaths in the emergent group, falsely lowering the cost, is avoided.

Follow-up data were collected from the out-patient clinic (control visit last 6 months) in only 10% of cases, from a questionnaire send to the family physician in 75%] and from telephone contact with relatives in 15%. An estimate of quality of life after discharge from the hospital (mean delay 6 months) was made by ascertaining the degree of independence and mobility, using a score relating the ability to perform daily activities.

Statistics

Relationships between demographic data, clinical risk category, or aneurysm characteristics, and operative outcome were analysed univariately with Pearson's chi-square test or Fisher's exact test when appropriate.

Cumulative survival curves are constructed according the Kaplan-Meier life table method [12].

Survival data for the age and sex matched Belgian population were obtained from the life tables published in 1991 by the National Institute of Epidemiology [3].

Results

From January 1984 to December 1996, 138 octogenarians (21 females, 117 males) were referred to our Department for AAA. This represents 8.9% of all (n = 1552) AAA referred during the same period. Their mean age was 82.9 years (range 80 to 94). Major patient characteristics are summarized in Table I.

Table I
Patient characteristics

Number	138 (21 F, 117 M)
Mean age	82.9 y (80-94)
Active smoking	35%
Hypertension	61%
Creatinine > 20 mg/l	26%
COPD	42%
FEVI < 1000 ml/s	14%
Previous myocardial infarction	45%
Congestive heart failure	11%
Previous myocardial revascularization	8%
Previous stroke	6%

At referral, 95 patients presented an asymptomatic AAA (mean diameter 64 ± 8 mm) (Group A), 15 were admitted with a painful AAA suggestive for impending rupture (mean diameter 70 ± 15 mm) (Group B), and 28 with a ruptured AAA (mean diameter 78 ± 16 mm) (Group C).

In 10 of the group C patients, the AAA was first recognized at the moment of rupture, while in 18 patients it concerned rupture of a documented, medically followed AAA. Of the 95 patients initially seen outside urgent situation (group A), only 37 benefited elective repair within 2 months of referral. For the 58 other octogenarians with an asymptomatic AAA (mean diameter 60 ± 20 mm), operation was initially not considered for one of the following reasons: small aneurysm size (diameter less than 50 mm) (n = 21), patient refusal (n = 10), unacceptable surgical risk or poor general condition (n = 27). For these patients, aneurysm size was monitored by serial ultrasonography or CT-scan every 6 to 12 months. Of the 58 observed patients, 34 ultimately came to surgery, after a mean delay of 39 months, because of aneurysm expansion to 50 mm or greater (n = 11) or a growth rate of 0,5 cm or more in 6 months (n = 4), aneurysm tenderness (n = 6) or aneurysm rupture (n = 13). This leaves 24 octogenarians who did not benefit aortic replacement during the study period (mean diameter 65 mm). This group includes 6 patients with small (< 50 mm) AAA, 5 patients who refused surgery and 13 patients deemed unfit for operation (2 patients suffered terminal malignancy, 2 patients had severe residuum from stroke, 2 were demented and 7 suffered incapacitating multisystem failure).

Overall 52 patients had early (n = 37) or delayed (n = 15) elective repair of their AAA. Their mean age was 81.5 years (range 80-86) and the mean transverse diameter of the AAA was 65 ± 19 mm. They all had a preoperative risk evaluation and respiratory preparation. Urgent operation was done for 21 patients with a painful AAA, including the 15 group B patients and the 6 group A patients who developed aneurysm tenderness during surveillance. Their mean age was 83.3 years (range 80-94) and the mean diameter 69 ± 20 mm. Emergent repair was done for 41 ruptured AAA, including 13 group A patients who ruptured a clinically followed AAA for which operation by first intention was declined. Thirty one patients had a known AAA, while in the ten other patients the AAA was not diagnosed before rupture. The mean diameter at the moment of rupture was 80 ± 13 mm and the mean age 83.5 years (range 80-90). There were 25 retained retroperitoneal ruptures and 16 free intraperitoneal ruptures. Severe hypotension (70 mmHg or less systolic blood pressure) occurred in 23 patients before aortic cross clamping, with in 12 cases episodes of unrecordable blood pressure, with a fatal cardiac arrest in 8. Two other patients who developed cardiac arrest intraoperatively could be successfully resuscitated. One of them survived to operation for 38 months. The other died on day 12.

A standard midline incision was used in 106 patients. In recent years, 8 patients had a retroperitoneal approach of their AAA by a left flank incision. A straight aortic tube was implanted in 16 patients, an aorto-biiliac bifurcation graft in 26, an aorto-ilio-femoral graft in 18 and an aorto-bifemoral graft in 46. Eight patients with ruptured AAA died before implantation of a graft. There was no significant difference in the operative technique (graft extension) between elective, urgent or emergent repair.

The operative outcome after elective, urgent or emergent repair is summarized in Table II. The median length of hospital stay was 15 (range 6 to 20), 32 (range 2 to 60), and 19 (range 1 to 52) days for elective, urgent or emergent repair respectively. The in-hospital mortality after elective repair was 5.7% (3/52). Three patients died, 2 from multiorgan failure on day 18 and 20, 1 from myocardial infarction on day 4. Three patients suffered perioperative myocardial infarction (1 fatal), 9 had postoperative respiratory failure (pulmonary infection in 7, adult respiratory distress syndrome (ARDS) in 2), 3 patients developed transient impairment of renal function (serum creatinine increase with 20% or more of the preoperative value), 1 evolved to haemodialysis (he finally died in multiorgan failure), and 1 patient suffered colon infarction, necessitating hemicolectomy (he finally died in sepsis and multiorgan failure).

For urgent repair, the in-hospital mortality was 28%. Six patients died after a mean of 19 days (range 1 to 60). The cause of death was cardiac in 2, respiratory in 1 and multiorgan failure in 3. One patient suffered ischaemic colitis that was medically managed, 3 had perioperative myocardial infarction (1 intraoperatively), 9 had respiratory failure, necessitating tracheotomy in 2.

Table II

Operative outcome

	Elective repair (n = 52)	*Urgent repair (n = 21)*	*Emergent repair (n = 41)*
Operative morbidity			
Myocardial infarction	3 (6%)	3 (14%)	7 (17%)
Respiratory failure	9 (17%)	9 (43%)	26 (63%)
(necessitating tracheotomy)	—	*2 (9.5%)*	*4 (10%)*
Renal insufficiency	4 (8%)	4 (19%)	19 (46%)
necessitating haemodialysis)	—	*1 (5%)*	*8 (19.5%)*
Ischaemic colitis	1 (2%)	1 (5%)	5 (12%)
(necessitating colectomy)	*1 (2%)*	—	*2 (5%)*
Operative mortality	**3 (5.7%)**	6 (28%)	**28 (68%)**
Cardiac	**1**	2	5
Multiorgan failure	**2**	3	10
Respiratory	-	1	4
Hypovolaemic shock	-	-	9
5-year survival	**47%**	30%	20%

Table III

Cost-effectiveness (charges for elective (n = 20) and emergent repair (n = 20) (1990-1996) divided by number of survivors)

	Ruptured AAA (R1) (n = 20) (7 survived)	*Elective AAA repair (R2) (n = 20) (19 survived)*	*Ratio R1/R2*
Hospitalization	474,854*	138,990*	3.4
Pharmaceutics	197,699	36,871	5.4
Transfusion products	70,879	7,104	10.0
Medical fees	480,598	163,456	2.9
Overall	**1,226,404**	**348,236**	**3.5**

* (costs are in Belgian francs).

For emergent repair of ruptured AAA, the mortality rate was 68% (28/41). There were 10 intraoperative deaths. It concerned 2 fatal myocardial infarctions after graft implantation and 8 cardiac arrests on uncontrollable hypovolaemic shock before aortic cross clamping. Multiorgan failure was the main cause (10/18) of the 18 postoperative deaths (1 to 19 days postoperatively). Twenty-six patients presented postoperative respiratory failure (bronchopneumonia in 18, shock lung in 8). Twenty required mechanical ventilation beyond 3 days and 4 had tracheotomy. Five patients suffered ischaemic colitis necessitating hemicolectomy in 2 of them. Preoperative hypotension (< 70 mmHg, n = 23) was significantly correlated to operative mortality (18 died at hospital, $p < 0.01$). The mortality of emergent repair was further analysed for patients who ruptured a previously known AAA (n = 31). In 25 of them, coexistent medical problems provided an argument for medical surveillance. The mortality in that subgroup attained 80%. In other words, 5 (20%) of these patients previously turned down for elective repair on medical grounds, survived emergent operation after AAA rupture.

Cost evaluation for elective and emergent AAA repair is represented in Table III. For the 20 electively operated patients, the hospital stay averaged 14 days (extremes 2 and 31) versus 30 days (extremes 1 and 65) for ruptured AAA. Mortality attained 5% (1/20) for elective repair and 65% (13/20) for emergent repair. Half of the deaths occured within 48 hours. This high early mortality falsely lowers the cost of emergent repair. Therefore, an estimate of the price to save one ruptured AAA was made by calculating the cost effectiveness of the procedure. Overall, the price to save one ruptured AAA is 3.5 times that of elective repair. Most important differences are the duration of stay in the

intensive care unit and the tremendous requirement for transfusion products (fresh frozen plasma and concentrated red blood cells) in case of ruptured AAA.

Some complications influenced the mortality rate. Twenty of the 44 patients who developed respiratory failure died ($p < 0.01$). Only 1 of the 3 patients with transmural colon ischaemia survived ($p < 0.05$). Nine patients necessitated reoperation for continuous bleeding (3 splenic injuries, 3 anastomotic leaks, 3 diffuse bleeding). Four of these patients ultimately died ($p < 0.01$).

The results of aneurysm repair in octogenarians in the last 7 years of the study period (1990-1996) were compared with those of the earlier period (1984-1989) (Table IV). The most considerable improvement was obtained for elective surgery (4.7% operative mortality versus 10%, p .0.05). The high operative mortality for ruptured AAA was unchanged (66% and 70%). For comparison, the recent results of aneurysm repair in the younger age group (less than 80 years) are also listed in Table IV.

The operative mortality was further analysed in function of the graduated risk category (Table V). For low-risk, otherwise healthy octogenarians, elective surgery could be performed with no operative mortality, versus a 10% mortality for elective AAA repair in high risk patients ($p < 0.01$). Urgent and emergent repair was characterized by a considerable operative mortality (28% and 68% respectively), whatever patient's risk profile. Age, considered alone, was not significantly determinant for operative mortality.

As already stated, for 58 patients of group A, a strategy of "watchful waiting" was adopted at initial assessment. In this group, 27 patients were considered to be at excessive risk for operation, and for them a deliberate decision had been made to pursue non-operative management. Of these 58 observed AAA, 13 were ultimately operated in emergency for rupture, with 8 operative deaths (6 of the deaths belonged to the group of high-risk patients). Another 6 fatal ruptures (mean initial diameter 68 ± 21 mm, mean follow-up of 39 months) occurred in the group of 24 never operated patients. Three of them belonged to the group of high-risk patients, for whom elective repair was denied, and 3 were intermediate risk patients who refused ($n = 2$) or waited for ($n = 1$) elective operation. Overall, 9 of the 27 observed high-risk patients (believed unsuitable for operation when first seen) ultimately died from AAA rupture during surveillance.

Late follow-up for the 77 survivors and the 24 non-operated patients averaged 43 months. Three patients were lost to follow-up at 6, 9 and 15 months. For the 52 electively operated patients, the 3- and 5-year survival (operative mortality included) is 71% and 47% respectively. Their survival curve is depicted in Figure 1 with, for comparison, the estimated survival of the age and sex matched Belgian population (3-year and 5-year survival of 78% and 63% respectively). For the 21 urgently and the 41 emergently operated patients, the 5-year survival (operative mortality included) is 30% and 20% respectively. An initial dramatic decline in the survival curve of these groups is due to an elevated

Table IV

Operative mortality rate

	Mortality ≥ 80 years 1984-1989 (n = 36)	*Mortality ≥ 80 years 1990-1996 (n = 78)*	*Mortality < 80 years 1994-1996 (n = 380)*
Elective repair	10% (1/10)	4.7% (2/42)	3.6% (12/334)
Urgent repair	33% (3/9)	25.0% (3/12)	5.8% (1/17)
Ruptured AAA	70% (12/17)	66.0% (16/24)	44.0% (18/41)

Table V

Results versus risk profile

	Mortality			*5-year survival*
	Elective repair	*Urgent*	*Ruptured*	
Low risk* (n = 17)	0% (0/10)	33% (1/3)	75% (3/4)	66%
Moderate risk* (n = 58)	6.25% (2/32)	18% (2/11)	60% (9/15)	25%
High risk* (n = 39)	10.00% (1/10)	43% (3/7)	73% (16/22)	19%
Overall (n= 144)	5.70% (3/52)	28% (6/21)	68% (28/41)	

* For definition of "low risk" "moderate risk" and "high risk" categories, see MATERIAL AND METHODS in the text.

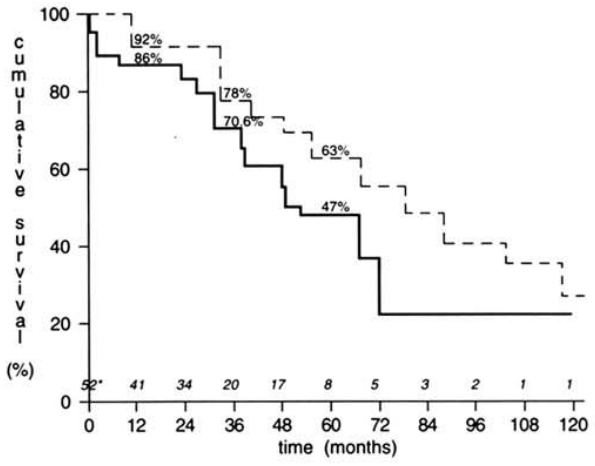


Fig. 1

* Number of patients at risk at a given time.
— Survival curve for 52 octogenarians electively operated for AAA.
--- Age and sex matched Belgian population (> 80 y).

in-hospital mortality. During follow-up, 53 patients died. Late mortality was cardiac related in 38%, cancer in 19%, respiratory or multiorgan failure in 11%, stroke in 7%, AAA rupture in 11% (6 patients of the 24 who did not undergo surgical correction of their AAA) and miscellaneous causes in 5%. For 4 patients (9%), we were unable to determine the cause of late death (Table VI).

Table VI

Causes of late death

Cardiac	20	(38%)
Cancer	10	(19%)
Stroke	4	(7.5%)
Respiratory	6	(11%)
AAA surveillance and rupture	6	(11%)
Miscelaneous	3	(5.5%)
Unknown	4	(7.5%)

Complete data for estimation of quality of life after discharge from the hospital were available for 59 of the 77 survivors. Ninety percent (53/59) returned to a comparable or even better mental status and physical activity. The results were even better after elective surgery (quality of life assessed in 42 of the 49 survivors) : 93% (39/42) declared to enjoy an identical or even better life-style. After repair of ruptured AAA, convalescence was longer (13 survivors, quality of life assessed in 7) and 70% (5/7) regained a reasonable quality of life (able to care for themselves). Of the 77 survivors, 20 benefited convalescence in a nursing home (temporary for 11, definitely for 9). For the 24 non-operated patients, 5-year survival was 33%. During follow-up, 6 of them suffered fatal rupture of their AAA. Survival rates for low, intermediate and high-risk patients are 66%, 25% and 19% respectively at 5 years (p.0.05).

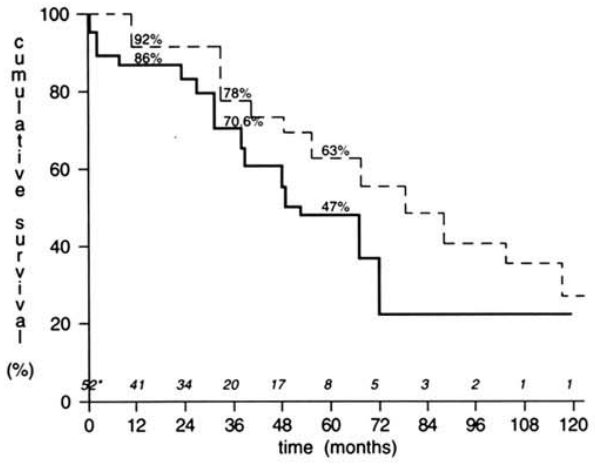
Discussion

Repair of AAA in selected octogenarians can be done with a reasonably low morbidity and mortality. At our department, elective repair in octogenarians has a mortality rate that is 1.3 times higher than that for non-octogenarians (4.7% versus 3.6% for the period 1990-1996). The same is true for urgent or emergent repair (Table IV). Most contemporary reports on elective AAA repair in patients aged 80 years or older mention an operative mortality that is approximately two times higher than in younger patients [13-18]. (Table VII). For emergent repair or ruptured AAA, the difference in results obtained in the two age groups is less evident. Outstandingly good results as obtained at the Cleveland Clinic [19] (3.8% and 29% mortality for elective and emergent repair in octogenarians, versus 1.2% and 26% in the younger age group) are not at the reach of all centers. Elderly patients often present concomitant systemic disease (Table I). The risk profile allows to evaluate the relative operative risk (Table V). For "otherwise healthy" octogenarians with

Table VII

Published series

Author (ref.)	*Study period*	*Nb*	*Elective repair Mortality*	*5-y survival*	*Nb*	*Ruptured AAA Mortality*	*5-y survival*
Treiman (16)	1963-1981	35	8.6%	14%	17	59%	29%
Johnston (32)	1986	51	10.0%	—			
Robson (17)	1980-1988	14	0%	—	14	93%	
Glock (14)	1972-1987	29	67%	64%	7	71%	30%
Dean (13)	1985-1992	18	5.5%	—	11	91%	—
Paty (18)	1978-1991	77	3.0%	60%	25	24%	58%
O'Hara (19)	1989-1993	53	3.8%	41%	7	29%	
This series	*1984-1997*	*52*	*5.7%*	*47%*	*41*	*68%*	*20%*



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no vital organ disease, elective AAA repair could be done with no mortality. For intermediate-risk and high-risk patients, mortality rates increase to 6.25% and 10%. It is note worthy that in case of ruptured AAA, this difference in mortality between the different risk categories is no longer evident. This can be explained by the intrinsic fatality of ruptured AAA. Despite most recent progress in perioperative care, hypovolaemic shock is poorly tolerated by octogenarians, whatever their comorbid conditions. Critical hypotension involves multiorgan failure, a leading cause of postoperative death [13, 14, 19, 20].

We have put the limit of AAA size at 50 mm for elective repair in fit octogenarians. AAA rupture before the diameter reaches 50 mm is less frequent [8, 20]. Two randomized trials are currently underway to compare operative management of small (40 to 55 mm) AAA versus clinical observation [22, 23]. The Joint Council of the Society for Vascular Surgery and the North American Chapter of the International Society of Cardiovascular Surgery published in 1992 recommended indications for operative repair of AAA [6]. The influence of advanced age and systemic vital organ disease (heart, lung, kidney) was evaluated. Compared to the optimal results obtained for elective repair in younger age groups (mortality rate less than 5%), there is a 1 to 2% added mortality for low-risk patients (*for risk criteria, see Material & Methods*). For moderate risk patients (about 30% of the octogenarians belong to that risk category), a 3 to 5% added mortality should be considered in the risk evaluation. For high-risk patients (15% of the elderly belong to that category), the operative risk increases with 7 to 10%. Pulmonary complications and respiratory failure are leading causes of postoperative morbidity in elderly patients [17]. A 3-day course of preoperative pulmonary preparation with spirometry and bronchodilatators, as well as early extubation and postoperative nasotracheal suction, intensive chest physiotherapy and adequate pain-control with continuous epidural analgesia could obviate most of this respiratory morbidity and aid surgical recovery [19, 24-28]. Preoperative coronary revascularization as well as haemodynamic optimization as guided by pulmonary artery catheter monitoring allow to minimize postoperative adverse cardiac events and renal functional impairment. In some frail elderly patients with severe comorbidity, the predicted operative mortality for aortic replacement approaches the estimated rate of AAA rupture. This constitutes the grey area of indecision for elderly patients at risk, who would not derive a clear advantage from surgery. The Joint Committee suggested a "wait and see" policy for high risk patients with AAA less than 60 mm in diameter. Once the aneurysm enlarged beyond the 60 mm limit, the risk of rupture becomes considerable. Repair of such huge AAA should be considered since the assumed benefit in terms of rupture prevention outweighs the potential operative risk. As already stated, rigorous preoperative medical support and optimized perioperative care allow to achieve reasonable results for elective repair in these high-risk patients (mortality rates of 5 to 10%) [24-28]. The only causes for whom non-operative management is justified are unfit patients with a very poor outlook, a life expectancy of less than two years due to overwhelming medical problems, such as advanced metastatic malignancy or end-stage vital organ disease, and entirely dependent bedridden or demented patients with an unacceptable low quality of life [6, 14, 19].

A "wait and see" policy entails some inherent risks. There is an unpredictable but likely exponential growth of the AAA over time [8, 21, 29] while patient's general condition will deteriorate by the ageing process. Leaving a 50 to 60 mm AAA unoperated exposes the patient to a considerable aneurysm related mortality [5, 21]. In our series, 24% (14/58) of the 58 medically followed AAA (mean diameter 60 $\pm$ 20 mm, 37 measuring 50 mm or more) ended up with fatal rupture over a mean observation period of 39 months. We recently published the outcome of 114 medically followed AAA [29]. Over a 26.8-month surveillance period, there were 19 (17%) ruptures, of which 6 were fatal. Even for small AAA, the risk of rupture exists. In the series of Brown [21], the annual risk of rupture of a 50 to 60 mm AAA was 3.4% in the group of 155 medically followed patients, deemed at unacceptable surgical risk. Cronenwett [7] observed 6 (9%) ruptures among 67 non-operated patients followed over 36 months for small ($<$ 55 mm) AAA.

Octogenarians suffering AAA ask for special consideration. In our series, 20% of the AAA were ruptured at referral. Of these 28 patients, 18 had a documented AAA that was conservatively managed by their family doctor. This reflects the reluctance of general practitioners to refer their elderly patients with a documented asymptomatic AAA for elective repair. Primary care physicians often make an incorrect assessment of operative risk and grossly underestimate life expectancy of the elderly and the rupture rate of AAA. They erroneously assume that advanced age is a contraindication to surgery.

Controversy about management of ruptured AAA in elderly still exists [13]. A decade ago, an official recommendation in England (Confidential Enquiry into Perioperative Deaths — CEPOD [30]) suggested to withhold surgery for AAA rupture at 80 years or older. None of the octogenarians operated for rupture in the district hospitals survived. Also argued was the tremendous cost for the health insurance. At our institution, the cost to save one patient with ruptured AAA

was evaluated at 1.2 million BF, versus 350.000 BF for successful elective AAA repair [11]. Dean [13] and Johansen [20] calculated analogous costs in both groups. In a more recent report from the Mayo Clinic, no single preoperative criterion could be identified justifying withholding care from patients with a ruptured AAA [24]. Forty-four percent of their octogenarians, operated on for rupture, survived.

An ethical problem and dilemma raises when an AAA ruptures in a patient who had already been turned down for elective repair on medical grounds or because of patient refusal [31]. In such an emergency situation, there is no time left for risk evaluation or open discussion with the patient or his family. The surgeon should take his full responsibility : no resuscitation at all or an aggressive approach. We are proponents of surgery for all ruptured AAA reaching the hospital alive, since it is the only reasonable chance of survival that can be offered to the patient [10, 24]. In case of preoperative cardiac arrest, the chances of survival are reduced, but not inexistant. At the Mayo Clinic, 20% of these patients survived to operation [24]. An opposite point of view is given by Dean [13] and Johansen [20], who did not see any realistic hope for recovery of patients in profound haemodynamic shock due to AAA rupture. Consequently, they questioned the appropriateness of a heroic attempt of surgical repair. Emergent repair of ruptured AAA for which elective repair was previously denied, deemed not to be fruitless, since in our series, 20% (5/25) survived. In the series of Piotrowski [31], 17% (3/18) of similar patients survived to operation for ruptured AAA. Only for patients in a preterminal condition prior to rupture, surgical abstinence in case of rupture can be considered [6, 13].

Long-term follow-up after elective AAA repair in elderly gives a 5-year survival of approximately 50%, what is lower than that of an age and sex matched general population (mean 5-year survival 63%) [19]. This illustrates that patients with AAA commonly suffer concurrent cardiac disease and are exposed to a higher heart-related mortality [2, 19, 32]. Other authors report a survival rate after successful elective repair that parallels that of the general population [13-15, 18]. Most survivors retain a gratifying quality of life [13, 15, 33, 34].

Direct graft replacement of AAA is the standard of care. Some less invasive alternatives to conventional AAA resection have been proposed, in an attempt to reduce procedure related mortality and morbidity, especially for patients at prohibitive operative risk. Wrapping of the AAA [35] is actually abandoned as an obsolete technique. Aneurysm exclusion by ligation of the neck or the iliac arteries, associated with subsequent axillo-bifemoral bypass, was very popular a decade ago. But the results of non-resective treatment (7.7% mortality rate [36]) are not superior to those of standard repair [26]. Even more important is the fact that the induced thrombosis is not a guarantee against ulterior rupture of the unresected aneurysm [37]. In the series of Pevec & Blaisdell [26 cases], 3 patients (11.5%) suffered subsequent rupture [36]. Today, its anticipated safety and efficacy is questioned and the technique has been disfavoured [26, 27, 37]. Endovascular stent-grafting is an attractive, innovative concept, clinically introduced by Parodi [38]. However, not all AAA are eligible for "endografting" (too short neck, tortuous or calcified iliac arteries). There is also a technique-related morbidity (cholesterol embolization [39], contrast induced nephropathy, graft overlap of the renal artery orifices, local vascular complications, such as iliac or femoral artery trauma by the relatively inflexible 24 F access sheath) and device-related malfunction (attachment fracture, migration, perigraft leak). In a recent series, a 3.2% mortality was reported for elective procedures [40]. This minimal invasive technique is still under investigation, and its long-term efficacy in preventing rupture remains to be proven [40-43].

We conclude that AAA repair can be performed safely in carefully selected octogenarians, even if the results are not so excellent as in the younger age group. Advanced age, in itself, should not be an exclusion criterion of AAA repair. Management of octogenarians with a non-ruptured AAA of 50 mm or more requires sound clinical judgement in each case, and fully information of the patient and his family. On the basis of our data, we recommend a straightforward surgery for "otherwise healthy" octogenarians with a 50 mm or more AAA, with a rapidly expanding AAA (growth rate exceeding 0.5 cm / 6 months) or with a symptomatic AAA. For "at risk" patients, we adopt watchful waiting and AAA surveillance up to 60 mm. For unfit individuals with life expectancy of less than 2 years and for mentally or physically severely disabled patients, no surgery should be performed.

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APPENDIX 3

Activated forms of MMP-2 and MMP-9 in abdominal aortic aneurysms. **Natzi Sakalihasan, Philippe Delvenne, Betty V. Nusgens,Raymond Limet, Charles M. Lapière.** *J Vasc Surg 1996;24:127-133*

Activated forms of MMP_2 and MMP_9 in abdominal aortic aneurysms

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Purpose: **The consistent observation of a reduction of the elastin concentration in abdominal aortic aneurysms (AAAs) has led us to investigate in AAA specimens two metalloproteinases that display elastase activity, MMP_2 (gelatinase A/72 kDa) and MMP_9 (gelatinase B/92 kDa).**

Methods: **Samples of full-thickness aortic wall, adherent thrombus, and serum were collected in 10 patients with AAAs. Samples of normal aortic wall and serum were taken from 6 age-matched control patients. Quantitative gelatin-zymography and gelatinolytic soluble assays after acetyl-phenyl mercuric acid activation were performed on serum and tissue extracts, and the results were expressed in units on a comparative wet-weight basis. Histologic analysis was performed in parallel to score the inflammatory infiltrate.**

Results: **The luminal and parietal parts of the thrombus contained, respectively, 20- and 10-fold more gelatinolytic activity than the serum. The predominate form was MMP_9. Although the total gelatinolytic activity was in the same range both in AAAs and in normal walls, a significantly higher proportion of MMP_9 was found in the aneurysmal aortic walls. Furthermore, a significant proportion of MMP_9 was under its processed active form, which was never observed in normal samples. A significantly higher proportion of MMP_2 was also present as processed active form in AAA wall. This latter parameter positively correlated with the inflammatory score.**

Conclusions: **The presence of activated MMP_9 and MMP_2 might contribute to the degradation of the extracellular matrix proteins that occurs during the development of aneurysms. (J Vasc Surg 1996;24:127-33.)**

Structural alterations of the aortic wall and degradation of matrix proteins consistently have been reported to occur in abdominal aortic aneurysms (AAAs) as compared with healthy aorta or with atherosclerotic occlusive aorta.[1-3] The analyses that we previously performed on tissue fragments collected from AAAs of increasing size demonstrated a reduction in elastin concentration that occurred during the early development of the lesion without alteration in collagen concentration, whereas an increased extractability of collagen was observed in the ruptured specimens.[4] These alterations might result from an increased proteolysis, decreased antiproteolytic activities, or both.[5,6] Increased levels of blood and tissue proteinase activities have indeed been reported in patients with AAAs; some of the previous studies described a serine-protease[7,8] as the main elastolytic activity, whereas other studies showed that the elastolytic activity presented features of the family of metalloproteinases (MMPs).[9-12]

The MMPs are connective tissue–degrading enzymes that participate in a variety of physiologic remodeling processes and in many diseases associated with excessive tissue degradation, such as arthritis, tumor invasion, periodontitis, and osteoporosis. The members of this family share a high level of gene characteristics and common features, such as a Zn-dependent catalytic site, requirement of Ca++ for activity, and secretion under a latent proenzyme form requiring a primary activation by proteolytic enzymes, organomercurials, or chaotropic agents fol-

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Table I. Material and methods

	Aneurysm diameter 66.8 ± 10.8 mm	*Control aorta <30 mm*	*Control serum*
Samples No.	10	6	6
Men	8	6	6
Age (yr)	70.5 ± 10.5	77.5 ± 10.0	69.0 ± 6.3

lowed by an autocatalytic activation by cleavage of the propeptide. Their activity in the tissues is regulated by specific tissue inhibitors of metalloproteinases (TIMPs).[13] MMP_2 (gelatinase A/72 kDa) and MMP_9 (gelatinase B/92 kDa) express a broad spectrum of activity and have been shown to display significant elastase activity, more potent than the stromelysins,[14] on purified substrate[15] and in organotypic culture of aortic fragments.[16] MMP_2 is produced by cells of mesenchymal lineage, whereas MMP_9 is secreted by neutrophils, macrophages, and macrophage-derived osteoclasts and likewise participate in the inflammatory response. The excessive degradation of elastin and, perhaps, other non–collagen matrix components and the presence of an inflammatory infiltrate in the wall of AAAs[2,17,18] led us to investigate the involvement of these MMPs in this disease. For this purpose, we identified and quantitated MMP_2 and MMP_9 and their extent of activation in the wall of AAAs as compared with normal aortic walls. As significant amounts of the two enzymes and their inhibitors (TIMPs) are present in the circulating blood,[19,20] they were evaluated on a comparative unit wet-weight basis in the serum and in the wall-adherent thrombus at a luminal and parietal location.

MATERIALS AND METHODS

Collection of specimens. All tissues and blood samples were obtained with the approval of the Institutional Ethics Committee of the Liège University Hospital. Fragments of full-thickness aortic wall and adherent thrombus were collected 4 to 5 cm distal to the renal arteries in 10 patients undergoing elective operative repair. The mean diameter of the AAAs was 66.8 ± 10.8 mm. A sample of serum was collected from each patient. Control aortic samples were collected at autopsy within 24 hours after death from six age-matched patients without known cardiovascular disease. Age-matched control serum samples were obtained from six volunteers. The mean age was 70.5 ± 10.5 years in the AAA group, 77.5 ± 10.0 years in the control aorta group, and 69 ± 6.3 years in the control serum group (Table I). A piece of material (0.5 to 3 cm^3) was dissected from the luminal and parietal side of the thrombus and from the aortic wall, quickly frozen, weighed (wet weight), crushed in liquid nitrogen, lyophilized, and weighed again (dry weight).

Extraction of MMPs. Aliquots of powdered thrombus and aortic wall were extracted (2 ml/100 mg dry weight) overnight at 4° C in 0.05 mol/L Tris HCl pH 7.5, 1 mol/L NaCl, 2 mol/L urea. After centrifugation at 15,000 rpm in the cold, the supernatant was collected and used for measurement of gelatinase activity by a soluble assay and by zymography.

Soluble assay of gelatinase activity. The gelatinolytic activity present in tissue extracts and serum was assayed by using ^{3}H-gelatin as substrate. Type I collagen was purified from fetal bovine skin and radiolabeled with ^{3}H-acetic anhydride as previously described[21] to a specific activity of 0.79 × 106 cpm/mg collagen. Before performing the assay, the ^{3}H-collagen was neutralized with 1 mol/L Tris (base) and diluted with 0.05 mol/L Tris-HCl pH 7.5, 0.2 mol/L NaCl, 0.5% Triton X-100, 5 mmol/L $CaCl_2$, 3 mmol/L NaN_3 to 5,000 cpm per 50 µl of substrate solution, denatured to ^{3}H-gelatin by heating 10 minutes at 60° C and supplemented with 5 µl of 2.5 mmol/L solution of protease inhibitors, N-ethylmaleimide (NEM) and phenylmethane sulfonylfluoride (PMSF).

Serial dilutions (1/10 to 1/800) of the tissue extracts and of the serum, the latter being brought to 4 mol/L urea before activation, were incubated for 15 minutes at 25° C in the absence or presence of 0.3 mmol/L acetyl-phenyl mercuric acid (APMA) to activate the gelatinases. After APMA activation, some samples were supplemented with ethylenediamine tetraacetic acid (EDTA) to 10 mmol/L to inactivate the MMPs and served as blank values. Nonactivated, activated, and EDTA-inhibited samples were incubated overnight with 5,000 cpm ^{3}H-gelatin at 37° C, trichloroacetic acid–precipitated (12% final) in the cold and centrifuged at 2,000 rpm, and the TCA-soluble radioactivity (fragments of gelatin) was measured by liquid scintillation spectrometry. The activity was calculated from the serial dilutions fitting in the linear part of the kinetics curves. The results were expressed in units per mg wet weight (tissue) or per µl (serum), one unit being the activity able to degrade 1 µg of gelatin (=790 cpm) in 16 hours at 37° C.

Zymography assay. Serial dilutions of tissue extracts and serum were incubated for 1 hour at room temperature in 0.01 mol/L Tris HCl pH 6.8, 4% sodium dodecylsulfate (SDS) before performing polyacrylamide slab gel electrophoresis according to the technique of Laemmli[22] using a separation gel con-

Table II. Amount of gelatinolytic measured by soluble assay and by zymography and distribution between MMP_2 and MMP_9

	Tissue samples					
	Serum		*Thrombus*		*Wall*	
	Control (n = 6)	*AAA (n = 10)*	*Luminal (n = 10)*	*Parietal (n = 10)*	*AAA (n = 10)*	*Control (n = 6)*
Total activity						
Soluble assay*	3.0 ± 0.9	2.8 ± 0.8	41.9 ± 51.3	13.1 ± 15.8	11.1 ± 7.8	5.5 ± 4.8
Zymography†	2.7 ± 0.5	5.6 ± 2.5‡	125.4 ± 93.0	68.4 ± 56.4	71.4 ± 39.4	53.6 ± 44.6
Distribution (in%)						
MMP_2	55 ± 13	56 ± 11	19 ± 16	33 ± 17	49 ± 16	71 ± 8
MMP_9	45 ± 13	44 ± 11	81 ± 16	67 ± 17	51 ± 16‡	29 ± 8

Results are expressed in units per μl of serum or per mg of wet weight of tissue, *one unit in soluble assay corresponding to enzyme activity degrading 1 μg of gelatin in 16 hours at 37° C and †one unit in zymography assay corresponding to bleaching of 1 arbitrary volume of gelatin containing acrylamide gel.
‡Significantly different from control values, $p < 0.02$.

taining 1% gelatin. After migration, the gels were washed two times in 2% Triton at 30° C and incubated at 37° C overnight in 0.05 mol/L Tris HCl pH 7.6, 10 mmol/L $CaCl_2$ to allow for gelatin degradation. The gels were stained with Coomassie Blue, the gelatinase bands appearing as white on a blue background. The intensity of the bands was recorded with a LKB Ultrascan XL laser scanning densitometer. The enzyme activities were calculated from the linear part of the regression curves relating the intensity of the bands and serial dilutions of the tissue extract or serum.

Histologic analysis. The specimens of aortic tissue and thrombus, luminal and parietal, were fixed in 3.5% saline-buffered formaldehyde and processed with standard techniques for paraffin embedding. Hematoxylin and eosin–stained sections were used to evaluate the density, localization, and nature of the inflammatory cells. The intensity of the inflammatory reaction was scored as mild (+), moderate (++), or severe (+++).

Statistical analysis. Statistical analysis was performed by Student's t test, and the distribution of each variable was characterized by the mean and standard deviation. Results were considered to be significant at the 5% critical level ($p < 0.05$).

RESULTS

The patterns of distribution of MMP_2 and MMP_9 display obvious differences in the various samples (Fig. 1). They are representative of the quantitative results in Tables II and III.

Table II illustrates the gelatinolytic activity measured by soluble assay and by zymography, expressed in units per mg wet weight of extracted tissue or per μl (serum), in the serum, extracts of luminal and parietal fragments of the thrombus and of the aortic wall from the 10 patients with AAAs compared with six normal age-matched serum samples and six samples of normal aortic wall from age-matched individuals. The distribution of gelatinolytic activity between MMP_2 and MMP_9 is recorded from zymograms and reported as the sum of the zymogen and of the processed forms of each of the two enzymes.

All reported activities were inhibited by EDTA, a property characteristic of the Ca-dependent MMPs and resistant to the thiol-proteinases inhibitor, NEM, and to the serine proteinases inhibitor, PMSF.

Gelatinase activity in the serum. By using the soluble assay after APMA activation, the total gelatinase activity measured in the serum was similar in AAAs and normal controls whereas two times more activity was found in AAA serum by zymography (Table II). The gelatinolytic activity was almost equally distributed between the MMP_2 and MMP_9 in the serum of control and in AAAs. It must be noted that in five of the 10 samples of AAAs, around 10% of the MMP_9 was found under an activated processed form (Table III), whereas none of the control samples displayed such processing.

Gelatinase activity in the thrombus. A high level of gelatinolytic activity was observed in the luminal thrombus in contact with the blood and, to a lesser extent, in the parietal segment of the thrombus (Table II). As compared with the activity in the serum and expressed on the same basis (1 μl of serum = 1 mg wet weight of thrombus), the luminal part of the thrombus contained 15 (by soluble assay) to 25 (by zymography) times more gelatinolytic activity than the serum. These levels dropped in the parietal segment of the thrombus. This high gelatinolytic activity was mainly a result of MMP_9, representing

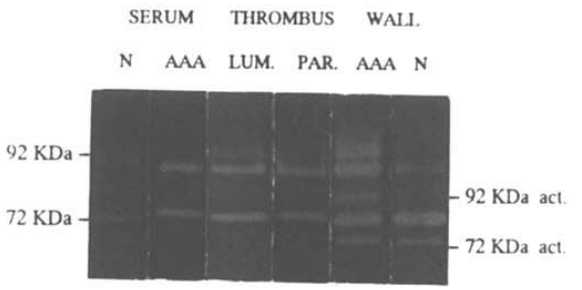

Fig. 1. Representative example of MMP_2 and MMP_9 activity under latent (92 and 72 kDa) or processed (92 and 72 kDa act) forms measured by zymography in serum and vessel wall *(WALL)* extracts of normal aorta *(N)* and AAAs. Extracts of samples from luminal *(LUM)* and parietal *(PAR)* parts of adhering thrombus are also shown.

80% and 70% of the total activity in the luminal and the parietal parts of the thrombus, respectively. In the luminal thrombus, no activated form was observed, whereas in the parietal thrombus 10% of the MMP_9 and 30% of the MMP_2 were present as activated processed enzyme (Table III).

Gelatinase activity in the aortic wall. The total gelatinase activity measured in the AAA wall was similar to that found in the parietal thrombus and did not significantly differ from the activity present in the normal aortic walls (Table II). Whereas the preponderant form of gelatinase in the normal aorta was MMP_2, a significantly higher proportion of MMP_9 was detected in AAA ($p < 0.05$). Moreover, a significant proportion of the MMP_9 appeared in the AAA wall as fully processed activated enzyme, which was never observed in the control aortic wall. The activated processed form of MMP_2 was also significantly increased, more than doubled in the wall of AAA as compared with controls (Fig. 1 and Table III). A gelatinolytic activity migrating above the latent pro-MMP_9 was also observed in most of the AAA wall samples (Fig. 1). It was not taken into account in the calculation of MMP_9 activity.

Histologic examination. Microscopic examination of the AAA wall showed medial and intimal fibrosis often associated with atherosclerosis, focal calcifications, perivascular sclerosis, and thickening of the vasa vasorum. The thrombi consisted of a fibrinous material infiltrated diffusely or locally by degenerated red cells and rare leukocytes. No obvious morphologic difference was found between the luminal and the parietal thrombus. Fig. 2 is a representative example of our series of specimens, showing the low density of the inflammatory infiltrate in the luminal (Fig. 2, *A*) and parietal (Fig. 2, *B*) side of the adherent thrombus and the preferential localization of inflammatory cells in the adventitia and the media of the aortic wall (Fig. 2, *C*). They consisted predominantly in mononuclear cells (lymphoplasmacytic cells and macrophages) beside some polymorphonuclear neutrophils. A linear regression relationship was tentatively established between the extent of the inflammatory cells infiltrate in the aortic wall and the level of expression and activation of the two gelatinases (Table IV). The only significant positive correlation ($r = 0.46$) was between the level of activated MMP_2 and the density of the infiltrate.

Table III. Activated forms of MMP_2 and MMP_9 gelatinases

	MMP_2	*MMP_9*
Serum control	0	0
Serum AAA		12 ± 12%*†
Thrombus luminal	0	0
Thrombus parietal	27 ± 24%	9 ± 10%
Wall AAA	35 ± 12%*	21 ± 15%*
Wall control	17 ± 5%	0

*Significantly different from control walls, $p < 0.05$.
†Results are expressed in percent of total MMP_2 or MMP_9 activity.

DISCUSSION

The enzyme activities measured in the serum and in the tissue extracts display an array of features specific of the MMPs. They are inhibited by EDTA, resistant to PMSF, and activated by NEM, organomercurials, and SDS. The latent forms and processed forms of these enzymes display adequate molecular size as compared with the enzyme secreted by A2058 cell line for the MMP_2[23] and 12-0-tetradecanoylphorbol-13-acetate–induced transformed endothelial cells ECV-304 for the MMP_9[24] (data not shown).

The two assays used for measuring gelatinase activity provide somewhat different information. In the soluble assay, the activation of the gelatinases is performed by an organomercurial reagent that does not dissociate the enzymes from their complex with the TIMPs. This technique therefore measures the total free MMP_2 and MMP_9 activities. By zymography, the gelatinases are activated by SDS and dissociated from their inhibitors by electrophoresis. This technique allows discrimination of the MMP_2 and MMP_9 as well as their processed activated forms by their molecular size and definition of a distribution pattern of each form. This distribution was used to calculate the gelatinolytic activity, measured by the soluble assay, attributable to each form. The difference between the values measured by zymography and by the soluble assay provides an indirect estimation of the TIMPs.

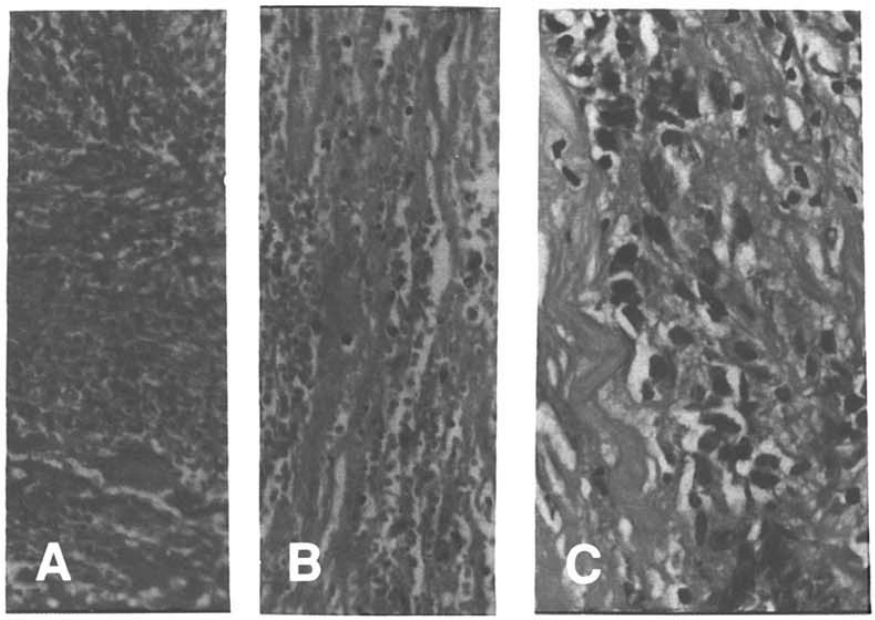

Fig. 2. Hematoxylin-eosin stained sections of luminal **(A)** and parietal **(B)** side of adherent thrombus, which consisted essentially of fibrinous material infiltrated by degenerated red cells and rare leukocytes. The aortic wall **(C)** shows medial and intimal fibrosis associated with mononuclear cell infiltrate predominating in adventitia and media.

Table IV. Relationship between inflammatory cells infiltrate in aortic wall and latent and activated forms of MMP_2 and MMP_9

		MMP_9 (units/mg)		*MMP_2 (units/mg)*	
Infiltrate	*No.*	*Latent*	*Activated*	*Latent*	*Activated*
+*	2	0.90 (0.78 to 1.17)	0.23 (0.18 to 0.27)	0.99 (0.24 to 1.75)	0.33 (0.17 to 0.50)
++	5	1.47 (0.30 to 2.95)	0.48 (0.03 to 1.47)	1.05 (0.64 to 2.50)	0.51 (0.22 to 0.99)
+++	2	2.86 (0.63 to 5.09)	0.25 (0.25 to 0.26)	1.62 (0.63 to 2.61)	0.83 (0.42 to 1.25)
r		NS	NS	NS	0.46

*Intensity of infiltrate was scored as mild (+), moderate (++), or severe (+++).
r, Correlation coefficient; *NS*, not significant.

The main conclusions that can be drawn from our investigations are (1) aneurysmal aortic walls contain a significantly higher amount of MMP_9 than control specimens, and (2) the activated processed forms of both MMP_2 and MMP_9 are significantly increased.

The two forms of gelatinolytic activity, MMP_2 and MMP_9, in the serum were almost equally represented, both in control patients and in patients with AAAs. Latent gelatinases are regular plasma components.[19] The MMP_9 probably originates from granulocytes. This observation is supported by the higher proportion of the MMP_9 in human serum compared with plasma,[25] as we observed in our samples, probably on its release during the in vitro clotting process. The MMP_2 originates from multiple cellular sources, as discussed by Moutsiakis et al.[20] The higher gelatinolytic activity measured in AAA serum by zymography as compared with the soluble assay suggests that circulating gelatinase activity might be partly inhibited by complexing with TIMPs. It is also noteworthy that five of the 10 patients with AAAs had a detectable amount of the activated processed MMP_9, which was never detected in the control group. By examining the clinical and biologic parameters in these five patients, results indicated that they differed by the size of the aneurysmal lesion. The mean aortic diameter of the patients who showed no processed MMP_9 form was 58.0 ± 5.7 mm (range, 50 to 65 mm), whereas that in the group of patients who showed detectable levels of

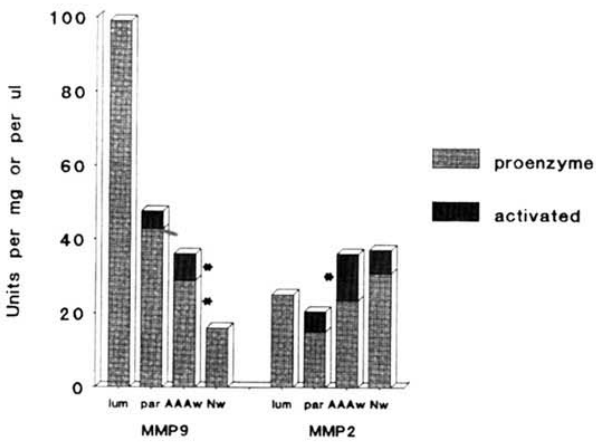

Fig. 3. Illustration of gradient of decreasing level of MMP_9 activity and increasing activation of both MMP_9 and MMP_2 from luminal thrombus toward aortic aneurysmal wall. *, Significantly different from control values.

activated MMP_9 was 75.6 ± 6.2 mm (range, 65 to 80 mm).

The large amount of MMP_9 activity in the luminal part of the thrombus could not be related to its sustained release by inflammatory cells because they were almost absent in this section of the thrombus. The enzyme might have been released during thrombus formation and sequestered by complex formation with other proteins. Both MMP_9 and MMP_2 are formed of structural domains that have been shown to participate in their binding to matrix components.[26] In addition to the propeptide, the catalytic, and the C-terminal hemopexin-like domain, the MMP_9 contains a fibronectin-like region and a type V collagen-homologous region that might participate in interactions with extracellular proteins such as fibrin. The MMP_2 was found as the predominating form in the aortic wall of the control group, whereas a significant shift toward MMP_9 was observed in the AAA specimen. MMP_2 is synthesized by the smooth muscle cells of the intima and the media of the wall as well as by the adventitial fibroblasts, as described by Herron et al.[10] It does not differ in absolute value in the control and AAA groups. The higher proportion of MMP_9 in the AAA is probably related to the presence of inflammatory cells known to produce this MMP. It was recently identified by immunohistochemical analysis[17] and in situ hybridization[18] in macrophages infiltrating the aneurysmal aorta. The MMP_9-positive cells represented, however, a subset of only 10% to 20% of the inflammatory cells.[18] This finding might explain why we were unable to find a significant correlation between the level of expression of the MMP_9 in AAA and the score of infiltrating cells. Other types of inflammatory cells might also produce the MMP_9, such as neutrophils that present the special feature to secrete the monomeric 92 kDa form and a heterodimer of 125 kDa where the MMP_9 is disulfide-linked with α2 microglobulin.[27] The gelatinolytic band migrating above the 92 kDa in our AAA wall samples might originate from polymorphonuclear leukocytes observed in the adventitia and the media of the AAA wall. Recent reports[28,29] identified in AAA, besides the MMP_9, the stromelysin 1 (MMP_3), interstitial collagenase (MMP_1) in high–molecular weight forms and the activated forms of MMP_9 and MMP_3. To our knowledge, our report is the first to demonstrate the presence of the activated form of MMP_2 in significantly higher proportion in the AAA than in normal aortic wall. The gradient of processing of both MMP_2 and MMP_9 observed in our study (Fig. 3) increasing from the luminal thrombus toward the AAA wall suggests that the activation process might originate in the wall.

Because the aneurysmal aortic wall contains a greater amount of MMP_9 with a significant proportion of activated form, unlike the enzyme in the normal aortic wall, and also a significantly larger amount of activated MMP_2, the possibility exists that these enzymes contribute to the degradation of extracellular matrix proteins observed in AAA.

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APPENDIX 4

TIMP-2 and PAI-I mRNA levels are lower in aneurysmal as compared to athero-occlusive abdominal aortas. **Olivier Defawe, Alain Colige, Charles Lambert, Carine Munaut,Philippe Delvenne, Betty Nusgens, Charles Lapière, Raymond Limet, Natzi Sakalihasan.***Cardiovasc Res 2003;60:205-213*

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TIMP-2 and PAI-1 mRNA levels are lower in aneurysmal as compared to athero-occlusive abdominal aortas

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Abstract

Objective: Significant alterations of the vascular wall occurs in abdominal aortic aneurysm (AAA) and atherosclerotic occlusive disease (AOD) that ultimately may lead to either vascular rupture or obstruction. These modifications have been ascribed to one or a group of proteases, their inhibitors or to the matrix macromolecules involved in the repair process without considering the extent of the observed variations. **Methods:** The mRNA steady-state level of a large spectrum of proteolytic enzymes (matrix metalloproteinases: MMP-1, -2, -3, -8, -9, -11, -12, -13, -14; urokinase plasminogen activator: u-PA), their physiological inhibitors (tissue inhibitors of MMPs: TIMP-1, -2, -3; plasminogen activator inhibitor: PAI-1) and that of structural matrix proteins (collagens type I and III, decorin, elastin, fibrillins 1 and 2) was determined by RT-PCR made quantitative by using a synthetic RNA as internal standard in each reaction mixture. The profile of expression was evaluated in AAA ($n=7$) and AOD ($n=5$) and compared to non-diseased abdominal (CAA, $n=7$) and thoracic aorta (CTA, $n=5$). **Results**: The MMPs -8, -9, -12 and -13 mostly associated with inflammatory cells were not or barely detected in CAA and CTA while they were largely and similarly expressed in AAA and AOD. Expression of protease inhibitors or structural proteins were only slightly increased in both pathological conditions with the exception of elastin which was reduced. The main significant difference between AAA and AOD was a lower expression of TIMP-2 and PAI-1 in the aneurysmal lesions. **Conclusions:** The remodeling of the aortic wall in AAA and AOD involves gene activation of a large and similar spectrum of proteolytic enzymes while the expression of two physiological inhibitors, TIMP-2 and PAI-1, is significantly lower in AAA compared to AOD. The repair process in the aneurysmal disease seems similar to that of the occlusive disease.

Keywords: Arteries; Atherosclerosis; Extracellular matrix; Gene expression; Inflammation

1. Introduction

Abdominal aortic aneurysms (AAA) and atherosclerotic occlusive diseases (AOD) arise from a common basic mechanism, atheroma, but their outcome is different. In AOD, the outer diameter of the vessel is preserved and the medial layer remains largely intact while a progressive narrowing of the lumen, resulting from intimal accumulation of lipids, matrix proteins and cells, ultimately interrupts the blood flow [1]. In contrast, AAA is characterized by a preserved blood flow, enlargement of the blood vessel and, eventually, its rupture which is related to a fragmentation and rarefaction of the elastic lamellae [1]. We previously showed that the elastin loss is an early event in the course of the aneurysmal disease while the collagen concentration remained unchanged reflecting a compensatory adventitial fibrosis [2]. The AAA is also characterized

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by a medial and adventitial infiltration by inflammatory cells while this infiltration occurs mainly in the intima of the AOD [3]. Matrix metalloproteinases (MMPs) can collegially degrade all the components of the extracellular matrix. Secreted as inactive zymogens, MMPs are processed into their active forms by other MMPs or serine proteases such as plasmin. Plasmin itself results from the activation of plasminogen by plasminogen activators (urokinase-type: u-PA; and tissue-type: t-PA), a reaction controlled by the plasminogen activator inhibitor (PAI-1). The MMPs activity is further controlled by physiological inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) [4,5]. The involvement of proteases/antiproteases in AAA and AOD is well documented in aneurysmal progression and plaque instability [6]. Data are however sometimes conflicting and most often restricted to one or a few of the potential actors in the pathomechanism of the lesions.

The extensive remodelling of the aortic wall seen in AAA and AOD involves both degradation and synthesis of structural matrix proteins. The biomechanical properties of the vessel largely depend on the adequate proportion of collagen, responsible for the tensile strength of the wall, and elastic fibers forming its extensile network [7]. A compensatory repair process has been documented in human AAA and AOD [8] as well as in animal models [9].

In order to evaluate the respective implication of proteolysis, antiproteolysis and repair processes in normal aorta and in AAA and AOD, we devised a sensitive and quantitative reverse transcription-PCR (RT-PCR) procedure to compare in the same series of samples the mRNA steady-state level of a panel of the most representative proteolytic enzymes, their inhibitors and the main structural matrix macromolecules. Large differences occurred in the pathological samples as compared to control tissues. Besides a decreased level in elastin mRNA in AAA and AOD, a similar increase in the expression of all the tested genes is observed in both diseased aorta except for a significantly decreased expression of two proteases inhibitors, PAI-1 and TIMP2, in AAA and a complete absence of active or latent MMP-9 observed by zymography in AOD.

2. Methods

2.1. Patients characteristics

Full-thickness infrarenal aortic tissue was obtained during elective surgery for degenerative AAA from seven patients (mean age, 67 years; range 55–82 years). All patients were asymptomatic at the time of surgery. The mean size of the aneurysms was 58 mm (range 39–80 mm). Atherosclerotic occlusive aortas were harvested during surgery for infrarenal occlusive disease in five patients (mean age, 63 years; range 50–70 years). Thoracic aorta presenting weak or not clinically observable atherosclerosis was obtained during coronary bypass surgery in seven patients (mean age, 65 years; range 54–74 years). Clinically normal abdominal aortas were collected from seven patients deceased of non-vascular disease (mean age, 60 years; range 51–74 years). The study was approved by the local Ethic Committee and conforms with the principles outlined in the Declaration of Helsinki.

2.2. RNA isolation and quantitative RT-PCR procedure

After isolation [10], RNA concentration was measured by a fluorimetric assay (SpectraMax, Gemini-XS). Pairs of RT-PCR primers (see Table 1) were selected according with the following criteria: (i) a high and similar annealing temperature; (ii) minimum complementarity between primer sequences; and (iii) localization on different exons. For each investigated mRNA, a synthetic RNA (sRNA) was generated, according to previously published works [11–13], in order to monitor in each tube the efficiency of both the reverse transcription and the amplification reactions. The sRNAs give rise to products of a size slightly different from that of the endogenous mRNA (Table 1 and Fig. 1). RT-PCR was performed under non-competitive conditions in an automated system (GeneAmp PCR System 9600, Perkin-Elmer) using the GeneAmp Thermostable rTth Reverse Transcriptase RNA PCR kit (Perkin-Elmer), specific pairs of primers (5 pmol each), 5 ng of total cellular RNA and a known copy number of sRNA per 25 μl reaction mixture. The RT step (70 °C for 15 min) using the antisense primer in presence of Mn^{2+} was followed by addition of the sense primer in presence of Mg^{2+} (i) 2-min incubation at 95 °C, (ii) PCR amplification for the adequate number of cycles and (iii) a final elongation step of 2 min at 72 °C. The PCR conditions for the amplification of most cDNA were: 94 °C for 15 s; 66 °C for 20 s; and 72 °C for 10 s. For MMP-3, -11 and -13, conditions were: 94 °C for 15 s, 63 °C for 30 s and 72 °C for 30 s. The RT-PCR products were resolved on 10% polyacrylamide gels and quantified (Fluor-S-MultiImager, BioRad) after staining (GelStar dye, FMC BioProducts). Each sample was analysed in duplicate. The optical density of the endogenous RNA was normalized by the value of the sRNA (see Fig. 1) and expressed in arbitrary units per unit of 28S ribosomal RNA.

2.3. Zymographic analysis of the gelatinases MMP-2 and MMP-9

The analysis of tissue homogenates of CAA, AAA and AOD was performed by the procedure described earlier [14].

2.4. Statistics

Statistical differences among groups were tested by

Table 1
Sequence of forward and reverse primers used for RT-PCR amplification of the target RNA and length of the RT-PCR products

RNA species	Forward (5′–3′)	Reverse (5′–3′)	Length (bp) of the RT-PCR product from	
			Endogenous RNA	sRNA
MMP-1	GAGCAAACACATCTGAGGTACAGGA	TTGTCCCGATGATCTCCCCTGACA	185	267
MMP-2	AGATCTTCTTCTTCAAGGACCGGTT	GGCTGGTCAGTGGCTTGGGGTA	225	271
MMP-3	GATCTCTTCATTTTGGCCATCTCTTC	CTTCCAGTATTTGTCCTCTACAAAGAA	246	272
MMP-8	CCAAGTGGGAACGCACTAACTTGA	TGGAGAATTGTCACCGTGATCTCTT	200	267
MMP-9	GCGGAGATTGGGAACCAGCTGTA	GACGCGCCTGTGTACACCCACA	208	266
MMP-11	ATTTGGTTCTTCCAAGGTGCTCAGT	CCTCGGAAGAAGTAGATCTTGTTCT	155	268
MMP-12	ACATTTCGCCTCTCTGCTGATGAC	CAGAAACCTTCAGCCAGAAGAACC	196	245
MMP-13	ATGATCTTTAAAGACAGATTCTTCTGG	TGGGATAACCTTCCAGAATGTCATAA	203	270
MMP-14	GGATACCCAATGCCCATTGGCCA	CCATTGGGCATCCAGAAGAGAGC	221	269
TIMP-1	CATCCTGTTGTTGCTGTGGCTGAT	GTCATCTTGATCTCATAACGCTGG	170	271
TIMP-2	CTCGCTGGACGTTGGAGGAAAGAA	AGCCCATCTGGTACCTGTGGTTCA	155	269
TIMP-3	CTTCTGCAACTCCGACATCGTGAT	CAGCAGGTACTGGTACTTGTTGAC	210	269
uPA	ACTACTACGGCTCTGAAGTCACCA	GAAGTGTGAGACTCTCGTGTAGAC	199	245
PAI-1	AGGGCTTCATGCCCCACTTCTTCA	AGTAGAGGGCATTCACCAGCACCA	191	269
α1(I)	CCCACCAATCACCTGCGTACAGA	TTCTTGGTCGGTGGGTGACTCTGA	214	267
α1(III)	GAGATGTCTGGAAGCCAGAACCAT	GATCTCCCTTGGGGCCTTGAGGT	207	265
Decorin	CCTGAAAGGACTGAATAATTTGGCTA	GTTGCTGAAAAGACTCACACCCGAA	277	201
Fibrillin 1	GGTGAATGTACAAACACAGTCAGCA	ATAGGAACAGAGCACAGCTTGTTGA	275	210
Fibrillin 2	ATGGCTCTCGATGCATCGATCAGA	CATTGCCACTTGGGGCAAAGCCA	282	199
Elastin	CCGCTAAGGCAGCCAAGTATGGA	AGCTCCAACCCCGTAAGTAGGAAT	275	189
28S	GTTCACCCACTAATAGGGAACGTGA	GGATTCTGACTTAGAGGCGTTCAGT	212	269

MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase; u-PA, urokinase plasminogen activator; PAI, plasminogen activator inhibitor; α1(I), α1 chain of type I collagen; α1(III), α1 chain of type III collagen; 28S, 28S ribosomal RNA; sRNA, synthetic RNA used as internal standard for RT and PCR reactions.

one-way ANOVA after normalization using neperian Log. A probability value ≤ 0.05 was considered as significant.

3. Results

3.1. RT-PCR assay

The mRNA levels of the selected genes were determined using total RNA purified from seven control thoracic aorta (CTA), seven control abdominal aorta (CAA), seven abdominal aortic aneurysms (AAA) and five aortic occlusive diseases (AOD) in the same run of RT-PCR. The electrophoretic pattern of the RT-PCR products of MMP-9 mRNA, that of ribosomal 28S rRNA and their respective sRNA is illustrated in Fig. 1 as a representative example. Each sample was analyzed in duplicate. A large increase of MMP-9 mRNA in the diseased vessels (AAA, AOD) compared to control aorta (CTA, CAA) was observed while the signals for sRNA and cellular 28S rRNA were almost constant. This assay allows a quantitative and comparative titration of specific mRNA in specimens

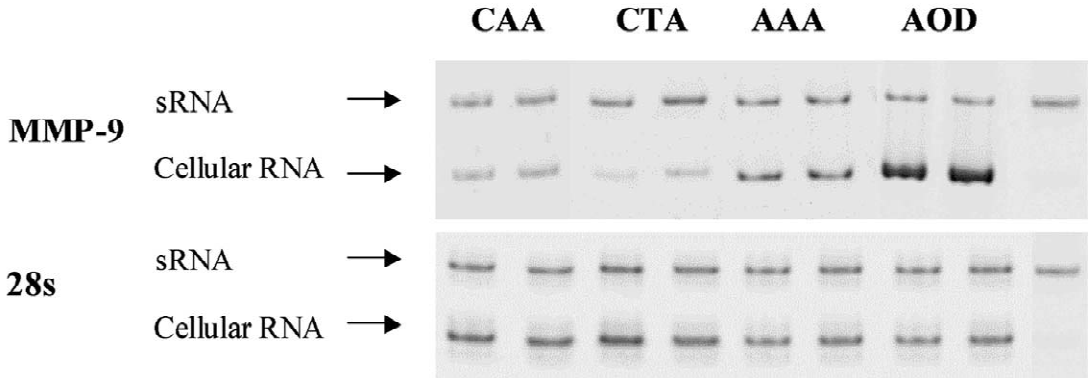

Fig. 1. Representative illustration of an electrophoretic pattern of RT-PCR products amplified from MMP-9 mRNA (32 cycles), 28S ribosomal RNA (28S) (18 cycles) and their respective synthetic RNA (sRNA). The assay was performed in duplicate using RNA from normal abdominal (CAA), thoracic (CTA) or aneurysmal (AAA) and occlusive (AOD) aorta. The sample migrated in the last right lane contained all the reagents including the sRNA but no cellular RNA.

displaying an extended range in level of expression. The accuracy and reliability of this procedure has been reported previously [11–13].

3.2. Comparative analysis of the mRNA steady-state levels in normal thoracic and abdominal aorta

The three groups of mRNA, coding, respectively, for proteolytic enzymes, their physiologic inhibitors and selected structural matrix macromolecules, were measured in clinically normal segments of aorta at two different locations, thoracic (CTA) and abdominal (CAA). No significant difference was observed between the two types of samples (Table 2). It is worth noting that MMP-3, as well as MMP-8, -9 and -12, most often associated with inflammatory cells, were generally not or barely detected as seen from the individual values illustrated in Fig. 2.

Since the efficiency of the amplification process lies within the same order of magnitude for each individual primer pair, as judged from results obtained from a known copy number of the various sRNA (data not shown), the relative abundance of each mRNA can be roughly estimated from the number of cycles of PCR required to obtain measurable amplification products (Table 2). This indicates that the mRNAs for structural proteins and for TIMPs, requiring less amplification cycles, are more expressed than most of the MMPs. The values reported in Table 2 can be considered as representative of the phenotype of the resident cells in non-diseased late adult aortic wall. Even though the pathological aortic samples will only be compared to CAA, the data concerning CTA are provided to support the reliability of the procedure and as information that might be useful to others.

Table 2
Steady-state levels of mRNA in control aorta

		Cycles (*n*)	CAA	CTA	CAA/CTA
Proteinases	MMP-1	37	5±6	3±4	1.7
	MMP-2	31	18±9	17±10	1.1
	MMP-3	37	0	0	n.a
	MMP-8	37	<1	<1	n.a
	MMP-9	32	<1	<1	n.a
	MMP-11	37	9±8	2±2	5.0
	MMP-12	31	<1	<1	n.a
	MMP-13	37	1±2	9±8	0.2
	MMP-14	29	12±6	9±10	1.4
	u-PA	31	4±3	5±6	0.8
Inhibitors	TIMP-1	29	24±21	8±4	2.8
	TIMP-2	28	32±14	15±6	2.0
	TIMP-3	27	28±15	14±10	2.0
	PAI-1	31	27±22	17±7	1.7
Structural proteins	a1(I)	28	13±7	13±14	1.0
	A1 (III)	27	13±8	13±10	1.0
	Decorin	31	9±6	11±6	0.8
	Elastin	27	27±36	23±19	1.3
	Fibrillin 1	31	20±8	11±6	1.7
	Fibrillin2	36	26±19	7±9	3.3

CAA, control abdominal aorta; CTA, control thoracic aorta.
*Mean ratio between the mRNA levels. Number (*n*) of amplification cycle. The values expressed in arbitrary units are means+S.D. n.a., not applicable.

3.3. Comparative mRNA expression in control, aneurysmal and occlusive abdominal aorta

The mRNAs from the diseased aorta (AAA and AOD) were reverse-transcribed, amplified and measured simultaneously with the control tissues (CAA and CTA) as illustrated in Fig. 1 for MMP9. Amplification products of the investigated mRNAs were detected in most samples of the pathological vessels. The exceptions were MMP-3 found in only two AAA while other MMPs were sporadically absent (Fig. 3). The mean mRNA level of all the MMPs and that of the inhibitors were always higher in AAA and AOD than in the CAA (ratio >1), except TIMP2 and PAI-1 in AAA (Table 3). The main and most significant differences, when compared to control tissue, were observed for MMP-8, -9, -11, -12, -13 and u-PA. In our groups of samples, MMP-3 is regularly undetectable, except in two AAA. MMP-8 is expressed in several pathological specimens and only in two controls. A non-significant increase of mRNA coding for structural proteins was observed similarly in both pathological (AAA and AOD) aorta by comparison with normal aorta except for elastin mRNA, which was reduced in both pathologies although to a non-significant level (Fig. 3). MMP2 and its activator MMP14 were also slightly increased (not significant) by a factor of two. The only differences that reached statistical significance between the expression profile of the mRNAs of AAA and AOD was a reduced level of TIMP-2 and PAI-1 in AAA.

3.4. Gelatinolytic activity

As illustrated in a representative zymogram (Fig. 4), a large amount of proMMP-9 is observed in AAA, largely more intense than in CAA. In AAA, a band of activated MMP-9 is also visible while absent in CAA. No proMMP-9 or activated MMP-9 is present in AOD. The amount of proMMP-2 and MMP-2 are similar in AAA and AOD and much larger than in CAA.

4. Discussion

4.1. Technological considerations

In order to reconcile some apparently conflicting data of the literature and to obtain a better and quantitative overview of the pathophysiological processes implicated in AAA and AOD, we measured the expression of three series of genes in clinically normal thoracic (CTA) and abdominal (CAA) aorta compared to samples of AAA or

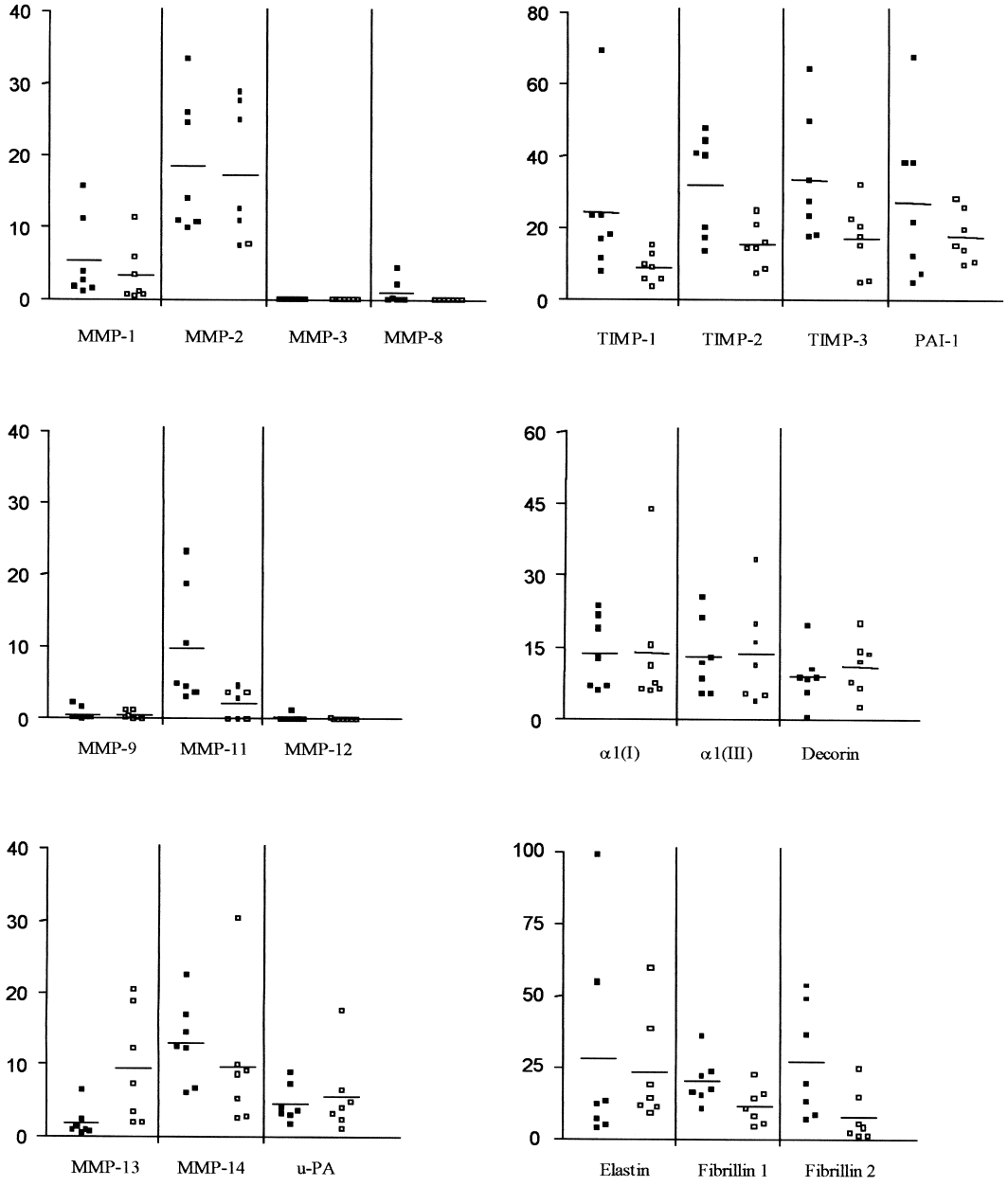

Fig. 2. Expression of mRNA of proteolytic enzymes (MMPs and u-PA), their physiologic inhibitors (TIMPs and PAI-1), and structural proteins (type I and type III collagen, decorin, elastin, fibrillin 1 and 2) in samples of control abdominal aorta (CAA, solid squares), and control thoracic aorta (CTA, open squares). The results are expressed in arbitrary units per unit of 28S rRNA allowing a comparative analysis between samples for each mRNA. No statistical difference between CAA and CTA was observed.

AOD. The mRNA level of proteolytic enzymes (MMPs, u-PA), their inhibitors (TIMPs, PAI-1) and that of the most abundant structural proteins found in the aortic wall was measured by RT-PCR. This procedure was made quantitative by adding in each sample a known copy number of specific synthetic RNAs as internal standards [11–13]. Such standards, co-reverse transcribed and co-amplified with the cellular mRNA using the same primers, allow to monitor the efficiency of both the reverse-transcription and the amplification steps, which is not the case with the cDNA standards such as those used by Carrell et al. [15]. Our sensitive procedure provided quantitative and comparative data for samples expressing an extended range in mRNA levels and permitted to estimate differences in expression more precisely than other assays [16].

4.2. Definition of the biosynthetic phenotype of normal aorta

The thoracic (CTA) and abdominal (CAA) aorta display a very similar biosynthetic phenotype with a significant expression of the structural proteins, including elastin and

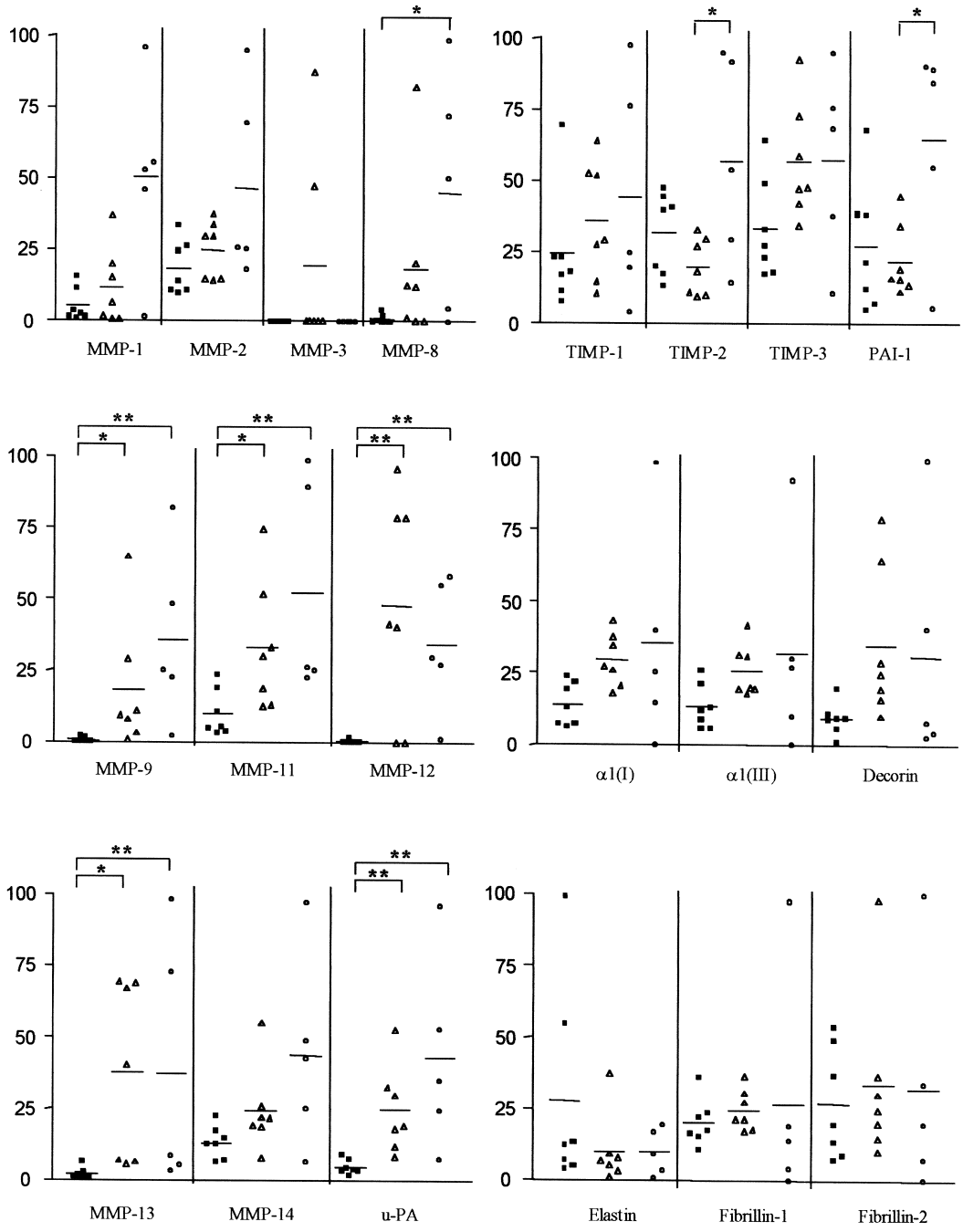

Fig. 3. Expression of mRNA of MMPs, u-PA, TIMPs, PAI-1, type I and type III collagen, decorin, elastin, fibrillin 1 and 2 in samples of control abdominal aorta (CAA, solid squares), abdominal aortic aneurysms (AAA, open triangles) and aortic occlusive disease (AOD, open circles). The results are expressed in arbitrary units per unit of 28S rRNA allowing a comparative analysis between samples for each mRNA. $^{**}P \leq 0.01$, $^{*}P \leq 0.05$.

fibrillins which are involved in the maintenance of the matrix in the vessel, in agreement with previous observations [17]. The mRNA of the MMPs known to be induced in the context of inflammation (MMP-8, -9 and -12) and that of MMP-3 are not expressed to a significant level in the control specimens. By contrast, the mRNAs of the MMP inhibitors (TIMPs) are produced as well as the plasminogen activator u-PA and its inhibitor, PAI-1.

4.3. Expression of the MMPs in AAA and AOD

The genes that are expressed at a significant level (≤31 cycles) in the control tissues (CAA and CTA) tend to be higher in AAA or AOD as are MMP-2 and -14, the TIMP-1 and -3 and the structural proteins. These genes participate in the biosynthetic phenotype of the resident cells in the normal tissue. Their tendency to increase in the

Table 3
Comparative variation of gene expression between AAA or AOD and CAA

		AAA/CAA	AOD/CAA	AAA/AOD
Proteinases	MMP-1	2.2	9.5	0.2
	MMP-2	1.3	2.5	0.5
	MMP-3	n.a	n.a	n.a
	MMP-8	19.8	48.7	0.4
	MMP-9	30.6	60.9	0.5
	MMP-11	3.4	5.3	0.6
	MMP-12	265.2	188.8	1.4
	MMP-13	21.4	21.2	1.0
	MMP-14	1.9	3.4	0.6
	u-PA	5.5	9.7	0.6
Inhibitors	TIMP-1	1.5	1.8	0.8
	TIMP-2	0.6	1.8	0.3
	TIMP-3	2.0	1.7	1.2
	PAI-1	0.8	2.4	0.3
Structural proteins	a1 (I)	2.1	2.6	0.8
	a1 (III)	2.0	2.4	0.8
	Decorin	3.8	3.4	1.1
	Elastin	0.4	0.4	1.0
	Fibrillin 1	1.2	1.3	0.9
	Fibrillin 2	1.2	1.2	1.0

n.a., not applicable.

diseased vessels might be related to an activation of the biosynthetic activity of the SMC. This activation is likely to be underestimated in the lesional tissues by dilution with RNA coming from blood born cells and by the rarefaction of SMC, at least in AAA. Disruption and degradation of elastic lamellae in the media is a prominent feature of AAA that occurs early in the course of the disease [2] and the implication of MMP-9 in its development is clearly supported by animal experimentation. Using mice with targeted gene deletion of MMP-9 or MMP-12 and a double MMP-9/MMP-12 KO only the animals with a deletion of the MMP-9 gene are protected against aneurysmal development [18]. The similarly increased [19,20] or an even higher expression of MMP-9 mRNA in AOD than in AAA as shown here, questions the specific role of this enzyme in aneurysmal dilatation. MMP-9 is produced by the macrophages infiltrating the aortic wall [20]. It is also produced by SMC at least in culture [21]. The larger expression of this mRNA in AOD while the density of the macrophages is lower compared to AAA [2] might indicate that the SMC might participate in the overexpression perhaps upon stimulation by the cytokines released from the inflammatory infiltrate.

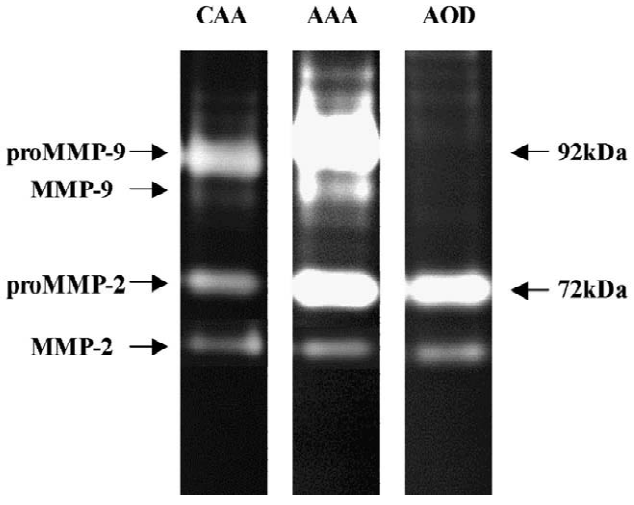

Fig. 4. Representative example of MMP-2 and MMP-9 activity under latent or activated forms measured by gelatin zymography in extracts of normal (CAA) and diseased aortic wall (AAA and AOD).

The tensile strength of the vessel is conferred by the cross-linked polymeric collagens, mainly the fibers of type I and III. Aortic dilatation in aneurysmal progression is linked to a profound remodelling of the fibrillar network of the vessel wall that requires collagenolytic activity. While collagenase-1 (MMP-1) was not much increased as compared to control tissue, the collagenase-3 (MMP-13) was elevated in AAA and AOD in agreement with a previous study [22] immunolocalizing MMP-13 in the SMC within the outer wall in AAA and in the intimal plaque in AOD. Besides its collagenolytic activity, MMP-13 can degrade a broad spectrum of matrix proteins and activate other MMPs [23] suggesting its central role during the evolution of these diseases. Collagenase-2 (MMP-8), a most effective collagenase to initiate type I collagen degradation not detected in the study of Carrell et al. [15], was present in eight out of our 12 patients, while barely detected in the controls. As polymorphonuclear neutrophils are almost absent in the AAA [14] and AOD [3], MMP-8 could be produced by the endothelial cells, the SMC or the macrophages, as supported by its recent detection in vulnerable atherosclerotic plaques and SMC in culture [24]. An intriguing observation in our samples is the very low amount of MMP-3, requiring the highest acceptable number of amplification cycles for detection, expressed in only two AAA samples, and absent in all control and AOD specimens. Although we basically agree with the conclusion of Carrell et al. [15] that MMPs are involved in the pathomechanisms of AAA development, our results do not support a specific role of MMP-3 in this process.

4.4. Imbalanced expression of the inhibitors

When compared to the strikingly similar overexpression of most genes in AAA and AOD, two gene products make the exception, TIMP2 and PAI-1. For both mRNAs, the statistically significant difference that we observed results of a non significant reduction in AAA compared to CAA and a non significant increase in AOD. Since u-PA and MMP-8, -9, -12 and -13 mRNAs are largely increased in both pathologies, whereas their inhibitors, PAI-1 and TIMP2 are lower in AAA as compared to AOD, it could

be suspected that the functional balance of proteolysis/antiproteolysis is altered in AAA. This consideration is supported by observations in the apoE^{-}/$^{-}$, plau^{-}/$^{-}$ (u-PA invalidated) double KO mice in which u-PA deficiency protects against media destruction and aneurysm formation [25]. Moreover, local overexpression of PAI-1 demonstrates the same type of protective potential and a decreased MMP9 activity [26]. The reduction of TIMP2 may also participate in the increased proteolysis in AAA. A significantly different polymorphic allele frequency in male AAA found in the coding region of TIMP2 is intriguing and deserves further investigations [27]. Furthermore, TIMP2 at low concentration participates in association with MMP14 to the activation of MMP2 [28]. We previously showed that a significant part of the MMP2 was present in the AAA wall under an activated form [14]. The role of stromelysin-3 (MMP-11), moderately but significantly increased in AAA and AOD, is putative since the only known substrate of this MMP is the α1-proteinase inhibitor (α1-AP) [29], a serine elastase inhibitor also known for its antiapoptotic activity [30]. Since patients with α1-AP deficiency present multiple aneurysms and emphysema by elastolysis in the lung [31], its potential significance in the aneurysmal disease also merits further investigation.

It has been observed that MMP-9 is found in its precursor and activated form in AAA [14] while neither the proMMP-9 nor the activated MMP-9 are detected by zymography in AOD ([32,33] and our data). Vine and Powell [34] have observed that the elastolytic activity of the homogenates of AAA and AOD increases largely upon treatment of the extracts by KSCN to release the proteases from their complex with α_2-macroglobulin (α_2M). The lack of MMP-9 (pro- and activated) in our samples of AOD might be related to a similar process. Beside its large amount of liver origin in the plasma, α_2M is also produced by macrophages mainly upon cytokine stimulation [35]. As observed in vitro the secreted proteinases might react with the secreted α_2M to form complex catabolized by the macrophages. If such a process occurs in AOD and not in AAA as suggested in our zymograms, it might explain the divergent evolution of the blood vessel wall in these diseases. Further work is progressing to clarify this issue.

4.5. Extracellular matrix proteins

The mRNA level of the structural proteins involved in the formation and maintenance of the resistant collagen framework of the wall was slightly increased in AAA and AOD by comparison to control aorta. It could represent the expression of a repair mechanism performed by the resident cells, fibroblasts and SMC [8]. The lower than normal expression of the elastin gene associated with an increased collagen and decorin expression suggests in both aortic diseases that the composition of the repair connective tissue may be inappropriate and perhaps responsible for alterations of the biomechanical properties of the vessel. Again, the lack of difference between AAA and AOD is striking.

The transcriptional profiling for the three groups of genes reported in this study has been performed under conditions of amplification that allow a direct comparison between control aortic samples and the two pathologies. In our procedure the range of variations, although quite extensive (in the order of a few hundred times between the lowest and the highest value for each mRNA), is much smaller than those observed by Carrel et al. (up to 10 000 times) [15]. This variability within each group of samples is multifactorial and potentially related to the genetic heterogeneity of the human race, additional pathologies or their therapy, and possible minor clinically non-apparent defects related to aging in the control samples. In the pathological specimens, the extent of heterogeneity is caused by similar factors probably enhanced by genetic differences in the transcriptional efficiency of some of the tested genes [36,37]. Furthermore, AAA and problably AOD are heterogeneous not only in terms of size but also in metabolic activity as we recently demonstrated by PET scan imaging [38]. Additionally, each of the specimens is not uniform but made of a mosaic of zones in which the pathological process displays different levels of activity. Our recent results (unpublished data) in one ruptured AAA show that the level of MMPs (-1, -2, -9, -12 and -14) decreases as a function of the distance in samples collected progressively away from the site of rupture while the m-RNA of MMP-3, -8 and -11 was either absent or significantly high in the same series of samples. This could also explain some of the differences between the published data and our work.

Our study investigated the simultaneous implication of three biological processes, proteolysis, anti-proteolysis and repair, in the AAA and AOD. Altogether, our results obtained by measuring mRNA to evaluate gene expression show that, when compared to the basal physiological phenotype of clinically normal age-matched aortas, both the aneurysmal and the atherosclerotic lesions are similarly characterized by a largely increased proteolytic repertoire which is not compensated by a similar increased level of proteases inhibitors and structural proteins involved in the repair process. The profile of mRNA expression of 18 genes is quite comparable in AAA and AOD, while only two genes, TIMP-2 and PAI-1, significantly differ between the two pathologies. These observations suggest a significant role for proteinase inhibitors during the divergent evolution of AAA and AOD.

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APPENDIX 5

Gradient of proteolytic enzymes, their inhibitors and matrix proteins expression in a ruptured abdominal aortic aneurysm. **O. Defawe, A. Colige, C.A. Lambert, P.Delvenne, C.M. Lapière, R. Limet, B. Nusgens, N. Sakalihasan.** *Eur J Clin Invest,2004;34.(7) :513-4.*

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Letter to the Editor

Gradient of proteolytic enzymes, their inhibitors and matrix proteins expression in a ruptured abdominal aortic aneurysm

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Sir,

Abdominal aortic aneurysm (AAA) is a chronic degenerative disease occurring with a high incidence, up to 10%, in the over 65-year-old population [1]. The aneurysmal aortic wall is characterized by increased levels of matrix metalloproteinases (MMPs) and urokinase plasminogen activator (UPA) [2–5], reflecting an activation of inflammatory and resident cells, coupled with an imbalance with their inhibitors, tissue inhibitors of metalloproteinases (TIMPs) and plasminogen activator inhibitor (PAI-1) [6]. The resulting damage to the vessel wall and its progressive dilatation lead to an increased tensile stress to the wall that, in coupling with the destruction of elastic lamellae, may result in rupture. Although several studies have analyzed the wall of ruptured aneurysms, few data concerning the site of rupture are available [7]. In this context, six tissue samples were collected from one ruptured AAA, adjacent to the site of rupture and at increasing distance from the rupture. The steady-state mRNA level for matrix proteins, proteolytic enzymes involved in matrix degradation, and their inhibitors was measured by a quantitative RT-PCR procedure [8,9]. The expression of MMP-1, -2, -9, -12, -14 and uPA was 5–10-fold higher at the site of rupture than in the distant tissue samples (Fig. 1a) while MMP-3 and MMP-8, high at the site of rupture, were sporadically expressed at distance of it (Fig. 1b). TIMP-1, -2, -3 and PAI-1 were also largely expressed at the site of rupture (Fig. 1c). The elastin mRNA was not detected in the site of rupture but expressed, at varying levels, in the distant samples (Fig. 1d). The level of type I and III collagen mRNA was high at the site of rupture and declined outside of it (Fig. 1d). The MMP-1, -2, -9, -12, -14, uPA and the

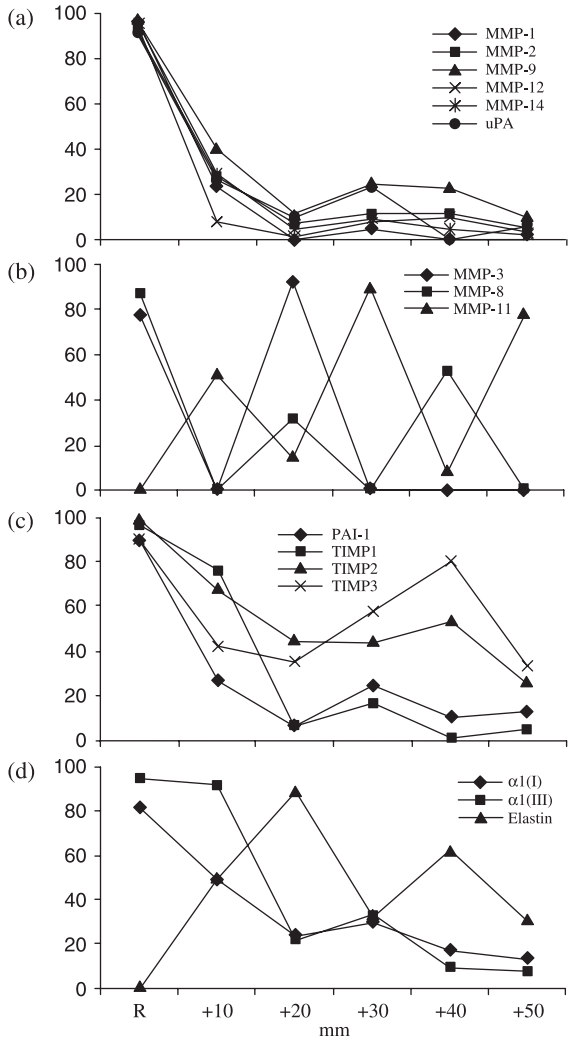

Figure 1 (a–d) Steady-state level of mRNA for matrix metalloproteinases (a,b), their inhibitors (c) and fibrillar matrix proteins (d) in tissues sampled from the site of rupture (R) and at increasing distance. The results are expressed in arbitrary units per unit of 28S ribosomal RNA. α1(I), collagen type I; α1(III), collagen type III.

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inhibitors TIMPs and PAI-1, highest in the rupture area and at +10 mm, may reflect an activation of the inflammatory infiltrate (macrophages, lymphocytes and polymorphonuclear neutrophils; data not shown) and resident cells by a network of cytokines and chemokines, and characterizes a hot spot in the aneurysmal wall that may be more susceptible to rupture. High metabolic activity spots have indeed been shown in the AAA wall using positron emission tomography (PET) [10,11]. In contrast, the expression of these genes at distance from the site of rupture (+20 to +50 mm) were similar to the levels observed in the wall of nonruptured AAA [6]. The sporadic expression of MMP-3, -8 and -11 suggests a heterogeneity in the metabolic activity of the cells, infiltrating the aneurysmal aortic wall. The undetectable level of elastin mRNA at the site of rupture can be explained by the depletion and the severely disturbed distribution of smooth muscle cells (data not shown), known as a major producer of elastin. It is opposed to the high level of mRNA for the two fibrillar collagens I and III at the same site, likely related to an activation of adventitial fibroblasts by inflammatory mediators and possibly by the high mechanical load that they support [8,9]. In conclusion, our study showed that the rupture of the AAA might occur at hot spots characterized by an altered expression of a panel of connective tissues genes.

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APPENDIX 6

Positron emission tomography (PET) evaluation of abdominal aortic aneurysm (AAA). **N. Sakalihasan, H. Van Damme, P. Gomez, P. Rigo, C.M. Lapière, B. Nusgens, R. Limet.**
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Positron Emission Tomography (PET) Evaluation of Abdominal Aortic Aneurysm (AAA)

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Background: *aneurysmal disease is associated with an inflammatory cell infiltrate and enzymatic degradation of the vessel wall.*
Aim of the study: *to detect increased metabolic activity in abdominal aortic aneurysms (AAA) by means of positron emission tomography (PET-imaging).*
Study design: *twenty-six patients with AAA underwent PET-imaging*
Results: *in ten patients, PET-imaging revealed increased fluoro-deoxy-glucose (18-FDG) uptake at the level of the aneurysm. Patients with positive PET-imaging had one or more of the following elements in their clinical history: history of recent non-aortic surgery (*n = *4), a painful inflammatory aortic aneurysm (*n = *2), moderate low back pain (*n = *2), rapid (>5 mm in 6 months) expansion (*n = *4), discovery by PET-scan of a previously undiagnosed lung cancer (*n = *3) or parotid tumour (*n = *1). Five patients with a positive PET scan required urgent surgery within two to 30 days. Among the 16 patients with negative PET-imaging of their aneurysm, only one had recent non-aortic surgery, none of them required urgent surgery, only two had a rapidly expanding AAA, and in only one patient, PET-imaging revealed an unknown lung cancer.*
Conclusion: *these data suggest a possible association between increased 18-FDG uptake and AAA expansion and rupture.*

Key Words: Tomography, emission-computed; Aortic aneurysm, abdominal; Metalloendopeptidases.

Introduction

The risk of rupture increases with the diameter of AAA,[1] however, rupture also occurs in small AAA; it would therefore be useful to define criteria of accelerated growth and impending rupture in smaller AAA. An increased number of inflammatory cells and elevated levels of cytokines within the aneurysm wall have been observed.[2] Cytokines may trigger an increased production of matrix metalloproteinases (MMP) by macrophages and smooth muscle cells. We observed a relationship between the level of inflammatory infiltrate and activation of MMP_2.[3] The risk of rupture can be correlated with the level of biologic markers (matrix metalloproteinases (MMP-2 and -9) and their tissue inhibitors (TIMP 1 and 2)).[4] We investigated the potential of whole-body positron emission tomography (PET) to detect increased metabolic activity of the aneurysm wall. Such hypermetabolism could possibly reflect changes in the aneurysm wall portending rupture.

Positron emission tomography (PET) is a diagnostic method that creates high resolution, three-dimensional tomographic images of the distribution of positron emitting radionuclides in the human body. The radiolabelled compounds used include substrates, ligands, drugs, antibodies, neurotransmitters and other biomolecules that are tracers for specific biological processes. The resulting PET images can be considered as "functional images" of these biochemical or physiological processes. Biochemical processes are altered in most diseases, and these alterations usually precede gross anatomical changes.

Historically, the initial PET studies focused on cerebral and myocardial metabolism. Now, PET is often used for oncological investigation. The most widely

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applied substrate is fluorodeoxyglucose, (18-FDG) a marker of glycolysis. 18-FDG uptake into malignant cells is enhanced by an increased expression of glucose transport molecules on the tumour cell surface. However, FDG uptake is not specific for tumors. FDG-PET scan can also be positive in inflammatory disease.[5] Within tumours, as well as in inflammatory lesions, part of FDG is taken up by macrophages and other blood cells, and up to 25% of the signal reaching the scanner could be due to glycolysis from macrophages within the tumour.[6] FDG uptake in atherosclerotic lesions has been described primarily in spumous cells of the atherosclerotic plaque.[7]

In this study, we analysed PET images in 26 patients with a documented AAA in order to correlate the clinical course of the AAA with the rate of 18-FDG uptake.

Material and Methods

Patients

The study population consisted of a non-consecutive series of 26 patients with AAA (23 males and 3 females) documented by CT-Scan, for whom a complementary investigation by PET imaging was performed between March 1999 and August 2001. The patients presented an AAA with one or more of the following characteristics: large size (70 mm or more) ($n=11$), painful AAA ($n=11$), familial history of AAA ($n=1$), inflammatory AAA ($n=4$), rapid expansion ($n=6$). PET-imaging was performed depending on the availability of the PET imaging system of the hospital.

Mean age was 72 (range 56–85) years. The mean diameter of AAA was 63 mm (range 45–78 mm).

Methods

Radiopharmaceutical

After a minimum 6 h fasting, 3.7 mBq F-18-FDG per kilogram body weight was injected into a peripheral vein.

PET protocol

Static whole-body PET was performed with either an ADAC E-PET or a GE Advance tomograph. Beginning 60 min after tracer injection, emission and transmission images were recorded at each couch position (5–8) for 4–5 and 2–3 minutes, respectively. Coronal, sagittal and transaxial images were based on the use of an ordered subset expectation maximization iterative reconstruction algorithm (OSEM) including post-injection segmented attenuation correction.

Image interpretation

Two experienced investigators interpreted the PET images. The images were reviewed on hard copy and on a computer workstation (SUNSparc, SUN Microsystem, Palo Alto, CA, U.S.A.).

Results

Among the 26 patients, PET scan revealed visible 18-FDG uptake over the infrarenal aorta in ten. The details are shown in Table 1.

Four of these patients (cases 4, 5, 6, 7) had a past history of a recent (within 6 months) operation

Table 1. Patient characteristics with positive FDG uptake.

Patients			Diameter of AAA		Delay between diagnosis and surgery	Remarks	
No.	Sex	Age	Initial	Last			
1	F	64	–	71	<1 month	Low back pain	
2	M	60	–	76	<2 months	Painful inflammatory AAA	
3	M	70	42	51	36 months	Painful inflammatory AAA, rapidly expanding	
4	M*	79	32	70	96 months	Rapidly expanding AAAy prostatectomy	Parotid tumour†
5	M*	73	60	64	6 months	Recent nephrectomy for hypernephroma, leaking AAA	
6	M*	77	35	70	24 months	Recent bilateral carotid TEA, rapidly expanding AAA	Pulmonary CA†
7	M*	82	54	60	6 months	Recent sigmoidectomy, rapidly expanding AAA	
8	M	74	–	70	<1 month	Low back pain	
9	M	69	–	70	2 days	Thoracic aneurysm, painful AAA	Pulmonary CA†
10	M	84	–	50	unoperated	Asymptomatic	Pulmonary CA†

* Patients who had emergency surgery.
† Lesions revealed by PET scan.

excluding aortic surgery (carotid endarterectomy, nephrectomy, prostatectomy, sigmoidectomy). Each of these four patients with positive PET imaging and a positive history of recent operation required urgent aneurysmectomy for rupture (case 6), leaking (case 5), severe back pain and rapid growth (cases 4 and 7). One of these four AAA patients (case 6), with a history of three previous vascular interventions, ruptured his aneurysm eight days after the PET and had emergent surgery (Fig. 1). Another patient (case 5), who underwent nephrectomy six months prior to PET imaging, had emergency surgery for leaking AAA 20 days after the examination. Another patient (case 4) with recent resection for prostatic carcinoma had a known AAA. He did not have elective surgery because of pulmonary disease, and had to be operated on one month later for back pain and an increase in size of the aneurysm of 12 mm (Fig. 2).

A positive PET image was also observed in two of the four inflammatory aortic aneurysms (IAAA) diagnosed on CT scan. Both IAAA were painful (cases 2 and 3).

In three other patients (cases 6, 9, 10) with positive 18-FDG uptake of their AAA, PET imaging also revealed a previously undiagnosed lung cancer. One of them (case 10) benefited from pulmonary lobectomy and will be followed further for his AAA. Another patient (case 9) underwent urgent AAA resection two days after PET imaging, because of severe back pain related to his 70 mm AAA. His lung cancer is treated by chemotherapy. In this patient, 18-FDG uptake was also observed at the level of an aneurysmal thoracic aorta (45 mm in diameter). In the third patient (case 6), the lung cancer showed rapid evolution after AAA resection and was managed by palliation. He died 4 months later.

The remaining two patients (cases 1 and 8) with positive PET imaging of the aneurysm had moderate low back pain preoperatively (Fig. 3). Their past history was unremarkable.

The data concerning the 16 patients with negative PET imaging are summarized in Table 2 (cases 11 to 26). Only one of these patients had recent (within 6 months) surgery (coronary artery bypass grafting three months prior). Two of the four inflammatory AAA revealed by CT scan showed no 18-FDG uptake (cases 16 and 22). These IAAA were asymptomatic. One of them presented with silent ureterohydronephrosis. In one patient (case 25), PET imaging was negative at the level of the aortic aneurysm, but revealed a stage IV lung cancer. He did not have surgery for the AAA.

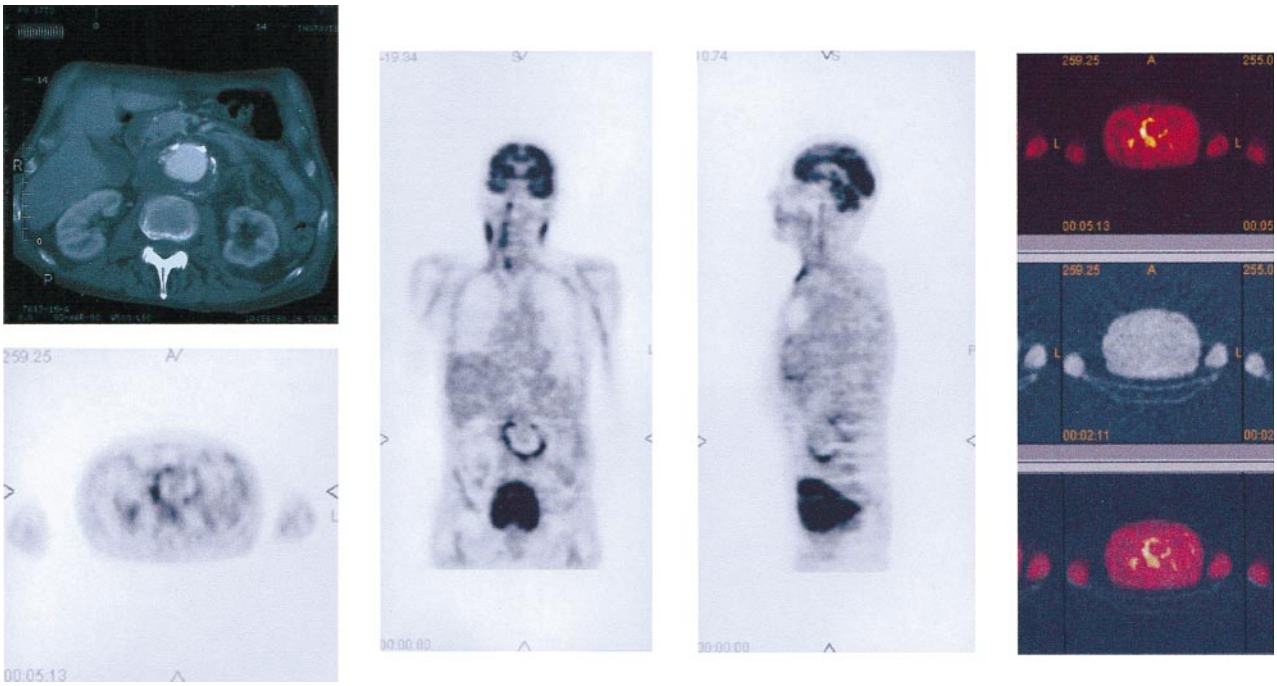

Fig. 1. This partially ruptured AAA is characterised by increased metabolic activity of the aneurysmal wall as evidenced by positive PET imaging. On the right side, the upper image corresponds to the emission image, the second one is a transmission image area; and the third one is a fusion of the two previous images.

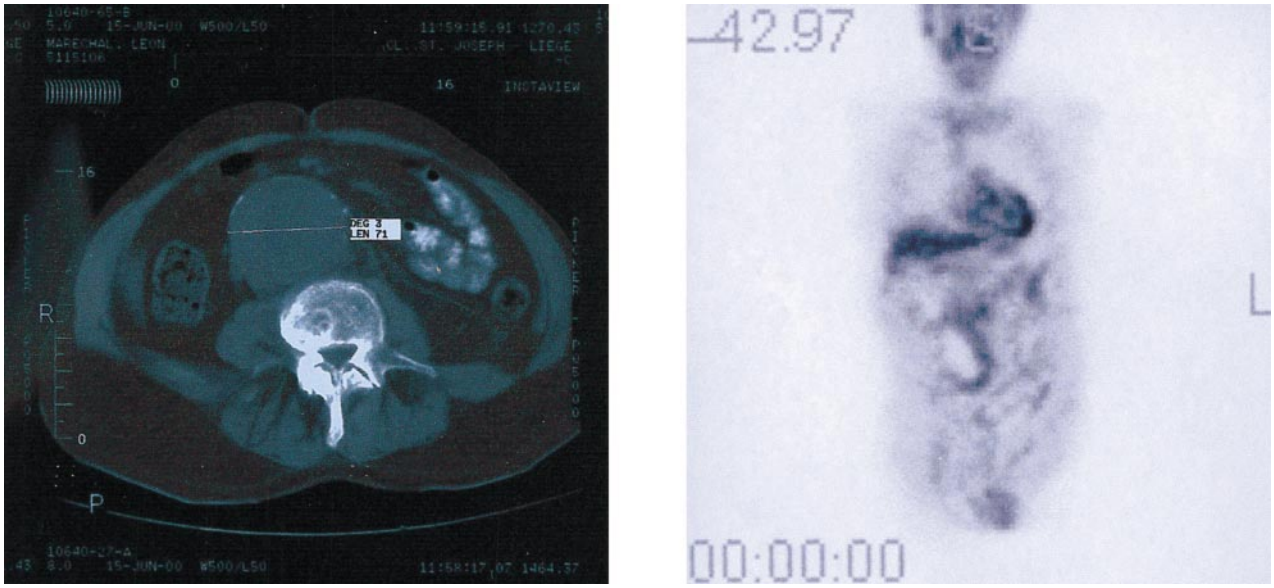

Fig. 2. This painful rapidly expanding aneurysm of 70 mm displays 18-FDG uptake at the level of the aneurysmal wall.

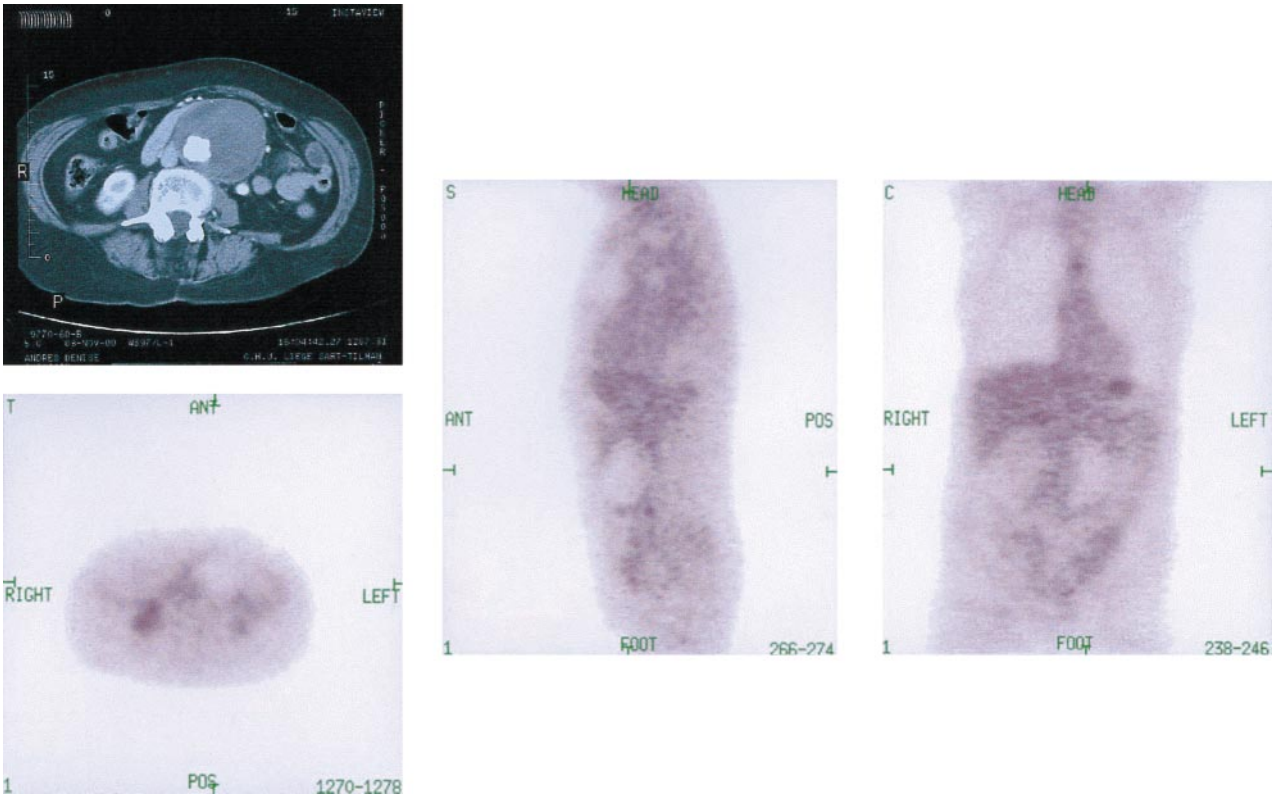

Fig. 3. This aneurysm is mainly filled with a parietal thrombus. The thrombus does not manifest FDG uptake, in contrast to the aneurysm wall where we observe moderate uptake of FDG.

Table 2. Patient characteristics without positive FDG uptake.

Patients			Diameter of AAA		Delay between diagnosis and surgery	Remarks
No.	Sex	Age	Initial	Last		
11	M	56	66	70	20 month	Asymptomatic
12	M	69	–	60	<1 month	Moderate back pain
13	M	78	56	60	7 months	Moderate back pain
14	F	73	–	45	<1 month	AAA with associated aortoiliac artery disease
15	M	68	50	70	17 months	Rapidly expanding AAA
16	M	60	50	50†	7 months	IAAA (asymptomatic)
17	M	70	40	52	36 months	Rapidly expanding AAA
18	F	77	–	65	<1 months	Moderate back pain
19	M*	66	30	47	60 months	Positive familial history for AAA
20	M	85	34	66	30 months	Moderate back pain
21	M	78	–	60	<2 months	Asymptomatic
22	M	77	–	51†	<2 months	IAAA (asymptomatic) with hydronephrosis
23	M	75	–	78	<1 months	Moderate back pain
24	M	64	–	60	<3 months	Asymptomatic, CABG 3 months before
25	M	74	–	70	unoperated	Pulmonary CA with metastasis
26	M	63	–	70	<1 month	Moderate back pain

∗ Familial AAA.
† Inflammatory AAA.

Discussion

This preliminary study of PET imaging of AAA suggests an association between 18-FDG uptake by the aneurysm wall, rapid expansion of the aneurysm, recent (within 6 months) surgery, and malignancy. Indeed, five of the nine operations among patients with positive PET imaging were done on an urgent or emergent basis. No urgent surgery was required among the 15 operated patients with negative PET imaging. In this series, malignancy was also more common among patients with positive PET-imaging of the AAA.

A surgical intervention may activate inflammatory cells, producing collagenase and elastase activity within the aortic wall. Swanson[8] was the first to report an increased growth of AAA as well as an increased tendency to rupture in the postoperative period of an unrelated operation. This interrelationship between AAA rupture and any operation has been well documented in other observational studies.[9]

Aneurysmal degeneration of the aortic wall and malignancy could have a common underlying histopathological process. An altered turnover of connective tissue proteins has been documented in AAA as well as in malignant tumors. Bernstein correlated tumor growth to degradation of the interstitial matrix.[10] Our group (and others) reported increased metalloproteinase (MMP) activity within the aneurysm wall.[3] Immunohistological analysis showed that these MMP are produced by inflammatory cells infiltrating the aortic wall.[11,12] The MMP positive cells however represent a subset of only 10–20% of the inflammatory cells within the aneurysm wall.[11] It is possible that this proportion increases in case of unstable aneurysms, prone to rupture (rapid growth and activation of inflammatory processes under certain circumstances). The triggering mechanism of this increased activity however remains unclear.

In inflammatory aortic aneurysms (IAAA), a lymphocytic infiltrate is present in the periadventitial tissue. These lymphocytes produce cytokines, known to regulate MMP expression by macrophages.[12–14] Some IAAA contain a dense macrophage infiltrate associated with the predominant lymphocyte infiltrate. In these cases, the regulation of MMP is altered and protein catabolism is initiated.[12–14] IAAAs with macrophage infiltrate are to be considered as unstable. Activated macrophages can be detected by PET imaging. In one recent report, PET imaging revealed 18-FDG uptake in unstable carotid atherosclerotic plaques. The authors compared the histology of the endarterectomised plaque with the PET imaging. Intraplaque haemorrhage correlated with 18-FDG uptake, indicating a focally increased metabolic activity.[15]

This report is preliminary and will be completed by investigations of the metabolic activity of the aneurysm wall. A comparison of PET imaging with morphological and biochemical analyses of specimens of excised aneurysm wall should provide more insight in the pathogenesis of aneurysmal disease. Positron emission tomography could also help the clinician to proceed to operation electively. However, this

specialised investigational procedure is not routinely available, and has not yet acquired a definitive place in the diagnosis or treatment of aortic aneurysms.

Conclusion

Our preliminary report shows the capacity of PET imaging to assess increased metabolic activity within the aneurysm wall. A subset of aneurysms shows an increased 18-FDG uptake, suggestive of a focally accelerated metabolism. This could predispose to rapid growth and/or imminent rupture.

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APPENDIX 7

Contribution of PET scanning to the evaluation of abdominal aortic aneurysm. **Natzi Sakalihasan, Roland Hustinx, Raymond Limet.** *Sem Vasc Surg,2004;17:144-153*

Contribution of PET Scanning to the Evaluation of Abdominal Aortic Aneurysm

By Natzi Sakalihasan, Roland Hustinx, and Raymond Limet

The size of abdominal aortic aneurysms (AAA) is the most usual predictor of the risk for rupture. Because chronic metalloproteinases production and activation by inflammatory cells causes degradation of elastin and collagen in the aneurysmal wall, the detection of an increased metabolic process preceding fissuration and rupture could be a more sensitive predictor of rupture risk. We investigated the metabolic activity of the aneurysmal wall by whole-body positron emission tomography (PET) in 26 patients with a documented AAA (mean diameter 63 mm, extremes 45 mm and 78 mm). A positive ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake at the level of the AAA was observed in 38% of the cases (10 of 26 patients). Nine of these 10 patients required emergent or urgent aneurysmectomy for ruptured (n = 1), leaking (n = 1), rapidly expanding (n = 2), or painful (n = 5) aneurysms; the negative ^{18}F-FDG uptake patients had a more benign course. This preliminary study suggests a possible correlation between ^{18}F-FDG uptake by the aneurysm wall and the triggering of processes leading to rupture. The ^{18}F-FDG uptake in the aneurysm wall may correspond to the accumulation of inflammatory cells responsible for the production and activation of degrading enzymes. PET scan seems useful in high-risk patients. Positive PET imaging in these cases would help us to decide to proceed with surgery, despite factors favoring a surveillance strategy.

IN THE NATURAL course of abdominal aortic aneurysm (AAA), the dilatation of the vessel is initially slow. Its growth rate may accelerate at a later stage, possibly leading to rupture.[1] However, the size of AAA is neither the sole nor the most accurate determinant for the risk of rupture. Currently, a conservative approach is often considered for patients with small AAA.[2] However, several reports have demonstrated the existence of a risk of rupture of AAA below 5 cm in diameter.[1,3-6] In elderly and/or high-risk patients bearing a larger-size aneurysm, the benefit of surgery is less evident because of higher operative risk. It would therefore be useful to define criteria of accelerated growth and impending rupture both for small AAA in normal risk patients and in critical size AAA in high-risk patients.

The process of expansion preceding fissuration and rupture seems to depend upon the release of matrix metalloproteinases (MMP) produced and/or activated by inflammatory cells,[7-9] causing degradation of elastin and collagen in the aneurysmal walls.[10] Elastin degradation and collagen remodeling depend on the activity of a variety of enzymes, including some elastases, plasminogen activators, and MMPs.[11] There is some evidence that aneurysms with a high inflammatory cell infiltrate and an increased expression of metalloproteinase activity are prone to rapid expansion and rupture (unpublished data).

A positive correlation between plasma's MMP-9 and large size and/or expansion of AAA has been reported.[12,13] The activity of MMP_2 and MMP_9 has been determined in the wall of asymptomatic and ruptured AAA. A higher proteolytic activity is observed at the site of rupture.[14]

However, previous investigations comparing the cellular metabolic activity in AAA wall in vivo and the evolution of AAA has to our knowledge not been reported. Whole-body positron emission tomography (PET) has been used to detect an increased metabolic activity of the aneurysm wall (Figs 1 and 2).

PET is a diagnostic method that creates high-resolution, three-dimensional tomographic images of the distribution of positron emitting radionuclides in the human body. The radiolabeled compounds used include enzymes substrates, receptor ligands, drugs, antibodies, neurotransmitters, and other biomolecules that are tracers for specific biological processes. The resulting PET images can be considered as "functional images" of these biochemical or physiological processes. Biochemical processes are altered in most diseases, and these alterations usually precede gross anatomical changes.

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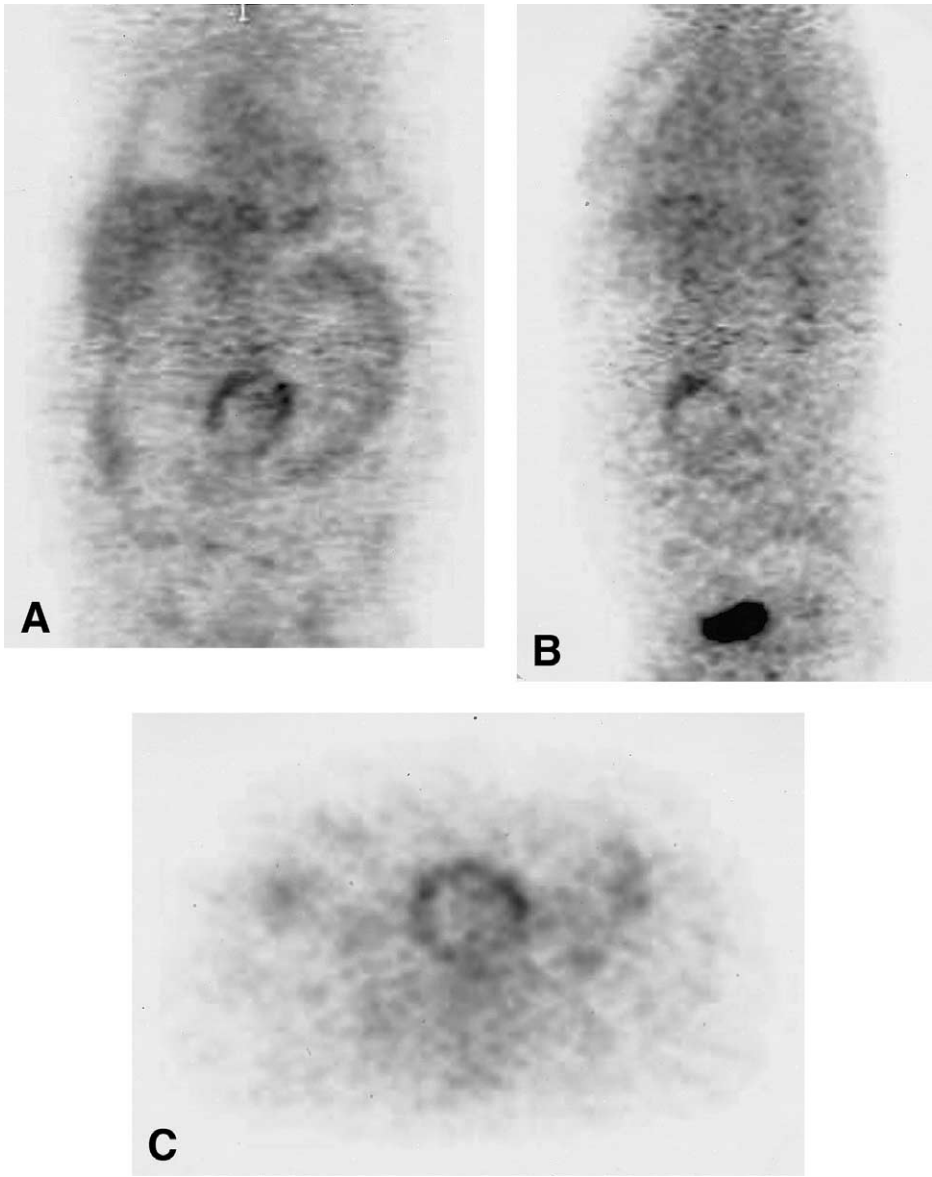


Fig 1. **^{18}F-FDG PET imaging of a 60-year-old patient with rapidly expanding AAA (10 mm in 1 month) showing an increased uptake of the tracer in the wall of the aorta. (A) Coronal, (B) sagittal, and (C) transaxial sections.**

Historically, the initial PET studies focused on cerebral and myocardial metabolism to detect zone of infarction. Now, PET is often used for oncological investigation searching for increased uptake. The most widely used substrate is ^{18}F-fluorodeoxyglucose (^{18}F-FDG), a marker of metabolic activity. ^{18}F-FDG uptake into malignant cells is enhanced by an increased expression of glucose transport molecules on the tumor cell surface. However, ^{18}F-FDG uptake is not specific for tumors. ^{18}F-FDG-PET scan can also be positive in inflammatory disease.[15] Within tumors, as well as in inflammatory lesions, part of ^{18}F-FDG is taken up by macrophages and other inflammatory cells. Up to 25% of the signal reaching the scanner could be due to glycolysis from macrophages within the tumor.[16] ^{18}F-FDG uptake in atherosclerotic lesions has been described primarily in the spumous cells of the atherosclerotic plaque.[17]

Hunter et al[18] described aortic blebs as a focal saccular deformation within the walls of aneurysm, which could also be detected by CT scan. The pres-

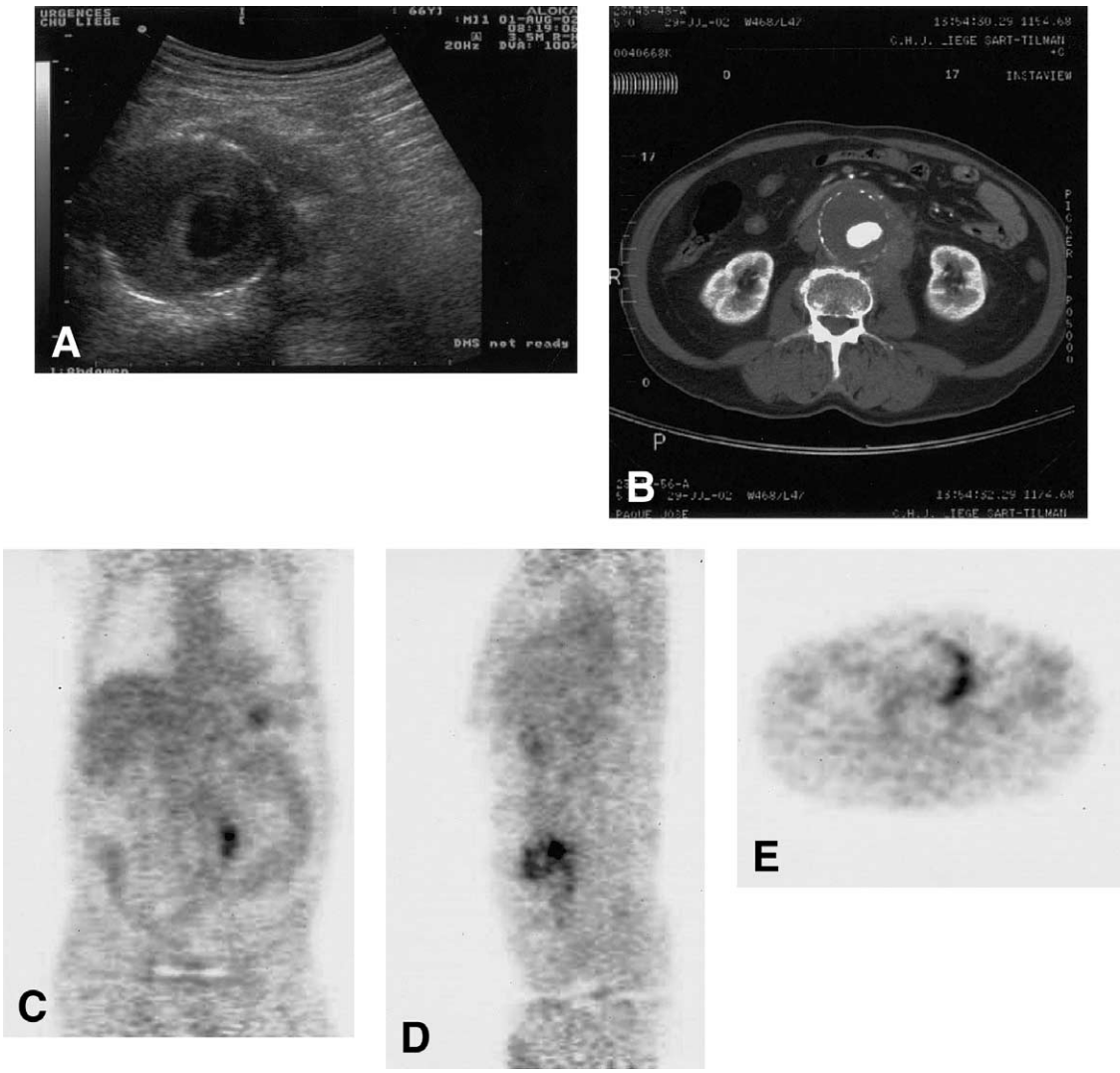


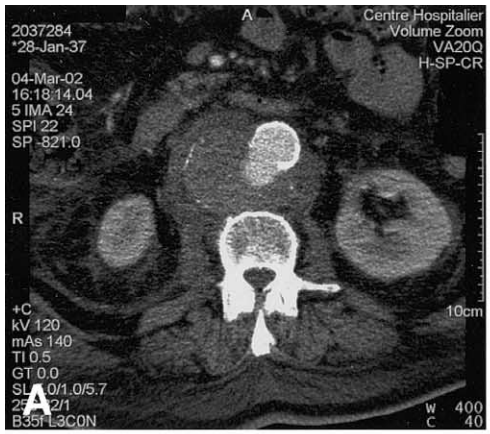
Fig 2. This 66-year-old male patient suffered from chronic back pain. (A) Ultrasonography discovered the presence of AAA with possible leaking of AAA. (B) Abdominal CT scan demonstrating aortic aneurysm and periaortic hematoma. (C) Coronal, (D) sagittal, and (E) transaxial sections of the PET examination showed an increased metabolic activity of the aneurysmal wall at the left side where leaking was suspected. Surgery confirmed a covered rupture of AAA.

ence of polymorphonuclear at the location of blebs of the aortic wall and at the site of rupture is also suggestive for a local inflammatory process preceding rupture. However, the CT scan images are not representative of the metabolic state of the lesion (Fig 3).

We recently investigated the potential of whole-body PET to detect a hypermetabolic state in the aneurysm wall, and correlated this information with the evolution of the disease.[19]

PET

PET imaging was developed in the mid-1970s, and has since benefited from major improvements in both diagnostic performances and practicality. Like any nuclear medicine imaging technique, it is based on the detection of photons emitted by the patient after administration of a radiolabeled tracer. Several physical characteristics of PET constitute a major advantage over monophotonic scintigraphy. Most importantly, the tracers are labeled with positron-emitters radionuclides. The two photons resulting from the disintegration of the positron are emitted in opposite directions (ie, at 180° from each other) and recorded in coincidence by the detectors surrounding the subject. A detailed description of the specific technical and methodolog-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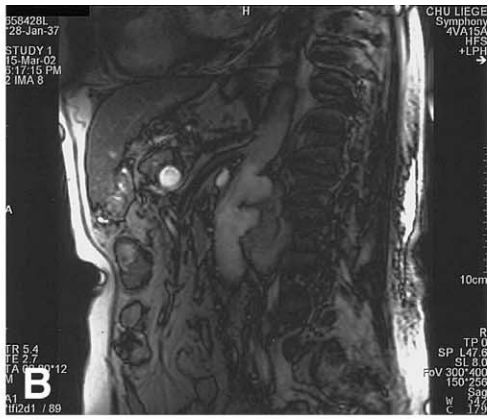
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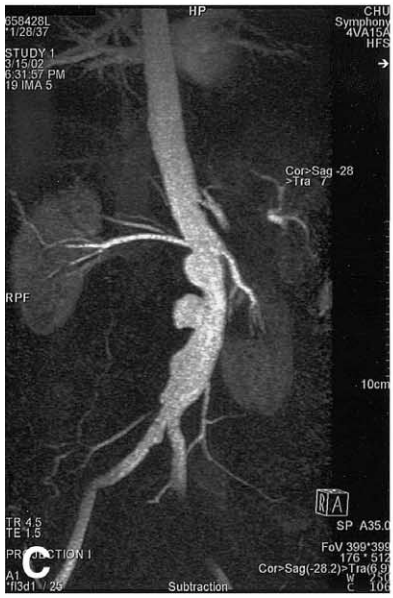
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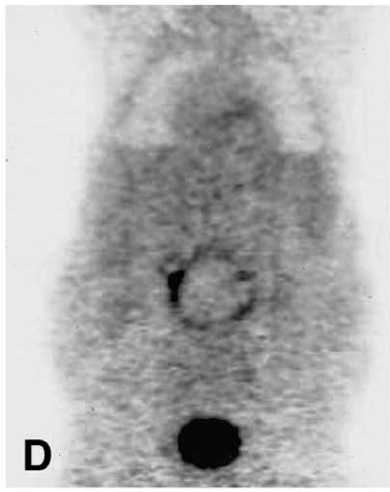
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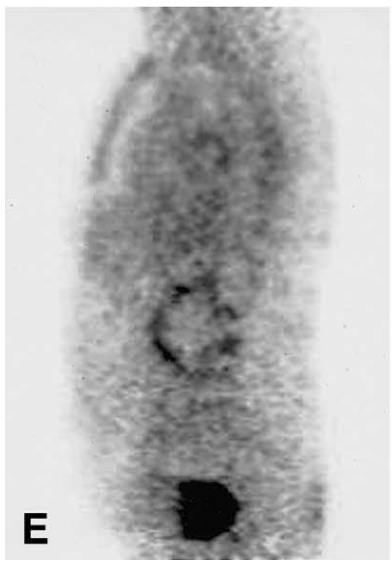
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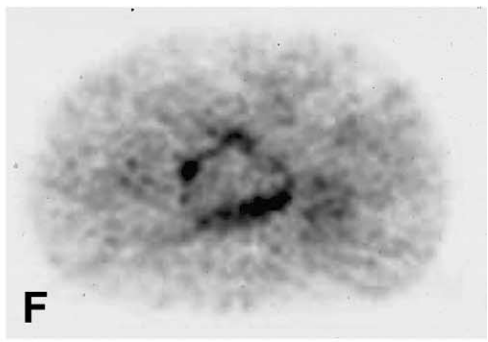
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ical features of PET imaging is obviously beyond the scope of this article and may be found in Phelps.[20] In short, PET imaging increases the count rates, that is the number of photons that are detected and improves the spatial resolution, ie, the lesion detectability. In addition, the images can be fully corrected, in particular for attenuation, which allows for an accurate and reproducible quantitation of the tracer distribution.

Depending on the radiotracer, a wide variety of physiological and pathological processes can be studied using this technique, at the molecular level. Blood flow, oxygen consumption, proliferation, protein synthesis, and even gene expression are examples of processes that can be iteratively and noninvasively evaluated using PET. However, in routine clinical practice, the vast majority of PET studies are performed using ^{18}F-FDG, which reflects glucose metabolism.

BIOLOGY OF ^{18}F-FDG

^{18}F-FDG is a glucose analogue. It enters the cells using the same membrane transporters as glucose. Once inside the cells, ^{18}F-FDG is phosphorylated into ^{18}F-FDG-6-phosphate, which is not a substrate for enzymes of the glycolytic chain. ^{18}F-FDG-PET is mainly used for cancer imaging. Glucose metabolism is significantly increased in most cancer types,[21] because of increased expression of membrane transporters, increased hexokinase activity, or both. Although the technique is now recognized as very useful in the management of a wide variety of cancers,[22] it must be emphasized that ^{18}F-FDG is not specific for tumors. Increased uptake is observed in many physiological and pathological, but nontumoral, conditions.[23] Usually, the level of ^{18}F-FDG uptake by inflammatory cells in resting state is low as compared with tumor cells. However, when activated, these cells may show a very high increase in glucose metabolism. This has been evaluated in various experimental settings, including skin transplantation,[24] turpentine-induced inflammation,[25] concavalin-A activated T lymphocytes,[26] bacterial abscesses,[27] or B lymphocytes after viral infection.[28] The lack of specificity for tumors results in a large number of potentially false-positive results when used in cancer patients[23] but it also provides a powerful tool for evaluating inflammatory and infectious diseases.[29]

Among these, an increasing number of reports suggest a potential role for ^{18}F-FDG-PET imaging in the assessment of large vessel vasculitis, such as giant cell arteritis and Takayasu arteritis.[30] It should be noted that ^{18}F-FDG uptake in the arterial wall is often seen in the absence of any known inflammatory vascular disease. Yun et al[31] evaluated two series of patients who underwent PET imaging for oncological or other indications. They found that the rate of positive vessels was close to 50%, and increased with age.[31] They also showed that hypercholesterolemia and age were the only parameters correlated with the presence of such uptake, among all major risk factors for atherosclerosis.[32] Using autoradiographic techniques, Rudd et al[33] showed increased tracer accumulation in the regions of the plaque with the highest density of macrophages. Further studies are needed to fully describe the PET semiology in arteritis and atherosclerosis (Fig 4). The pattern and intensity of uptake is probably different in the two entities, but this remains to be comprehensively studied and fully codified.

^{18}F-FDG-PET IMAGING: METHODOLOGY

With the advent of modern PET scanners, the procedure has been much shortened and simplified. Usually, patients are asked to fast for 6 hours prior to injecting ^{18}F-FDG, which is particularly important when investigating inflammatory processes, as glucose loading significantly decreases glucose transporters expression (and ^{18}F-FDG uptake) in inflammatory lesions.[34] ^{18}F-FDG (200 to 600 MBq) is injected through an indwelling catheter, depending on the scanner type. An uptake period of approximately 60 minutes, generally allowed for

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Fig 3. This 65-year-old insulin-dependent diabetic man with known AAA (for 7 years with initial diameter of 35 mm) was referred in our department with painful AAA. In his history we observed hepatic cirrhosis (stage child BIO) and esophageal varicosity. (A) CT scan showed a large fusiform AAA associated with a saccular aneurysm of the upper site of the infrarenal aorta. (B) MR and (C) MRA of abdominal aorta confirmed the presence of saccular AAA. Therefore, we performed PET imaging. The coronal (D), sagittal (E), and transaxial (F) sections revealed markedly increased ^{18}F-FDG uptake in a voluminous aneurysm. The bladder is also seen on the coronal and sagittal slices (urinary excretion of the tracer). Two days after PET examination, MR examination was performed because of increasing intensity of pain. This examination showed an increased diameter of AAA (10 mm) in 1 week. The patient underwent emergency surgery. During operation we observed a large anterolateral retroperitoneal hematoma.

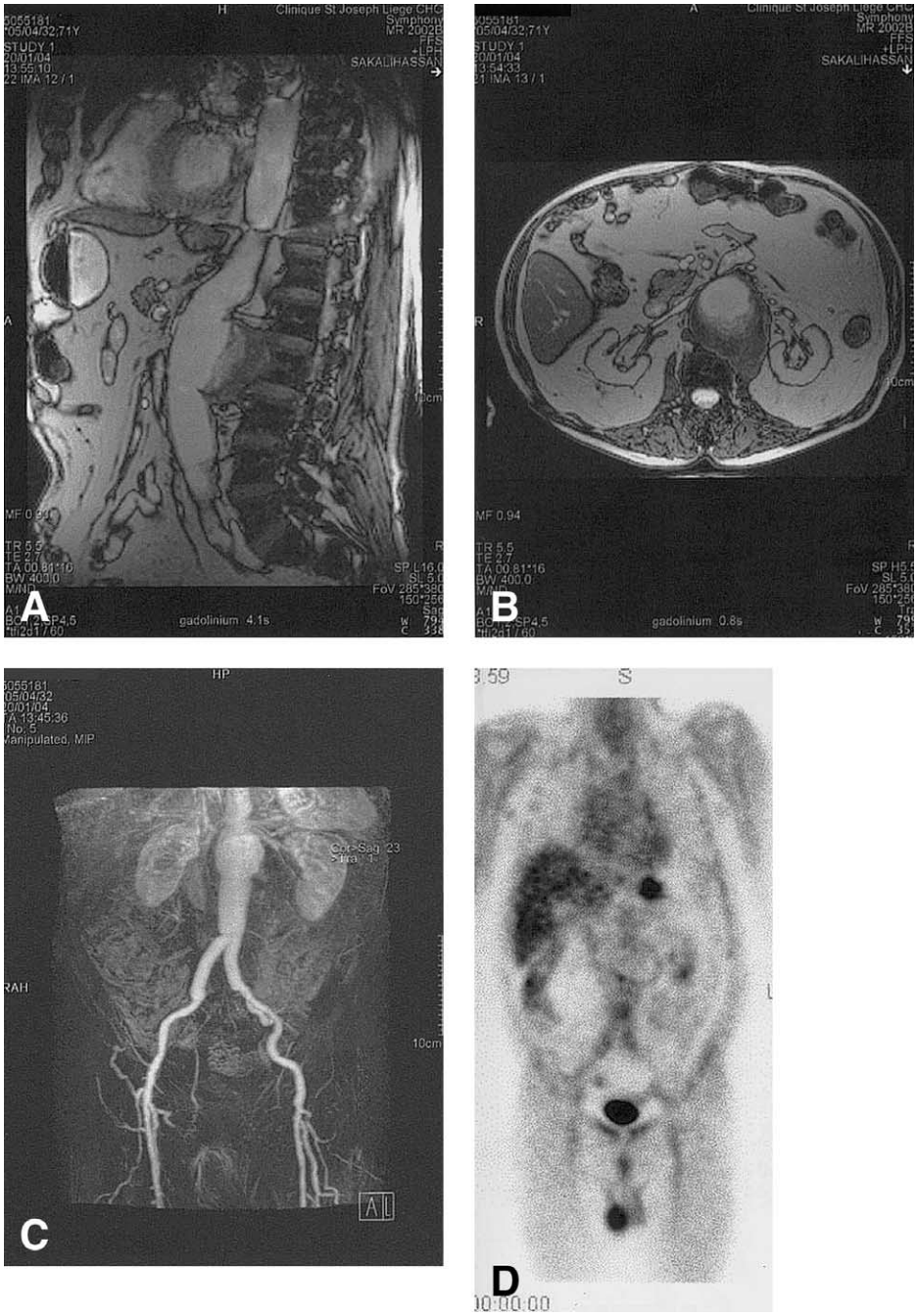

Fig 4. This 71-year-old man had been operated on for ruptured AAA 10 years prior by aortobiliac bypass. He had severe back pain (left side) for 3 weeks and was treated for nephritic colitis. Despite medical treatment the back pain persisted and abdominal MRI was performed. (A,B) MRI revealed a recurrent aneurysm of the infrarenal aorta with covered rupture. (C) MRA confirmed the diagnosis of recurrent AAA at the level of juxta renal arteries. (D) PET showed a mildly increased uptake at the level of the wall of supra renal aorta, recovered aneurysm, aortic prosthesis, and at the level of the iliac and femoral arteries.

oncological studies, could potentially be reduced to half an hour for evaluating inflammatory diseases. Images are then acquired, usually both an emission scan (collection of photons emitted from the subject) and a transmission scan (using an external gamma or x-ray source), which is used for attenuation correction. A classical acquisition, from the base of the skull to the inguinal folds (the so-called

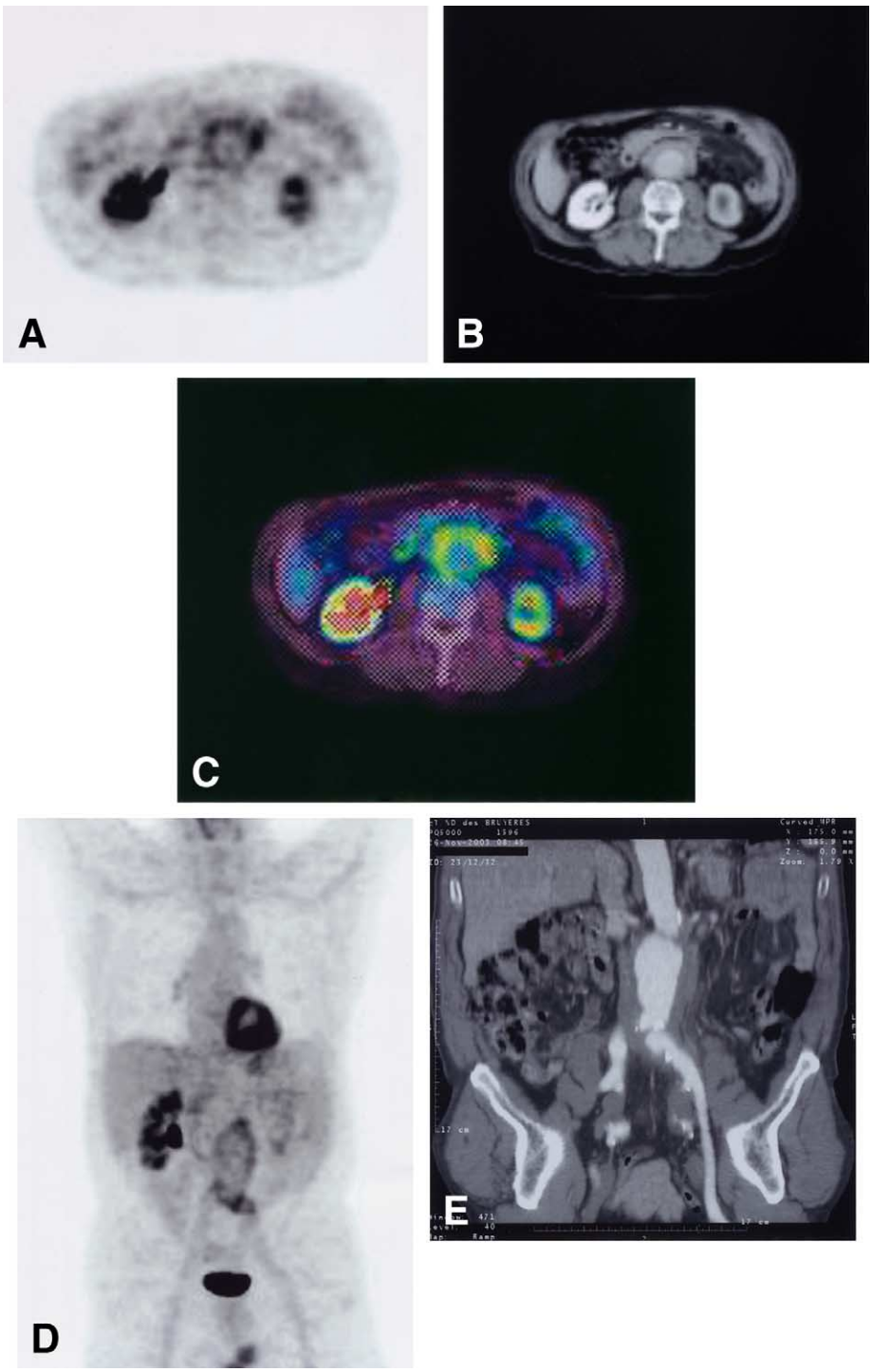

Fig 5. This 71-year-old man with known IAAA was referred to our department because of persisting lombalgia. CT scan performed in our institution confirmed the presence of IAAA. (A) ^{18}F-FDG-PET transaxial section showing increased uptake in the aortic wall. Note the high urinary activity in the right kidney. (B) Corresponding CT slice. (C) Fused PET/CT images. (D) ^{18}F-FDG-PET study (three-dimensional projection image) showing increased ^{18}F-FDG uptake in the aneurysm wall. There is also a high physiological uptake in the myocardium, the right kidney and the bladder. No urinary excretion is seen in the left kidney, suggesting impaired function. Note the moderate uptake in the iliac and subclavicular arteries. (E) Corresponding CT imaging with reconstruction of abdominal aorta.

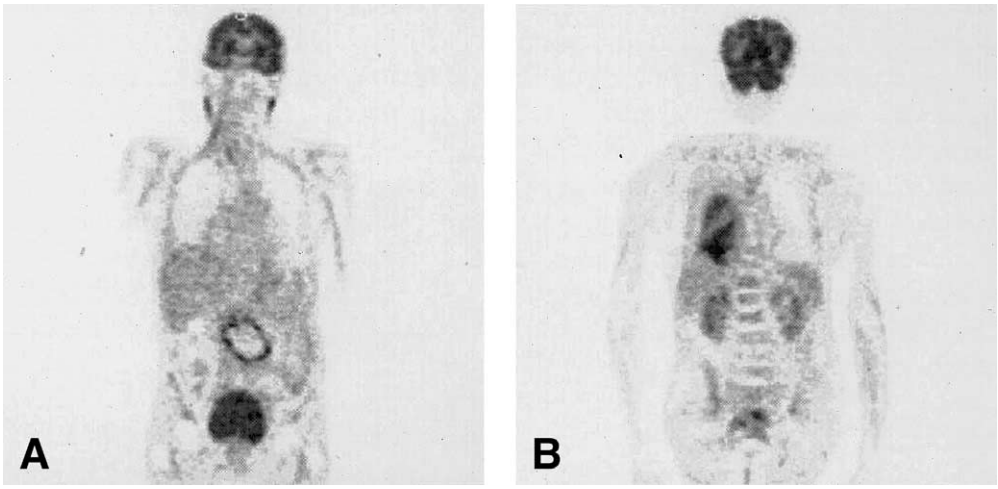

Fig 6. This 77-year-old man was followed for known AAA. Last ultrasonography examination revealed an increase in AAA diameter (about 15 mm in last 6 months). PET examination shows evidence of increased metabolic activity of the aneurysmal wall (A) with lung carcinoma (B). The patient underwent surgery because of leaking AAA 1 week after PET examination.

whole-body study) is obtained within 20 to 30 minutes using modern devices. Recent technical developments combine a PET scanner and a CT scanner in the same gantry. The PET/CT device thus allows collecting whole-body metabolic and anatomic images in a single session. The effective dose associated with ^{18}F-FDG is 0.027 mSv/MBq, which is very limited and allows the routine clinical use of the procedure.

PRELIMINARY CLINICAL REPORT

In a preliminary report of PET imaging in 26 AAA, we observed in 10 patients a positive ^{18}F-FDG uptake. In these 10 patients, there was a trend to forthcoming rupture (n = 1), leakage (n = 1), rapid expansion of the aneurysm (n = 2), or increasing back pain as observed before rupture (n = 5). These patients were all operated on without undue delay, except for one who had a large lung carcinoma. The sixteen negative PET patients had a more benign course.[19]

PET AND INFLAMMATORY AAA

The so-called "inflammatory abdominal aortic aneurysm" (IAAA) is characterized by a marked thickness and a white glistening of the aortic wall.[35] The majority of the inflammatory cells found in the wall of AAA are lymphocytes. These lymphocytes produce cytokines, known to regulate MMP expression by macrophages.[8,36,37] These cells might contribute to matrix degradation during the progression of AAA. Interleukin-4 and interferon-γ are secreted by T lymphocytes and act to downregulate macrophage MMP expression and increasing the production of tissue inhibitors of metalloproteinases.[8,36,37] The regulation of MMP could be altered if dense macrophages and lymphocytes infiltration is present on the wall of IAAA. These IAAAs could be considered as unstable. The activated macrophages can be detected by PET imaging (Fig 5). This might explain why visible ^{18}F-FDG uptake was observed in only some patients with an important macrophage activity.

MALIGNANCY ASSOCIATED WITH AAA AND PET IMAGING

Malignancy is unusually common among patients with positive PET imaging of the AAA. Whole-body PET scan imaging revealed an unknown tumor in 5 of the 26 AAA patients is indeed surprising (Fig 6). Furthermore, three patients had a past history of tumor resection within 6 months preceding the PET scan. Aneurysmal degeneration of the aortic wall and malignancy could share a common underlying histological process. An altered turnover of connective tissue proteins has been documented in AAA as well as in malignant tumors. Bernstein correlated tumor growth to degradation of the interstitial matrix.[38]

NONANEURYSMAL SURGERY AND RAPID EXPANSION OF AAA

Some hypotheses have been proposed to explain the relationship between increased metabolic ac-

tivity of the AAA and recent surgery. Collagenase and elastase activity increase in the wall of aneurysmal aorta because of the activation of inflammatory cells after surgery. When the rate of collagen degradation exceeds the rate of collagen synthesis, the tensile strength of the aortic wall declines and the risk of rupture increases. In his anecdotic report, Swanson et al[39] was the first who observed an increased tendency to rupture in the postoperative period of nonvascular surgery. This relationship between AAA rupture and any operation (coronary artery bypass graft, hernia repair, lung resection, colectomy) has been documented in other observational studies.[40]

CONCLUSION

A positive PET imaging represents a diagnostic argument to proceed to surgery without undue delay despite patient's age, size of the AAA, or operative risk. More data are needed to better define the criteria for implementing PET scan in the evaluation of AAA and for refining its bearing on the treatment.

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APPENDIX 8

Distribution of F-fluorodeoxyglucose in Abdominal Aortic Aneurysm : High Accumulation in Macrophages Studies by PET imaging and Immunohistology**. O.D. Defawe, M.S.,R. Hustinx, JO.Defraigne, R. Limet, N.Sakalihasan.** Clin Nucl Med (in press)

Distribution of F-18 Fluorodeoxyglucose (FDG) in Abdominal Aortic Aneurysm: High Accumulation in Macrophages Seen on PET Imaging and Immunohistology

Olivier Damien Defawe, MS,† Roland Hustinx, MD, PhD,‡ Jean Olivier Defraigne, MD, PhD,* Raymond Limet, MD, PhD,* and Natzi Sakalihasan, MD, PhD**

Abstract: A 68-year-old man was hospitalized for unstable angina and underwent emergency coronary artery bypass surgery. During the operation, a pulsatile large abdominal aortic aneurysm (AAA) was discovered. To define the optimal treatment of the abdominal aneurysm, after bypass surgery, CT scans and positron emission tomography (PET) were performed, as we routinely do. PET imaging combined with immunohistologic examination showed a region of increased F-18 FDG uptake corresponding to an inflammatory infiltrate in the aortic wall in contrast to the thrombus in the aneurysm (devoid of inflammatory cells). The luminal area showed midlevel F-18 FDG uptake corresponding to circulating mediators.

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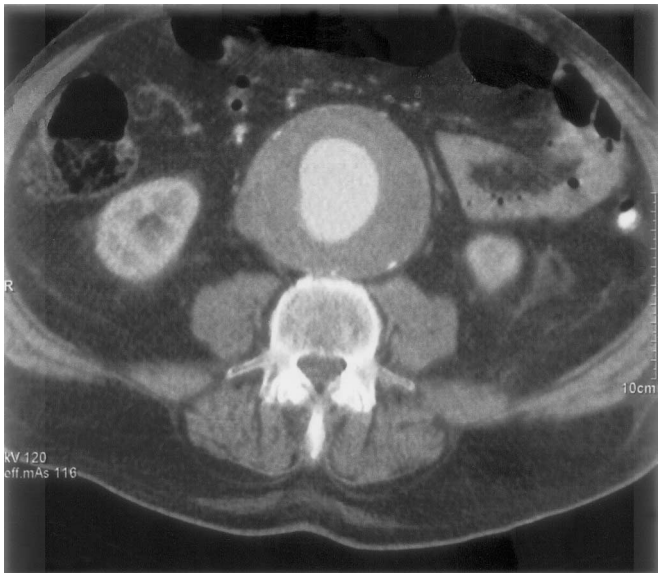

FIGURE 1. An abdominal transverse CT shows an abdominal aortic aneurysm, which was 97 mm in its largest transverse diameter.

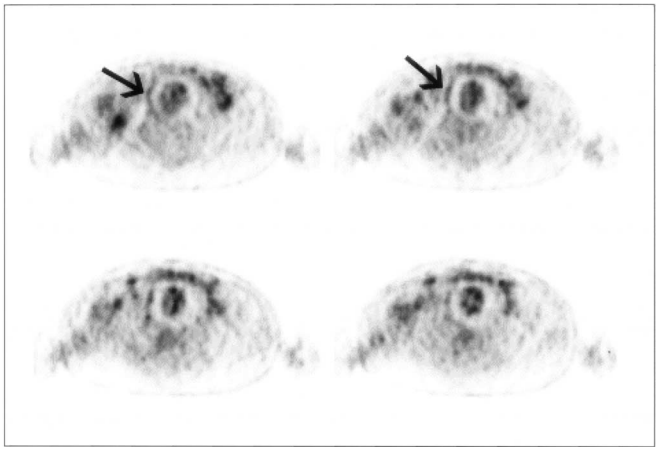

FIGURE 2. PET imaging is not routinely performed in the diagnosis or treatment of AAA. However, the authors previously showed an association between increasing F-18 FDG uptake and AAA expansion and rupture.[1,2] In this patient, a transaxial image shows a thin area of F-18 FDG uptake (arrow) corresponding to the aneurysmal wall. Inside, a rim without significant uptake corresponds to parietal thrombus devoid of inflammatory cells[3–6] whereas the luminal area shows mild uptake of F-18 FDG possibly associated with activated macrophages primed for several days by circulating mediators released at the time of surgery, as described previously.[7,8]

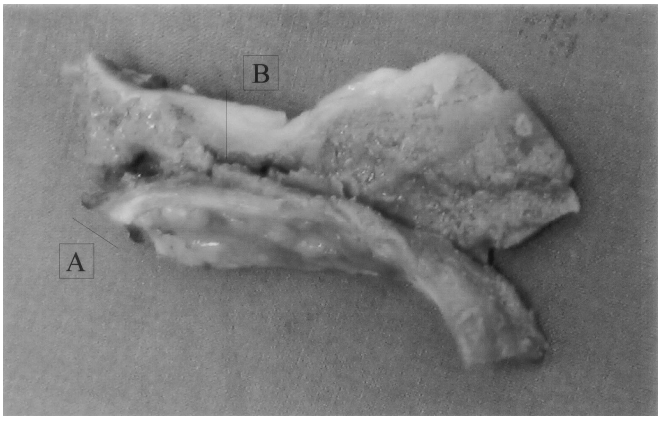

FIGURE 3. Macroscopic feature of sample collected in the abdominal aortic aneurysm wall. (A) Vessel wall. (B) Parietal thrombus.

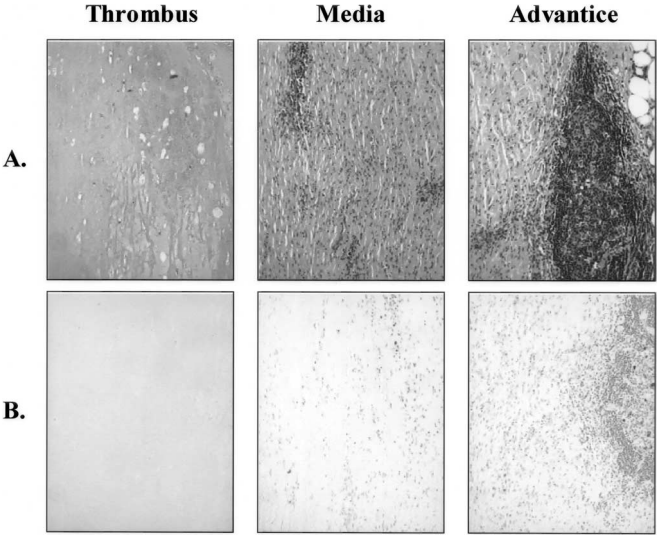

FIGURE 4. Microscopic features of sample collected in an abdominal aortic aneurysm wall. (A) Hematoxylin–eosin (200×) staining shows a gradient of inflammation from the adventitia to the parietal thrombus with some focal clusters of inflammatory cells. (B) Macrophage staining (CD68, 200×) shows a gradual distribution of macrophages from the adventitia to the media and the absence of macrophages in the thrombus.

APPENDIX 9

Decrease of plasma vitamin E (α-Tocopherol) levels in patients with abdominal aortic aneurysm**. N. Sakalihasan, J. Pincemail, J.O. Defraigne, B.Nusgens, C.M. Lapière, R. Limet.** *Ann NY Acad Sci 1996;800:278-282*

Decrease of Plasma Vitamin E (α-Tocopherol) Levels in Patients with Abdominal Aortic Aneurysm

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INTRODUCTION

Recent data suggest that the development of abdominal aortic aneurysms (AAA) is influenced by several factors, among which genetic factors and proteolytic enzymes have emerged. For example, the degradation of the extracellular matrix protein by metalloproteinases released by activated polymorphonuclear leukocytes (PMNs) has been demonstrated.[1] In a previous study we found increased amounts of metalloproteinases in the wall of AAA, reflecting an activation of PMNs.[2] Once activated, PMNs also generate toxic oxygen species through the activity of their membrane NADPH oxidase and release myeloperoxidase. Several oxidant species are thus produced: superoxide anion, hydrogen peroxide, hydroxyl radical, and hypochlorous acid, which may result in lipid peroxidation.[3]

The mammalian cells contain endogenous defenses against free radicals. *In vivo*, the consumption of vitamin E is considered as a specific although indirect index of *in vivo* peroxidative processes.[4] For example, significant decreases of vitamin E have been observed in situations where increased free radical production is suspected, such as ischemia-reperfusion syndrome, adult respiratory distress syndrome (ARDS), septic shock.[3,4]

The aim of our study is to test the hypothesis that in patients presenting with AAA the activation of PMNs may induce an oxidant stress and therefore lipid peroxidation. To test this we investigated the plasma vitamin E (α-tocopherol) concentration in patients with AAA.

MATERIAL AND METHODS

Three groups of male patients were screened: (1) patients undergoing elective operative repair of AAA reaching more than 4.5 cm in transversal diameter (AAA group, $n = 19$, mean age 72.5 $\pm$ 6.6 years), (2) atherosclerotic patients referred to our department for coronary bypass artery surgery (CAB group, $n = 18$, mean age 61.2 $\pm$ 7.4 years), and (3) healthy volunteers (healthy volunteers group, $n = 13$, mean age 35.2 $\pm$ 16.3 years). None of these patients were taking vitamin E

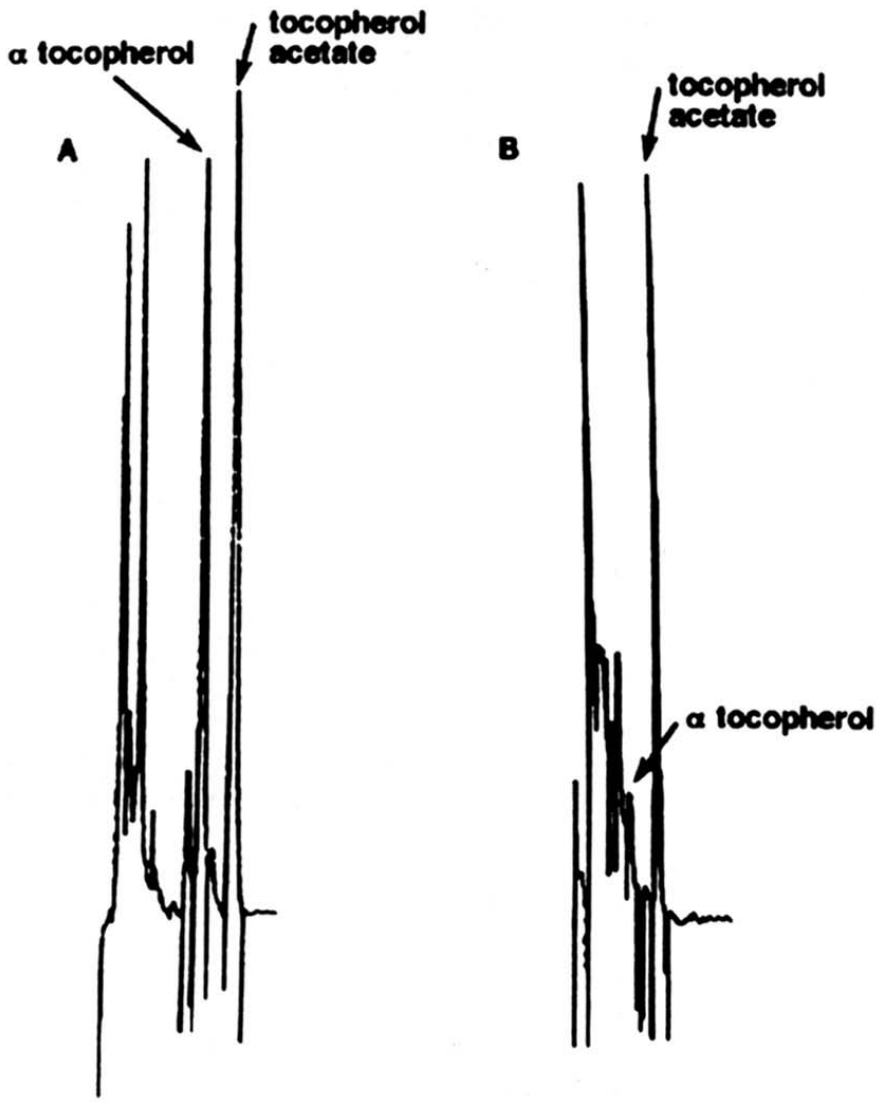

FIGURE 1. HPLC profiles of plasma taken from patients with coronary artery bypass graft (spectrum **A**) and abdominal aortic aneurysm (spectrum **B**) for vitamin E (α-tocopherol) determination.

supplement. Blood samples were taken the day before surgery. The vitamin E plasma levels were determined by high-performance liquid chromatography (FIG. 1). Values were expressed as μg/mL. Total lipids in plasma were measured by gravimetry, and vitamin E status was also expressed as vitamin E/total lipids ratio (mg/g). Results in the different groups were compared using Kruskal-Wallis nonparametric method because of small sample sizes and censored observations. Results were considered to be significant at the 5% critical level ($p < 0.05$).

RESULTS

The results are presented in TABLE 1. No remarkable difference was found between the total plasma lipids levels of patients with aneurysmal disease, coronary artery disease, and healthy volunteers.

TABLE 1. Vitamin E Level, Total Lipid Level, and Vitamin E/Total Lipid Ratio in Plasma[a]

	Control Groups		
	Volunteers (n = 13)	CABG (n = 18)	AAA (n = 19)
Total lipids (mg/mL)	5.39 ± 0.81	5.09 ± 1.84	6.87 ± 2.83
Vitamin E (μg/mL)	10.90 ± 3.12	11.00 ± 4.79	1.63 ± 2.44[b]
Vitamin E/total lipids (mg/g)	2.01 ± 0.47	2.51 ± 1.60	0.26 ± 0.37[c]

CABG, coronary artery bypass graft; AAA, abdominal aortic aneurysm.
[a] Data expressed as mean ± SD.
[b] p <0.001 versus CABG patients and volunteers.
[c] p <0.001 versus CABG patients and volunteers.

The plasma vitamin E level was below sensitivity limit (<0.6 μg/mL plasma) in 6 of 19 patients of the AAA group. In this group, the mean value of vitamin E concentration reached 1.63 ± 2.44 μg/mL plasma. This value was significantly lower than the plasma vitamin E levels observed in the CAB group (11.00 ± 4.79 μg/mL, $p < 0.001$) and in the healthy volunteers group (10.90 ± 3.12 μg/mL, $p < 0.001$). Similarly, the vitamin E/total lipid ratio was significantly lower in the AAA group (0.26 ± 0.37 mg/g) versus the CAB group (2.51 ± 1.6 mg/g, $p < 0.001$), and the healthy volunteers group (2.01 ± 0.47 mg/mL, $p < 0.001$).

DISCUSSION

Increased oxidative stress is thought to favor several human diseases. Activated oxygen species present a high reactivity toward the biomolecules and, particularly, the lipoproteins resulting in lipid peroxidation. Oxidized plasma lipoproteins are suggested as a contributing factor for the development of atherosclerotic lesions.[5,6] Activated oxygen species including free radicals are by-products generated at a low concentration during normal metabolism. As a consequence, living aerobic organisms possess endogenous antioxidant defenses for the neutralization of free radicals. An important defense against lipid peroxidation is represented by liposoluble antioxidants. Among these, vitamin E (α-tocopherol), which localizes in the hydrophobic area of biological membranes or in the phospholipid "coat" of plasma lipoproteins, seems to be the most important chain-breaking lipid-soluble antioxidant in humans.[7]

In our study, we found that patients with AAA exhibit significantly lower plasma vitamin E concentration than do patients presenting with another form of atherosclerosis who are free from AAA, and healthy volunteers. These differences are not related to variations in the total plasma lipid concentration. This decrease of the vitamin E concentration appears specific in patients with AAA, because the concentration measured in the patients with coronary artery disease and in healthy volunteers were not significantly different.

Recent studies suggested an inverse relation between vitamin E status and

the incidence of arterial disease.[8,9] Verlangieri *et al.*[10] observed that monkeys submitted to a lipid diet and supplemented with vitamin E developed less arterial stenosis than did monkeys receiving placebo. Several studies have investigated the potential role of vitamin E in humans. In the prospective study of Rimm *et al.*[8] and Stampfer *et al.*,[9] some evidence indicated an association between a high intake of vitamin E and a lower risk of coronary artery disease in men and women.

In our study, we did not find any significant difference in plasmatic vitamin E level between volunteers and CAB patients, despite the difference in age. In contrast, dramatic depletion in vitamin E was observed in our AAA patients, when compared to patients presenting for coronary artery bypass grafting and to healthy volunteers. At the present time, no other role than the neutralization of lipid peroxidation is devoted to vitamin E. Therefore, the decrease in plasma vitamin E concentration might be indicative of an increased oxidative stress in patients with AAA. Because the total lipid level was similar in patients with AAA and in control groups, the results with the vitamin E/total lipid ratio confirm that the decrease of vitamin E might be attributed to excessive vitamin E consumption and does not result from a decrease of the concentration of the plasma vitamin E carriers.

In summary, we observed evidence of an association between low plasmatic levels of vitamin E and the presence of an AAA. This decrease of the plasma vitamin E level is specifically associated to the development of AAA, because patients with coronary artery disease do not exhibit such a deficiency. Whatever the cause of this decrease, a low plasma vitamin E is associated with a diminution of endogenous defense against oxidative stress, and this decrease may increase the postoperative risk. Thus, vitamin E should be regularly monitored in patients at risk for development of AAA, and vitamin E supplement could be administered to prevent deficiency and to maximize protection against injury by toxic oxygen species that can occur during the development of aneurysm.

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APPENDIX 10

Aneurysms of the abdominal aortic aorta : familial and genetic aspects in three hundred thirteen patients **A. Verloes, N. Sakalihasan, L. Koulischer, R. Limet.** *J Vasc Surg 1995;21:646-655*

Aneurysms of the abdominal aorta: familial and genetic aspects in three hundred thirteen pedigrees

A. Verloes, N. Sakalihasan, L. Koulischer, and R. Limet, *Liège, Belgium*

Purpose: **Familial clustering of abdominal aortic aneurysm was first noticed in 1977.**
Methods: **Through questionnaire and phone inquiry, familial data on 324 probands with abdominal aortic aneurysms allowed the establishment of 313 multigenerational pedigrees including 39 with multiple affected patients.**
Results: **There were 276 sporadic cases (264 men, 12 women); 81 cases belonged to multiplex pedigrees (76 men; 5 women). We compared familial and sporadic male cases; the ages at diagnosis were 64.1 ± 7.9 years and 66.0 ± 7.3 years ($p < 0.05$), respectively, the ages at rupture were 65.4 ± 6.6 years and 75.2 ± 8.6 years ($p < 0.001$), and the rupture rate was 32.4% and 8.7% ($p < 0.001$). Survival curves were computed. Relative risk for male siblings of a male patient was 18. We performed a segregation analysis with the mixed model, the most likely explanation for occurrence of abdominal aortic aneurysm in our families was a single gene effect showing dominant inheritance. The frequency of the morbid allele was 1:250, and its age-related penetrance was not higher than 0.4.**
Conclusion: **This analysis indicates the preeminence of genetic factors on multifactorial/environmental effects of the pathogenesis of abdominal aortic aneurysm. (J VASC SURG 1995;21:646-55.)**

Abdominal aortic aneurysm (AAA) is a common disease with an estimated incidence of 20 to 40 cases per 100,000 persons per year.[1,2] Its prevalence in adult autopsy series lies between 1% and 6%. In a recent English study its prevalence was 2.6% in men aged 60 to 64 years, 6% for those aged 65 to 74 years, and 9% for those older than 75 years.[3] In an epidemiologic survey held in 1992 in England and Wales, AAA caused 1.9% of all death in men and 0.7% of all death in women 60 years or older. The overall survival rate in case of rupture was 18% including preoperative and perioperative mortality.[4] Although AAA is frequent in the elderly, the familial clustering of cases has only recently attracted attention. Since the first case report by Clifton was published,[5] several series have been published. They confirm that AAA is one of the most common "familial" diseases. Although some descriptive statistics are available on the familial aspects and on the natural history of the disease, the pathogenesis and the genetic background remain obscure. Each mode of inheritance (dominant, recessive, X-linked, multifactorial) was advocated in turn.

Most studies of common diseases assume that genetically determined factors are numerous and give an equal and individually small contribution to the phenotype (polygenic models), hence limiting the possibility of formal genetic analysis to the computation of heritability. The question of whether a single identifiable locus accounts for a significant amount of the phenotypic variation in a population may be addressed through the methods of segregation analysis.

In this article we present the results of a pedigree analysis of more than 300 probands with classical epidemiologic and statistical tools. We explored the mixed model of Morton and McLean[6] as modified by Lalouel and Morton.[7]

Definition of AAA. A consensus definition of AAA does not exist. Depending on authors, minimal infrarenal aortic diameter varies from 30 mm to 40 mm, and the minimal ratio infrarenal diameter/suprarenal diameter varies from 1.5:1 to 2:1. We considered a patient to be affected, if he or she had a dilatation of the infraaortic aorta higher than 30 mm or a ratio infrarenal diameter/suprarenal diameter higher than 1.5:1.

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MATERIAL

Between 1986 and 1991, 520 patients (489 men and 31 women) were surgically treated in our department for asymptomatic AAA or symptomatic (ruptured or not) nonsyndromal AAA. We excluded from this series the patients with Marfan syndrome (all of them with thoraco-abdominal aneurysms) or with Ehlers-Danlos syndrome. No systematic ultrasound screening was performed or even recommended for the siblings of the patients during the study period. We sent a written questionnaire oriented to personal and familial history to the 520 patients irrespective of any familial or surgical particularity. The questionnaire included names, birth date, address or phone number, and history of vascular problem in parents, siblings, and children of the probands. The patients who filled out the questionnaire (or their spouses, for deceased probands) were interviewed by the phone, and relatives were then contacted in the same way to obtain the most accurate and best cross-validated pedigrees. Anamnestic data were compared with surgical files. Because only a fraction of the affected patients from our referring population were enrolled in this study, and because some families were ascertained more than once, the ascertainment mode was multiple and incomplete.

METHODS

Descriptive statistics. Descriptive statistics were performed with classical methods. Comparison of proportions was done by the chi-squared test (with Yates' correction when sample was small), and comparison of means was done by unpaired *t* test.

Relative risks. Relative risks were estimated by the ratio between the observed number and the expected number of AAA in the nonproband subjects for each age class, and confidence interval for the risk was computed by the method suggested by Everitt.[8] We used a slightly edited version of the cumulative age incidence published by Majumder et al.[9]; our version was based on the AAA survey of Bickerstaff et al.[1] The modification was to set the incidence to 0 for men younger than 30 years (Table I).

Survival functions. Survival functions were computed by the classical Kaplan-Meier product limit. This method estimates the survival function from the continuous survival or failure times. It allows the computation of survival curves when a proportion of the studied patients "fails" (either because they are lost to follow-up or because the experiment stops before they are affected). Relatives alive at time of study or dead without evidence of AAA were considered to be censored, and relatives with AAA were considered to be noncensored. The "survival" function (in fact the survival time before a diagnosis of AAA) was estimated for the age at diagnosis. To compare survival curves among several subgroups, we used both Gehan's generalized Wilcoxon test and Cox-Mantel test. Cox-Mantel test is usually considered more powerful when samples come for exponential distributions, or when samples are small (<50); Gehan's test is used in the other circumstances. Results were considered to be significant at $p < 0.05$. All statistics were performed with the Statistica for Windows v4.0 (Statsoft Inc., Tulsa, Okla.) package.

Table I. Relative risk of AAA for siblings of affected patients

Age (yr)	*Male*	*Female*
<30	0	0
30-49	0.00015	0.00001
50-59	0.00152	0.00009
60-69	0.00482	0.00094
70-79	0.00773	0.00232
≥80	0.00893	0.00434

Cumulative incidence of AAA by sex (modified from Majumder et al.[9]).

Segregation analysis. Segregation analysis is basically the comparison of the observed proportion of affected siblings and offspring with the expected proportion according to a particular genetic hypothesis. To assess evidence of a major gene effect in the presence of other sources of correlation (polygene, sociocultural factors, etc.), pedigrees were analyzed with the pointer strategy, which was developed by Lalouel and Morton[7] as a tool for multigenerational pedigrees analysis.

Coding of the pedigrees. Pedigrees were partitioned in nuclear families. A nuclear family—the unit of analysis—is made of the two parents and their children. Three types of nuclear families differing by the ascertainment mode were enrolled: families where the proband was a child (multiple incomplete selection of proband's siblings), families where the proband was one of the parents (complete selection of proband's children), and families where none of the affected patients was a proband (truncated selection of the siblings). In the latter case "pointers" had to be added to the nuclear family. A pointer is an affected individual outside the nuclear family who contributed to the selection of this family. A maximum of three pointers is allowed, one to the father, one to the mother, and one to the children. Each pointer is defined by the relationship to the pointee (cousin, nephew, etc.).

Table II. Descriptive statistics of 315 nuclear families with respect to their family history, sex, and position (subjects younger than 30 years excluded)

	n	*AAA*	*Mean age of patients with AAA ± SD (yr)*	*Mean age of unaffected patients ± SD (yr)*	*Rupture (%)*	*Age at rupture ± SD (yr)*
Total	1597	357	66.4 ± 7.8	68.1 ± 13.4	52 (14.6)	–
Familial subgroup						
Fathers	39	8	73.3 ± 7.6	71.6 ± 14.0	4 (50.0)	69.0 ± 8.9
Mothers	39	5	73.0 ± 7.7	71.4 ± 12.7	3 (60.0)	70.8 ± 8.0
Brothers	104	68	64.1 ± 7.9*	64.8 ± 11.5	22 (32.4)†	65.4 ± 6.6‡
Sisters	44	0	–	66.4 ± 10.6	–	
Sporadic subgroup						
Fathers	276	0	–	69.9 ± 14.2	–	–
Mothers	276	0	–	74.4 ± 12.7	–	–
Brothers	546	264	66.0 ± 7.3*	63.8 ± 11.5	23 (8.7)†	75.2 ± 8.6‡
Sisters	273	12	68.0 ± 12.5	66.4 ± 12.2	0 (0.0)	–

*$p < 0.013$.
†$p < 0.001$.
‡$p < 0.001$.
Age statistics are given based on censoring age or age at death for unaffected subjects and age at diagnosis for patients with AAA.

Table III. Age distribution of AAA among brothers in several subgroups with calculation of relative risk

Age (yr)	*30-49*	*50-59*	*60-69*	*70-79*	*≥80*	*Total*
Relative risk	**0.005/33**	94.3	15.1	4.6	4.0	17.9
(95% confidence interval)	**0**	(0-425)	(2.9-27.3)	(0-17.9)	(0-86.5)	(12.8-22.9)

The study was reiterated with multiplex pedigrees only. This selection, limited to familial cases, biased the sample. Accordingly a sampling correction was applied to families with two affected siblings by defining the proband as a pointer (with a "sibling" degree of relationship), whereas his or her siblings were treated by truncated selection.[10]

Ascertainment probability. Ascertainment of our pedigree was multiple (some pedigrees had more than one proband) and incomplete. Ascertainment probability p is the probability that an affected person in the population is a proband. In our sample p was 0.248 as calculated by a multiple ascertained sibling method and was 0.2 to 0.4 as estimated by the comparison of the number of annual AAA operated with the expected number of cases in the Liège area; an incidence of 4 per 100,000 per year was assumed. A value of 0.25 was used for all computations.

Segregation model. Segregation analysis was carried out with the personal computer version of the computer program POINTER.[7,11] This software implements the unified version of the mixed model of Morton and McLean[4] modified by Lalouel and Morton.[7] It incorporates the transmission frequencies studied by Elston and Stewart.[12]

Analysis was limited to the mixed model. It assumes that a phenotype (expressed as a discrete or continuous value) results from the independent and additive contributions of three effects on a liability scale measured in SD units: a major monogenic biallelic effect, a multifactorial (genetic or acquired) transmissible effect, and a normally distributed residual. Variation of the phenotype for each major genotype is assumed to be normally distributed. Its variance is the sum of two components: a part resulting from the multifactorial component and an unexplained residual environmental variance. Note that heritability H represents the ratio of the multifactorial component of the variance to the total phenotypic variance. Two parameters define the phenotype: the overall variance V (set to 1 for qualitative traits) and the overall mean u (set to 0 for qualitative traits). The polygenic component has two parameters, the polygenic heritability in children H and the parent-to-child heritability ratio Z. The parameter of the monogenic component is formed by the frequency of the pathologic allele at the major locus q, the distance, or displacement, between the two homozygous genotype means on the liability scale t, and the position d of the heterozygous mean relative to the two homozygous means (equal to 0, 1, or 0.5 for a recessive, a dominant, or a codominant

pathologic allele, respectively), and three transmission probabilities τ_1, τ_2, and τ_3, which are the probabilities for a subject of genotype AA, Aa, or aa, respectively, to transmit the allele A. Under the mixed model, which assumes mendelian inheritance of the major effect, $\tau_1 = 1$, $\tau_2 = 0.5$ and $\tau_3 = 0$.

Computation and statistical tests. Parameters of the model were estimated by POINTER by maximization of the likelihood of the phenotypes of the siblings conditional on the phenotype of the parents and the pointers. Competing nested models were built by fixing some parameters. Nested models were compared by likelihood ratio test. The difference between -2 ln (likelihood) is asymptomatically distributed as a chi-squared analysis with the degrees of freedom equal to the difference in the number of estimated parameters. Nonnested models were compared by the Akaike information criterion, which is two times the number of estimated parameters -2 ln (likelihood). The best model has the smallest Akaike information criterion.

RESULTS

Questionnaire. We obtained answers for 324 patients (62% of our original sample), allowing a two- or three-generational pedigree to be drawn. Four questionnaires were filled out by the spouses of deceased patients. Those 324 probands (312 men and 12 women, sex ratio 26:1) came from 313 large pedigrees. The sex ratio of our original sample of 520 patients with AAA was 15.7:1. The higher mean age (at operation) in the women in the original sample compared with that of the men (74.07 ± 9.04 vs 68.2 ± 7.9 years) could be a reason for their reduced answer rate compared with that of the men and a rise of the sex ratio. Whenever possible relatives of the proband (usually siblings), including all relatives suspected to have a vascular problem, were interviewed by phone. The questionnaire appeared to be a very reliable tool for familial enquiry, because only minor discrepancies were found by this cross-referencing procedure except for some confusion between correction of AAA and aortofemoral bypass. Because of the rarity of aneurysms in young people, we excluded the relatives younger than 30 years from the study.

Descriptive statistics of the sample. The total number of patients with AAA in our 313 large pedigrees was 357 (340 men and 17 women, sex ratio 20:1). For 276 probands (264 men and 12 women, sex ratio 22:1) no positive familial history was elected, but 68 male patients belonged to 39 multiplex pedigrees (12.5%). In the latter families 33 new patients with AAA were found (28 men and 5 women), leading to a total of 81 patients with familial AAA: 76 men and five women (sex ratio 15:1). Based on 313 large pedigrees we constructed 582 nuclear families including 2695 subjects aged 30 years or older. Those nuclear pedigrees were used for segregation analysis.

Table IV, A. Comparison of survival curves for sex and familial history

	Male siblings, sporadic	*p Value*
Male siblings, familial	GW −3.59	0.00016
	CM −3.23	0.00061
Nonprobands male siblings, familial	GW −0.74	0.227
	CM 0.188	0.43
Female sporadic	GW −10.14	<0.00001
	CM −11.92	<0.00001

GW, Gehan's Generalized Wilcoxon test; *CM*, Cox Mantel statistics.

In the familial group all affected women were found among the parents of the probands. Among familial cases 23 pedigrees showed affected siblings and healthy parents, 10 showed an affected parent and an affected child, and six showed more complex structures (affected cousins, uncle, and nephew, etc.). The sex ratio was not significantly different among familial and sporadic subgroups (Yates corrected chi-squared analysis = 0.06, $p = 0.80$). In six families' patients with cerebral aneurysms were observed. Because aneurysms in the central nervous system are hard to dismiss among patients with "sudden death," we did not take those cases into account for the analysis.

To avoid duplicate use of subjects (a case being a parent in one nuclear pedigree and a child in another) the descriptive analysis of the pedigree was limited to the 315 nuclear families in which the probands appeared as children. Those nuclear pedigrees included 630 parents and 967 children. The 1597 subjects were 965 men and 632 women. After one proband was removed, the sex ratio of our sample was close to 1:1 (625 men and 615 women). Table II presents the numeric data regarding the patients with AAA and their unaffected relatives. They are partitioned in two groups: simplex and multiplex pedigrees. No significant differences in age were seen between patients with and without AAA. The age at diagnosis was significantly different among affected brothers of the two groups (t test = 2.50, $p = 0.013$) and also among affected fathers and affected children in the familial subgroup

(t test = 3.15, p = 0.002). The latter phenomenon was attributed either to a true "anticipation" of the diagnosis resulting from better medical awareness or to the use of more accurate and advanced methods for diagnosing AAA during the last 10 years. Another hypothesis was that nonrecording or bad diagnosis of some early cases (in the 1950s) led to an undue rise of the mean age at diagnosis. Finally, this phenomenon could reflect the natural increase in age-specific prevalence of this condition.[13] The rupture ratio was much higher in the familial subgroup than in the sporadic one (chi-squared analysis = 23, $p < 0.0001$). The mean rupture age was significantly different among affected brothers of the two groups ($p < 0.001$). In the familial group the proportion of rupture in men and women was not significantly different (chi-squared analysis = 0.71, p = 0.040).

Relative risk by sex We used only the group of brothers for this analysis. Occurrence in the siblings (after removal of one proband per sibling relationship) was compared with the expected recurrence assuming random occurrence of AAA. Table III shows the relative risk by age groups. The overall relative risk was 17.9 (95% confidence interval 12.9 to 22.9). Relative risk appeared to be major in the 50 to 59-year-old subgroup and declined for elder subgroups but with very wide confidence intervals so that no definite conclusion could be drawn.

Survival curves The Kaplan-Meier method was used on several subpopulations of our sample. Fig. 1 shows the survivorship function obtained with the 967 siblings (including 357 patients with AAA) coming from our nuclear pedigrees. To compare effects of sex and of positive familial history, several subgroups of siblings were extracted: men of the multiplex pedigrees, male probands and nonprobands of the multiplex pedigrees, male siblings of the sporadic pedigrees, and women of the sporadic pedigrees. To test whether the differences between the survivorship function reached statistical significance, we applied both Cox-Mantel and Gehan's Wilcoxon tests to the survivorship curves (Table IV, A). A highly significant difference was seen between men and women with sporadic AAA. Male siblings of multiplex pedigrees were affected significantly earlier than male siblings in the sporadic pedigrees. A significant difference was seen between the two groups of men; a more rapid decrease was seen in the survival curve of the familial subgroup (Gehan's Wilcoxon test: 3.6, p = 0.00016; Cox-Mantel test: 3.23, p = 0.00061) (Fig. 2). This difference was no longer observed when the sibs of sporadic cases were compared with the sibs of familial cases after the probands were removed. This finding indicates a possible bias to the earlier diagnosis of AAA in the familial cases. To further explore this phenomenon we compared male probands; a clear difference persists, although of borderline significance, when male probands of the multiplex pedigrees are compared with male probands with sporadic pedigrees. Finally, a significant difference appeared to exist between affected probands and affected nonprobands in the familial group (Table IV, B).

Segregation analysis. The 582 nuclear families were analyzed with POINTER. Of those families 101 belonged to the multiplex pedigrees. They were studied separately after recoding. Eight models (sporadic, multifactorial, polygenic, dominant, recessive, codominant, mixed, and mendelian) were evaluated.

The results of the analysis of the full sample are given in Table V, A. The subset of pedigrees with a positive familial history is shown in Table V, B. Comparison of the models was done as follows. The sporadic model of Table V, A has a $-2\ln L$ parameter of 637.23 and the dominant model a $-2\ln L$ of 503.25. The difference (133.98) as calculated by chi-squared analysis with 3 degrees of freedom was highly significant, indicating that the dominant model is significantly more likely. When the chi-squared test is not significant, the best of the two models is the model with the lesser free parameters (the most parsimonious one). When two models have the same degree of freedom (as in comparison of the dominant, recessive, or codominant models), the smaller Akaike criterion indicates the best model.

Analysis of the full set shows that a sporadic model is strongly rejected. When a purely mendelian inheritance of AAA is assumed, the best fit is obtained with a dominant model even when the dominance parameter is set free. No significantly better fit is obtained for a mixed model when a combined effect of dominantly inherited mutation and a weak multifactorial component is assumed, although this situation gives the best likelihood. The analysis of the familial subgroup shows almost similar results; the mixed model does not give a significantly better fit than a purely monogenic model with dominant inheritance (Table V, B).

The most parsimonious way to explain the segregation of AAA in our multiplex pedigrees is to suspect the action of a single dominant gene, for which the frequency of the morbid allele is 1 : 250 and in which the sex-dependent penetrance slowly increases with age to reach a maximum of 0.3 in women

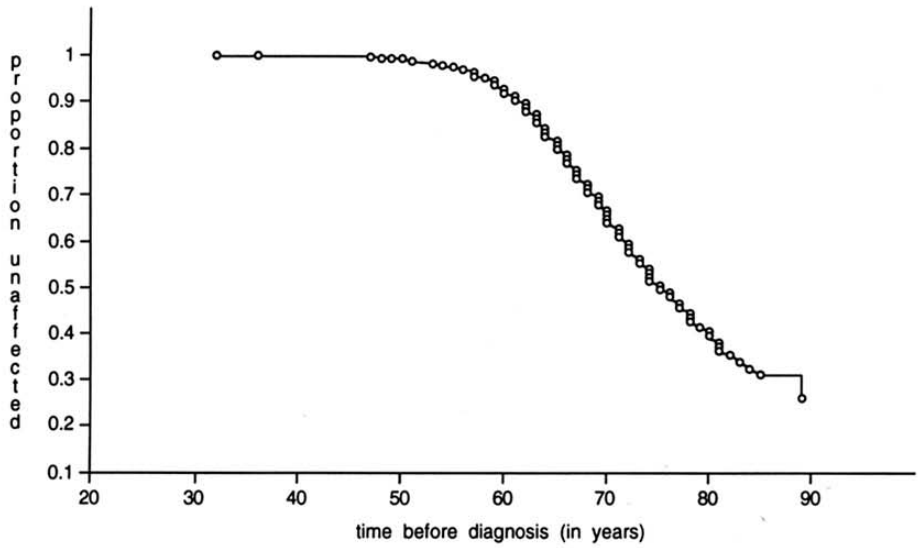

Fig. 1. Kaplan-Meier "survivorship" function for siblings (age at distribution) (n = 967; affected = 343).

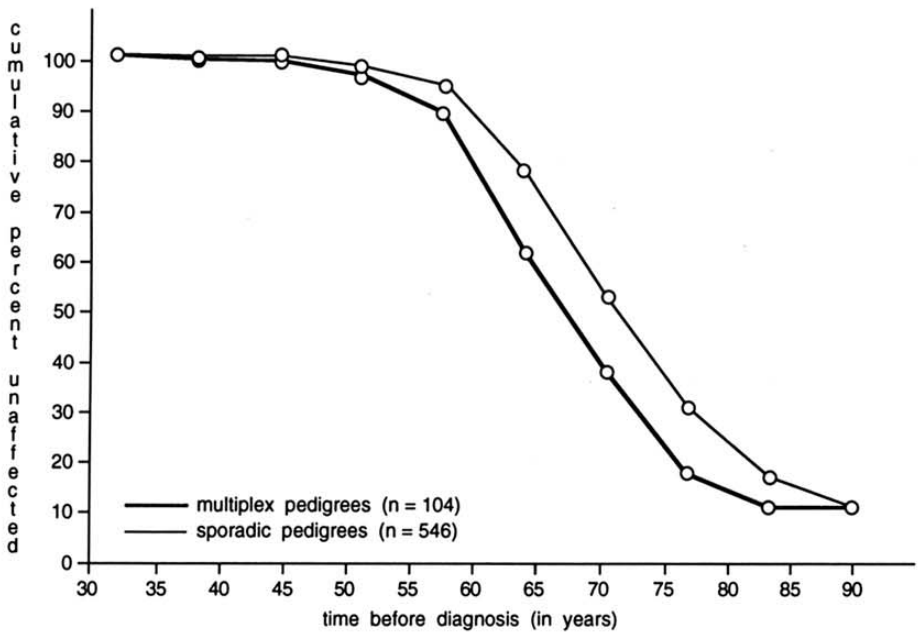

Fig. 2. Comparison of survival curves of male siblings depending on their familial history.

older than 80 years and 0.4 in men older than 80 years. This low penetrance even in the elderly intuitively explains why AAAs are so often sporadic and why generations seem to be skipped in multiplex families. With the mixed model the frequency of the gene is almost similar, and the heritability is only 2%. Note that the heritabilities obtained with multifactorial models (0.7 and 0.79) are similar to the heritability computed by Powell and Greenhalgh.[14]

DISCUSSION

Familial aspects. Since the seminal report by Clifton[5] on three siblings with AAA was published,

Table IV, B. Comparison of survival curves in three subpopulations coexisting exclusively of AAA

	Familial male patients	*p Value*	*Familial male probands*	*p Value*	*Familial male nonprobands*	*p Value*
Male probands, sporadic	GW −2.09	0.018	GW −1.76	0.039	GW −1.76	0.039
	CM −0.32	0.010	CM −1.62	0.053	CM −1.62	0.053
Familial male probands					GW 0.36	0.36
					CM −0.32	0.37

the familial aspect of AAA was addressed by a few authors. Norrgard et al.[15] retrospectively studied 87 pedigrees out of an initial series of 200 cases. Cole et al.,[16] in a retrospective study, gave data on 305 pedigrees. Darling et al.[17] presented a prospective study of 542 cases including 84 familial observations (15.1%). Johansen and Koepsell[18] gave data on 250 pedigrees. Webster et al.[19] thoroughly studied 91 pedigrees both on a descriptive basis and on a more formal genetic basis (Majumder et al.[9]). We can add to these reports the study of 50 multiplex pedigrees by Tilson and Seashore.[20] Table VI attempts to compare our series with the six previous ones. Our percentage of familial cases appears similar to those of other published series. Norrgard et al.[15] noted the coincidence of cerebral and aortic aneurysms in the same family. Whether this coincidence was fortuitous or whether it indicates a more generalized predisposition to arterial dilatation was left to debate. In our series at least 2% of our probands had a relative with central nervous system aneurysm, but no patient had both disorders. This point obviously requires further experimentation.

Webster et al.[21] found 16.2% of familial AAA history based on anamnesis in 43 consecutive patients with AAA. After prospective ultrasonography screening of the relatives, the number of familial cases was raised to 27.9%. In male siblings of patients with AAA, Bengtsson et al.[22] found 29% of AAA after ultrasonography screening.

Genetic aspects. Genetic aspects of AAA have been the subject of very few studies. Norrgard et al.[15] presented 19 patients (18% of their sample) with familial AAA but did not discuss etiology. Tilson and Seashore[20] showed 50 families including three pairs of identical twins. Those families were collected by various teams, and no data were given on the mode of ascertainment or on the isolated AAA from the same population. Twenty-nine of 50 were single-generational, 18 showed simple "vertical" transmission, and three were "complex." In an empiric approach of the results they favored a frequent X-linked dominant form and a less common autosomal/dominant or a multifactorial model. They excluded recessive inheritance because of the high frequency of parent-to-sibling transmission and questioned the shift of the sex ratio. Assuming that AAA is a multifactorial disease, Powell and Greenhalgh[14] calculated for a series of 60 patients (25 with positive family history) a 70% heritability by the method of Falconer.[23] In their set, eleven (8.6%) of 128 parents and 14 (7.3%) of 192 siblings of the probands had AAA. Separate heritabilities were not computed for those two subsets. Recently Majumder et al.[9] made an extensive segregation analysis based on 91 probands including 13 familial cases (10 single-generational). The mode of selection of the families was not reported, but systematic screening was not used. They concluded that susceptibility to AAA can be accounted for by the presence of a major gene, that it does not require a multifactorial component, and that this gene behaves as a recessive factor.

Our results, like those of Majumder et al.,[9] indicate that the importance of the genetic factor in the pathogenesis of AAA compared with the multifactorial or environmental effects. Nevertheless our final conclusions are in disagreement. Several factors may explain this discrepancy. Our population differs by the sex ratio. The ascertainment of our sample is quite different. The mode of selection of Majumder et al.'s patients[9] was not clear. The number of familial cases was very small, and their sample could have included, by chance, fewer pedigrees with subjects affected in two generations. Moreover their methods were also different. The definition of AAA was an aortic diameter greater than 5 cm; we used a less stringent definition. Majumder et al.[9] counted as "affected" only patients with operated AAA and used age at operation for analysis. They rejected patients who were discovered by systematic screening, whereas we considered all patients with known AAA, whatever the reason for their discovery (although we had no policy of systematic screening before 1992). It should be noted that the patients with AAA included in the study by Bickerstaff et al.[1] to compute incidences were gathered from all sources including diagnosis "by chance" and necroscopic discovery of patients not thought to have AAA. Although it is

Table V, A. Segregation analysis of the full sample

	d	*t*	*q*	*H*	*z*	*−2lnL*	*Akaike IC*	*df*
Sporadic	–	–	[0]		[0]	[0]	637.23	0
Polygenic	–	–	[0]	0.700 (0.045)	[1]	518.16	520.16	1
Multifactorial	–	–	[0]	0.796 (0.0488)	0.360 (0.117)	509.97	513.97	2
Recessive	[0]	2.813 (0.179)	0.0751 (0.0103)	[0]	[1]	512.53	516.53	2
Condominant	[0.5]	4.085 (0.470)	0.0361 (0.0098)	[0]	[1]	509.03	513.03	2
Dominant	[1]	2.255 (0.123)	0.00424 (0.00134)	[0]	[1]	503.25	507.25	2
Mendelian	0.972 (0.7951)	2.320 (1806)	0.00425 (0.00134)	[0]	[1]	503.25	509.25	3
Mixed	<1>	2.213 (0.124)	0.00458 (0.00149)	0.0169 (0.0229)	[1]	501.38	506.38	3

Parameter estimation with their ± SD and likelihood of several segregation models.

Table V, B. Segregation analysis of the subset of pedigrees with a positive family history

	d	*t*	*q*	*H*	*z*	*−2lnL*	*Akaike IC*	*df*
Sporadic	–	–	[0]	[0]	[0]	145.45	145.45	0
Polygenic	–	–	[0]	0.892 (0.122)	[1]	111.44	113.44	1
Multifactorial	–	–	[0]	0.840 (0.123)	1.141 (0.191)	111.17	115.17	2
Recessive	[0]	2.926 (0.591)	0.122 (0.024)	[0]	[0]	118.15	122.15	2
Condominant	[0.5]	5.170 (0.532)	0.00686 (0.00193)	[0]	[0]	103.76	107.76	2
Dominant	[1]	2.593 (0.283)	0.00740 (0.00283)	[0]	[0]	103.32	107.32	2
Mednelian	0.810 (0.641)	3.197 (2.544)	0.00745 (0.0228)	[0]	[0]	103.30	109.30	3
Mixed	0.809 (0.635)	3.200 (2.524)	0.00749 (0.00230)	0.00491 (0.0172)	[1]	103.28	111.28	4

Parameter estimation with their ± SD and likelihood of several segregation models. Numbers in brackets are fixed parameters.

difficult to ascertain whether those differences account for the diverging conclusions, they at least indicate that our studies are not totally comparable in their methods.

Our sample shows a sex ratio much higher than that of other reported series. We have no definite explanation for this phenomenon. An excess of men could come from our ascertainment of cases; male subjects are more exposed to coronary problems or atheromatosis and so have a much greater chance to be diagnosed "by chance." The sex ratio of AAA varies with the age group; the ratio is higher in younger persons. If the population attending our hospital has a lower mean age compared with other institutions, we could expect a higher sex ratio. Finally, we cannot exclude genetic, sociocultural, or environmental effects, although these are not obvious.

Familial cases show a significantly earlier onset as observed by Darling et al.[17] Higher rupture rate also characterizes our familial sample. Various explanations for earlier diagnosis such as familial awareness of the risk may be hypothesized. But because penetrance of the gene appears age-dependent, we may suspect that intrinsic factors affecting penetrance may influence expressivity, for example, more pathogenic mutations of the putative AAA gene are more likely to be expressed earlier in several relatives and to lead them more rapidly to an aneurysmal rupture.

Kontusaari et al.[24] showed two single-base mutations in the type III procollagen gene in two families with AAA. In the first multigenerational family the mutation led to the replacement of glycine 619 by arginine. In the second two-generational family, which presented with AAA and easy bruisability, the single-base mutation G − > A in intron 20 was shown to induce aberrant splicing of the mRNA that reduces the synthesis of the α1(III) chain. The authors showed that the clinical spectrum in their families with AAA extended from isolated AAA to classical Ehlers-Danlos type IV disease (with prominent cutaneous findings). More interestingly they showed that Ehlers-Danlos type IV and isolated AAA were observed in families with type III procollagen mutations, suggesting that a collagen defect could account for a fraction of AAA, although no precise estimation of this fraction can be given at this time. This finding also gives additional support to the observations of Menashi et al.[25] on low content of type III collagen in a group of patients with familial AAA. In the two families described by Kontusaari et al.,[26] the mutation behaved as a dominant trait. This finding appears in contradiction with Majumder et al.'s[9] conclusion on the recessivity of AAA but rein-

Table VI. Comparison of different data of families observed in this study and in six preview studies

	Norrgard et al.	*Tilson and Seashore*	*Johansen and Koepsell*	*Cole et al.*	*Darling et al.*	*Webster et al.*	*This study*
No. of pedigrees	87 (initially: 200)	50	250	305	542	91	313
Multiplex pedigrees (%)	18 (20.6)	50	48 (19.2)	37 (12.1)	82 (15.1)	14 (15.3)	39 (12.4)
Horizontal pedigrees	10	28	18	18	?	11	23
Vertical/complex pedigrees	8	22	>19	19	?	3	16
AAA	103	127	≥307	?	669	108	357
AAA (familial subgroup)	38	127	≥105	91	209	31	81
Sex ratio	155:45 (3.75:1)	?	207:43 (4.81:1)	?	532:137 (3.88:1)	49/19 (4.68:1)	340/17 (20:1)
Sex ratio (familial)	30:8 (3.75:1)	11:16 (6.94:1)	?	56:35 (1.6:1)	136:73 (1.86:1)	20:10 (2:1)	76/5 (15:1)
Sex ratio (sporadic)	?	?	?	?	396:64 (6.19:1)	69:9 (7.67:1)	264:12 (22:1)
Age at diagnosis	67 (M66/F70)	?	72	?	?	M67.1/F69.2	M66.2/F69.5
Age at diagnosis (familial)	65 (n = 19)	?	?	?	M62.4/F71.2	?	M65/F73
Age at diagnosis (sporadic)	?	?	?	?	M67.8/F68.8	?	M66.6/F68
Rupture rate (%)	68/200 (Initial)	?	?	?	?	?	52/357 (14.6)
Rupture rate (familial) (%)	14/38 (36.8)	?	?	22/52 (42)	42/209 (20.1)	?	29/81 (35.8)
Rupture rate (sporadic) (%)	?	?	?	?	?	?	20/276 (8.3)

M, Male; *F*, female.

forces our own observations (although we have not proved that our cases of AAA have an abnormality of collagen type III).

AAA is a complex disease, and we cannot expect to find a single physiopathologic explanation for all cases. Our data at least suggest that a genetic factor could be of major importance in the onset of AAA. This factor has been shown to be an alteration of one of the collagen III genes in some families. Whether the major gene effect always results from one abnormal collagen gene or more likely from several dominant genes is still to be demonstrated. Further investigation on selected large families with AAA appears warranted.

AAA is a complex disorder with probably multiple pathogenetic pathways. In this article we presented a familial study of 313 AAA pedigrees selected without the use of systematic screening. Our series illustrates the importance of familial factors in AAA and raises the hypothesis, sustained by a familial genetic analysis, that AAA could be a mainly genetic disease. The major determinant factor in the appearance of AAA could be an inborn defect possibly of collagen type III or of other components of the connective tissue matrix. This defect behaves as a dominant trait with low age-dependent penetrance. Differences in the severity of the complications of AAA between familial and sporadic cases clearly appear. These differences could be related to the variable penetrance of individual mutations.

Systematic screening of AAA is an emerging issue. A common question is whether to apply AAA screening to a general population or to an "at risk" subgroup. We strongly recommend ultrasound screening of first-degree relatives aged 50 years and older, a method that now permits simple, noninvasive, and accurate detection and follow-up of AAA. Recently in our retrospective study of the determination of the expansion rate and incidence of rupture of abdominal aortic aneurysms, we found 12% of rupture in aneurysms smaller than 44 mm and 22% when the diameter exceeded 50 mm.[27] When the higher incidence of rupture in patients with positive family history and the risk of rupture even for small AAA (less than 50 mm) are considered, a more aggressive therapeutic attitude is mandatory. Ratio-

nale for a national screening program has been recently given by Law et al.,[4] who recommended one ultrasonography detection in men aged 60 years and older. As long as cost-effectiveness of those general policies has not been demonstrated, a reduced screening policy could be recommended at least for patients with other peripheral artery aneurysms and for first-degree relatives of patients with an AAA.

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APPENDIX 11

Familial abdominal aortic aneurysms: collection of 233 multiplex families. **Helena Kuivaniemi, Hidenori Shibamura, Claudette Arthur, Ramon Berguer, C. William Cole, Tatu Juvonen, Ronald A. Kline, Raymond Limet, Gerry McKean, Orjan Norrgard, Gerard Pals, Janet T. Powell, Pekka Rainio, Natzi Sakalihasan, Clarissa van Vlijmen-van Keulen, Alain Verloes, Gerard Tromp.** *J Vasc Surg 2003;37:340-345*

Familial abdominal aortic aneurysms: Collection of 233 multiplex families

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Objective: **This study investigated a large number of families in which at least two individuals were diagnosed with abdominal aortic aneurysms to identify the relationship of the affected relatives to the proband.**
Subjects and Methods: **Families for the study were recruited through various vascular surgery centers in the United States, Finland, Belgium, Canada, the Netherlands, Sweden, and the United Kingdom and through our patient recruitment website (www.genetics.wayne.edu/ags).**
Results: **We identified 233 families with at least two individuals diagnosed with abdominal aortic aneurysms. The families originated from nine different nationalities, but all were white. There were 653 aneurysm patients in these families, with an average of 2.8 cases per family. Most of the families were small, with only two affected individuals. There were, however, six families with six, three with seven, and one with eight affected individuals. Most of the probands (82%) and the affected relatives (77%) were male, and the most common relationship to the proband was brother. Most of the families (72%) appeared to show autosomal recessive inheritance pattern, whereas in 58 families (25%), abdominal aortic aneurysms were inherited in autosomal dominant manner, and in eight families, the familial aggregation could be explained by autosomal dominant inheritance with incomplete penetrance. In the 66 families where abdominal aortic aneurysms were inherited in a dominant manner, 141 transmissions of the disease from one generation to another were identified, and the male-to-male, male-to-female, female-to-male, and female-to-female transmissions occurred in 46%, 11%, 32%, and 11%, respectively.**
Conclusion: **Our study supports previous studies about familial aggregation of abdominal aortic aneurysms and suggests that first-degree family members, male relatives, in particular, are at increased risk. No single inheritance mode could explain the occurrence of abdominal aortic aneurysms in the 233 families studied here, suggesting that abdominal aortic aneursyms are a multifactorial disorder with multiple genetic and environmental risk factors. (J Vasc Surg 2003;37: 340-5.)**

Abdominal aortic aneurysms (AAAs) are frequently familial.[1-4] The first family with three brothers who were all diagnosed with AAA was reported by Clifton in 1977.[5] Four case reports on twins with AAA have also been published.[2] Tilson and Seashore[6,7] reported on two collections of families with AAA with 16 and 50 multiplex families, respectively, with at least two members with AAA. Interviews and ultrasonographic screening studies among relatives of patients with AAA have clearly shown the increased prevalence of AAA among first-degree relatives, with up to 18% of brothers and 5% of sisters having AAA.[2,8] Population-based ultrasonographic screening studies have also emphasized family history as an important risk factor for AAA.[9,10] Formal segregation studies have shown that AAAs are likely to be a genetic disease with autosomal, either dominant or recessive, inheritance pattern.[3,4]

The challenging question then is how to dissect the genetic components of AAA. The approaches used to date include analysis of candidate genes for mutations, genetic association studies, and development of animal models for AAA.[11-23] Such approaches revealed that about 2% of pa-

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tients with AAA have mutations in the gene for type III procollagen[14,15] and that one of the human leukocyte antigen alleles carries a susceptibility for AAAs.[11,12] In addition, it was found that mice lacking the gene for matrix metalloproteinase–9 had AAAs develop at a much lower frequency in the elastase-induced surgical aneurysm model than the wild-type mice.[16] Another mutant mouse model, namely the apolipoprotein deficient mice, had aneurysms develop with angiotensin II infusion.[17] These approaches, however, require that the investigators come up with one or more biologically plausible candidate genes to be tested with patient samples or in animal models, and the genes chosen for the study may or may not be the genes involved in the pathophysiology of the disease.

We are attempting to identify the genetic risk factors for AAA with families and an unbiased, comprehensive, genome-wide screen with highly variable repeat markers in a DNA linkage study. Here we report the first step of this approach to collect a large number of families with at least two affected individuals and suitable for genetic studies.

METHODS

Families with at least two members with AAAs were identified for the study at the following sites: the Department of Surgery, Wayne State University School of Medicine, Detroit, Mich; the Department of Surgery, Dalhousie University, Halifax, Canada; the Department of Surgery, University of Oulu, Oulu, Finland; the Department of Cardiovascular Surgery, University of Liège, Liège, Belgium; the Department of Vascular Surgery, Vrije University Medical Center, Amsterdam, The Netherlands; the Department of Surgery, Charing Cross and Westminster Medical School, University of London, The United Kingdom; and the Department of General Surgery, University of Umeå, Umeå, Sweden; and through our website at http://www.genetics.wayne.edu/ags.[24] The study was approved by the Institutional Review Board of Wayne State University School of Medicine and by the patient recruiting centers.

An arterial aneurysm definition by Johnston et al[25] was used. These standards have also been used by other investigators.[26-28] In the participating vascular surgery units, medical records were searched for AAA operations. These patients then were contacted, consent was obtained, and family histories were collected. There were slight variations in the methods used to obtain the records by the different units, but in all units, a certified vascular surgeon was leading the efforts to identify patients with AAA and affected family members. Family histories of any new patients for surgical repair of AAAs at these sites were obtained in interviews conducted by a research nurse specifically trained for this work.

Those individuals who responded to our website were asked to complete a family history questionnaire. The patients who indicated that they had at least one other family member diagnosed with AAA were included into the study; a detailed family history and life-style questionnaire was sent to them, and they were subsequently interviewed by a genetic counselor.

Table I. Nationalities of families with AAA

	No. of families
Canadian	80 (34%)
Belgian	59 (25%)
Dutch	47 (20%)
US American	37 (16%)
Finnish	5 (2.1%)
British	3 (1.3%)
Spanish	1 (0.4%)
Swedish	1 (0.4%)
Italian	1 (0.4%)
Total	233

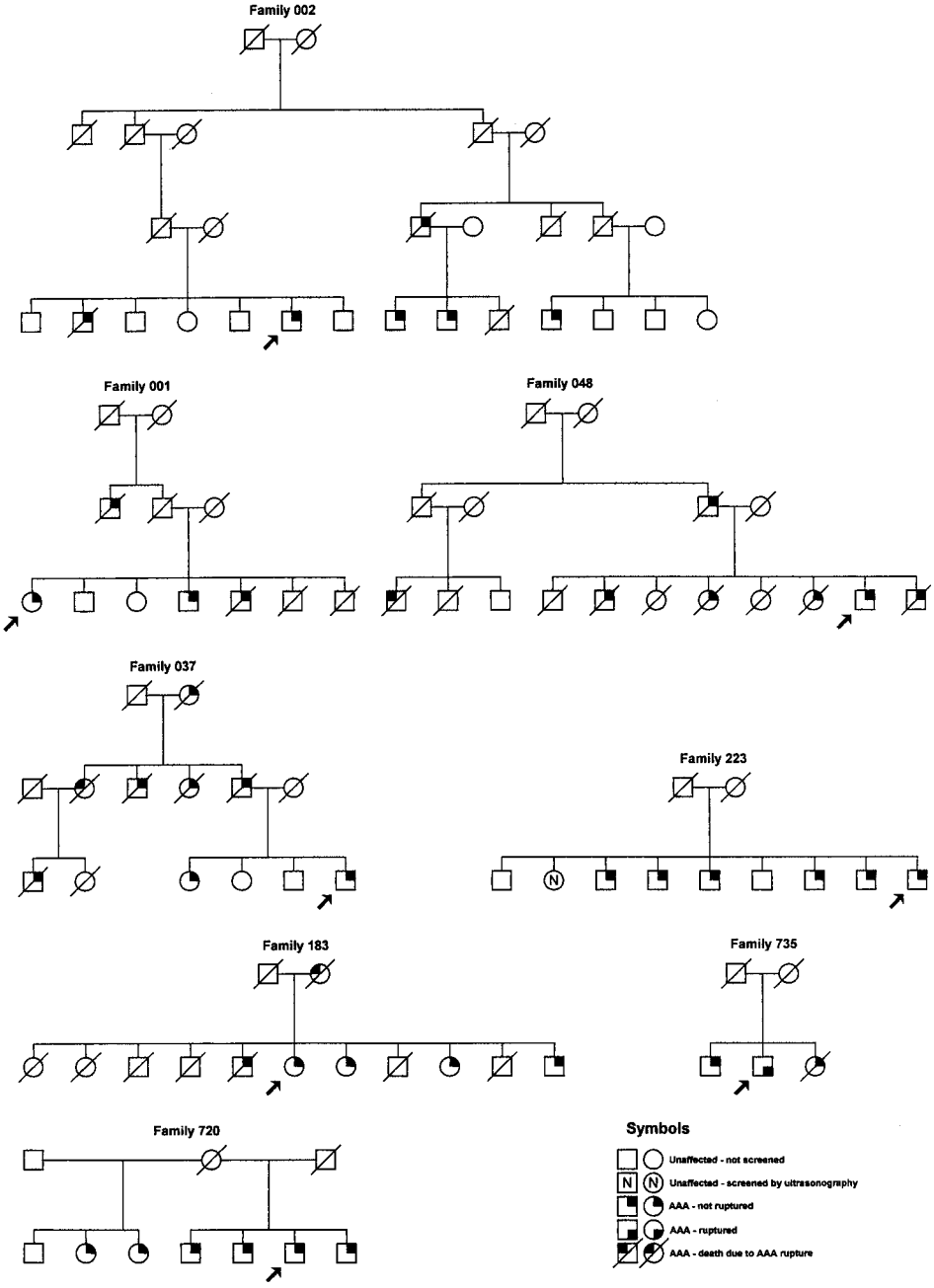
All patients with AAA were white.

In most cases, only a limited amount of information was available from family members who were second-degree or more distant relatives. We did not contact estranged family members. To identify individuals with heritable connective tissue disorders, such as Ehlers-Danlos syndrome type IV or Marfan's syndrome, a specific questionnaire was used assessing skin and skeletal manifestations characteristic for these disorders. Families with these disorders were excluded from the study. The family trees were drawn with Cyrillic software (Cherwell Scientific Publishing Limited, Oxford, Great Britain). Whenever possible, the AAA diagnosis of a deceased family member was verified by requesting the autopsy or medical records. Some family members, if 50 years old or more, had been examined with ultrasonography and were identified as affected if the infrarenal aortic diameter was 3.0 cm or greater, a cutoff point used by other investigators previously.[28] If the patient had isolated iliac artery aneurysm, it was noted; likewise, other aneurysms such as thoracic or thoracoabdominal aneuryms were noted but not included into the study.

RESULTS

We identified 233 families in which at least two members had an AAA (Table I; Fig). The families originated from nine different nationalities, but all were white (Table I). In 192 of the families (82%), the first person known to us to have an AAA (proband) was male, and in 41 of the families (18%), the proband was female. In addition to the probands, there were altogether 420 relatives with AAA, bringing the total number of AAA cases in the 233 families to 653, with an average of 2.8 AAA cases in each family. Although most of the families were small and had only two members with AAA, we identified six families with six affected individuals, three with seven, and one with eight (Table II). Tables II and III show the breakdown of different categories of relatives with an AAA. Most often the relative with an AAA was the proband's brother, and 74% of the families had at least one affected male sibling (Table III). Most (77%) of the affected relatives were male (Table IV).

Most of the families (72%) appeared to show autosomal recessive inheritance pattern on the basis of the fact that



Representative AAA families from our collection of 233 families. Proband in each family is indicated with *arrow*. *Slash* across symbol means death. Other symbols used are explained in insert to figure.

affected individuals had no affected parents, whereas in 58 families (25%), AAAs occurred in one parent of the affected individual and the inheritance mode was, therefore, consistent with autosomal dominant inheritance. In the remaining eight families (for example, families 002, and 048 in Fig), the familial aggregation could be explained by autosomal dominant inheritance with incomplete penetrance because some affected individuals in these families had an affected parent and others did not. Alternatively, these eight families could have autosomal recessive inheritance with a common disease allele.

In the 66 families where AAAs appeared to be inherited in a dominant manner and it was, therefore, possible to follow the transmission of the disease, 141 transmissions of the disease from one generation to another were identified and the male-to-male, male-to-female, female-to-male, and

female-to-female transmissions occurred in 46%, 11%, 32%, and 11% of the cases, respectively. In 57% of the transmissions, the disease came from the father, and in 43%, from the mother. In 111 of the 141 transmissions (79%), the disease was transmitted from a mother or a father to a son, and in only 21% of the transmissions, it was transmitted to a daughter in the family.

DISCUSSION

A large number of previous studies have identified family history of AAA as a significant risk factor for AAA development.[2-4,8] The specific genetic factors contributing to the susceptibility for AAA have, however, been difficult to identify because of the problems in obtaining large families for genetic studies. It is almost impossible to collect blood samples from family members in two or three consecutive generations because of the late age at onset and the high mortality rates associated with aneurysm rupture. Many of the pedigrees in our collection of 233 families with AAA (Fig) also show these features. Furthermore, it is not possible to predict the true phenotype of apparently unaffected individuals who might be completely asymptomatic today and show a development of AAA 5 to 10 years later, making it risky to use any genetic information from the unaffected person. In addition, AAAs do not consistently show any one mode of inheritance in the families, suggesting that they are a multifactorial disease with heterogenous etiology. Most of these characteristics are shared by many other adult-onset common diseases. The approach to study them must, therefore, take into consideration these factors. First, a large collection of families is necessary for initial and subsequent follow-up studies. Second, statistical methodology must be chosen carefully to take into consideration the fact that genetic information from currently unaffected individuals might be misleading because the person's phenotype could change over the years to come.

Our collection of 233 families with AAA had patients from nine different nationalities, but all of them were white. The underrepresentation of other ethnic groups has been noted by other investigators previously[29] and is in agreement with the hypothesis that genetic factors contribute to the disease.

Previous studies had suggested that although the prevalence of AAAs is lower in women than in men, AAAs might be more aggressive and perhaps more likely to be from accumulation of genetic susceptibility factors if present in women.[30-32] We therefore investigated the transmission of AAA from one generation to another to see whether females were more likely to pass on the disease to their offspring. To our surprise, we did not find a significant difference in the transmission of the disease between the father and the mother, and both genders seemed to transmit the disease at approximately equal frequency to their offspring. It was, however, noteworthy that in 79% of the observed transmissions, AAAs were transmitted from one of the parents to a son, and in only 21% of the cases, to a daughter.

The goal of our study was to collect as many families with AAA as possible to be later used in genome-wide DNA linkage studies. Many vascular surgery centers in the United States and abroad contributed to the study by identifying families, collecting family information, and drawing blood samples for future studies. We attempted to gather as much information about the affected family members as possible. There were, however, some limita-

Table II. Number of affected relatives in families with AAA

No. of affected individuals	*Families (%)*	*Relationship to proband**			
		M	*F*	*B*	*S*
2	131 (56)	8	14	89	14
3	56 (24)	7	10	69	16
4	25 (11)	5	6	43	8
5	11 (4.7)	2	3	27	5
6	6 (2.6)	2	3	12	4
7	3 (1.3)	1	1	12	3
8	1 (0.4)	0	1	0	1

*Number of relatives in categories indicated.
Other relationships found were child, cousin, aunt, uncle, nephew, niece, grandparent, and great grandparent (see Table III).
M, Mother; *F,* Father; *B,* Brother; *S,* sister.

Table III. Relationship of affected relative to proband

Relationship	*No. of families (n = 233) (%)*
Brother	172 (74)
Sister	46 (20)
Father	37 (16)
Mother	25 (11)
Child	2 (0.9)
Uncle	16 (7)
Cousin	14 (6)
Aunt	8 (3.4)
Grandparent	5 (2.1)
Niece	1 (0.4)
Nephew	2 (0.9)

Table IV. Gender of affected relatives*

Affected male relatives		*Affected female relatives*	
Relationship	*No.*	*Relationship*	*No.*
Brother	249	Sister	51
Father	38	Mother	25
Uncle	16	Aunt	10
Nephew	2	Niece	1
Male cousin	16	Female cousin	2
Grandfather	0	Grandmother	5
Total	321 (77%)		94 (23%)

*Only affected relatives of probands are shown here. Probands were not taken into account. Total number of affected relatives was 420. Relationships shown account for 99% of all affected relatives.

tions in our study, including the uncertainty about second-degree and third-degree relatives of the proband, because in many cases it was difficult to obtain further information about relatives who were not part of the immediate family. It is, therefore, possible that we missed some of the affected second-degree and third-degree relatives. The second limitation was the uncertainty about currently unaffected individuals to determine whether they were truly unaffected or would have AAAs develop a few years later. In particular, this is a problem with individuals who are still relatively young, below the age of 60 years. The third limitation was that the exact cause of death is often not determined, meaning that no autopsy was performed and a sudden death from aneurysm rupture could have been listed as an apparent heart attack if no further investigations were carried out. This could be especially true with the older generations in the pedigrees and could lead to an interpretation of the inheritance mode to be autosomal recessive rather than dominant. The inheritance pattern in many families was also consistent with pseudodominant inheritance (ie, apparent dominance from one parent being homozygous and the other heterozygous for a recessive allele). Pseudodominance is not probable for AAA, however, because it requires a high population frequency of mutant alleles. Such a high frequency may be possible if alleles at all loci had equal and additive effects, but that is inconsistent with the conclusion of a major gene effect from segregation studies.[3,4] In the light of these limitations, the results presented here should be considered conservative estimates about the number of affected relatives in each family and the mode of inheritance.

Our study was not designed to compare the familial AAAs to sporadic ones or to identify differences in their risk factors. The data on the 233 multiplex families presented here emphasize the value of routinely obtaining family history from patients with AAA and considering ultrasonographic screening of unaffected siblings to detect AAAs before rupture. It is important that primary care physicians realize that AAAs do run in families even when no signs of Ehlers-Danlos syndrome or Marfan's syndrome are present, making familial AAAs (OMIM 100070; Online Mendelian Inheritance in Man at www.ncbi.nlm.nih.gov/htbin-post/Omim) a separate disease entity deserving due attention.

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APPENDIX 12

Analysis of coding sequences for tissue inhibitor of metalloproteinases 1 (TIMP1) and 2 (TIMP2) in patients with aneurysms. **Xiaoju Wang, Gerard Tromp, C.William Cole, Alain Verloes, Natzi Sakalihasan, Sungpil Yoon, Helena Kuivaniemi.** *Matrix Biology 1999;18:121-124*

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MATRIX BIOLOGY

Analysis of coding sequences for tissue inhibitor of metalloproteinases 1 (TIMP1) and 2 (TIMP2) in patients with aneurysms

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Abstract

Aneurysms are characterized by dilation, i.e. expansion and thinning of all the arterial wall layers, which is accompanied by remodeling of the connective tissue. Genes involved in the regulation of tissue remodeling are therefore candidate genes. We analyzed TIMP1 and TIMP2 coding sequences in 12 individuals with abdominal aortic aneurysms (AAA), one individual with AAA and intracranial aneurysms (IA), four individuals with IA and two clinically unaffected individuals. We identified two nucleotide variants in both the TIMP1 and the TIMP2 coding sequences. All differences occurred in the third base positions of codons and were neutral polymorphisms. A significant difference was observed in the frequency of TIMP2 nt 573 polymorphism between 168 alleles from AAA patients and 102 control alleles.

Keywords: Aortic aneurysms; Connective tissue; Direct sequencing; Intracranial aneurysms; Polymorphism

1. Introduction

Rupture of an abdominal aortic (AAA) or intracranial (IA) aneurysm is a significant cause of mortality and morbidity, and 1–6% of the population in the USA and other industrialized countries harbor aneurysms (see Verloes et al., 1996). Despite the major advances in surgical treatment, the survival rate after a ruptured aneurysm is low. Early diagnosis of aneurysms is, therefore, important. If it were possible to predict who is at risk for developing an aneurysm, diagnostic efforts (ultrasonography, computerized tomography and magnetic resonance imaging) could be directed towards those at risk.

Familial predisposition to both AAAs and IAs is now well recognized (see Verloes et al., 1996; Ronkainen et al., 1997). The possible genetic factors involved in the development of aneurysms include (see Verloes et al., 1996): (a) structural components of arteries; (b) enzymes degrading the structural molecules; and (c) inhibitors of the proteolytic process.

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Abbreviations: AAA, abdominal aortic aneurysm; cDNA, complementary DNA; IA, intracranial aneurysm; PCR, polymerase chain reaction; TIMP1, tissue inhibitor of metalloproteinase 1; TIMP2, tissue inhibitor of metalloproteinase 2; nt, nucleotide

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DNA sequencing of 50 aortic aneurysm patients (Tromp et al., 1993) and 55 IA or carotid artery dissection patients (Kuivaniemi et al., 1993) revealed that mutations in type III procollagen are an infrequent cause of aneurysms.

The development of aneurysms is associated with remodeling of the extracellular matrix, including breakdown of structural components of the vascular wall (see Verloes et al., 1996). Collagenase activity and production of 92-kDa gelatinase (MMP9) are increased in ruptured aneurysmal aorta (see Verloes et al., 1996). The increased proteolytic activity could be due to overexpression of the enzymes or down-regulation of their inhibitors. In fact, decreased levels of tissue inhibitors of metalloproteinases (TIMPs) in AAAs have been reported (see Verloes et al., 1996). Furthermore, the ratio of matrix metalloproteinase (MMP) mRNA amount to TIMP mRNA was higher in AAA than in normal aortas (Tamarina et al., 1997). The relative TIMP deficiency could be due to local tissue conditions inhibiting the expression or mutations in the primary structure of the TIMP genes. There are at least four members in the TIMP family, all of which were cloned, sequenced and mapped onto human chromosomes (see Olson et al., 1998).

We studied the coding sequences of TIMP1 and TIMP2 in patients with AAA and/or IA to determine whether mutations in the TIMP genes are associated with aneurysms.

2. Materials and methods

This study was initiated at Thomas Jefferson University and was approved by the Institutional Review Committees of Thomas Jefferson University and Wayne State University School of Medicine. Skin biopsies obtained after written informed consent were used to establish fibroblast cultures. RNA was isolated, cDNA synthesized, PCR products purified and genomic DNA isolated as described previously (Tromp et al., 1993). Oligonucleotide primers based on the cDNA sequences of TIMP1 (TIMP1-I, CGCGAATTCAGATCCAGCGCCCAGAGAG; TIMP1-II, CGCGGATCCGGAAGAAAGATGGGAGTGGG; Docherty et al., 1985) and TIMP2 (TIMP2-II, TTTATTCATGCTGTTTCCAGGAAGGG; TIMP2-VI, CGGCCCCGCCCGCCCAGC; Stetler-Stevenson et al., 1990) were used in PCR to amplify all the coding sequences. Because of the high GC content in the 5′ end of the TIMP2, many PCR conditions failed, but Q-solution and Qiagen PCR kit were used successfully (Qiagen Inc., Santa Clarita, CA, USA).

The PCR products were sequenced directly using either Sequenase® (US Biochemical Corporation, Cleveland, OH, USA) and [α-^{33}P]dATP (NEN/DuPont), or Thermo Sequenase® (Amersham Life Science, Inc., Cleveland, OH, USA). The samples were electrophoresed on a Sequagel-6® (National Diagnostics, Atlanta, GA, USA) or on glycerol-tolerant gels (Amersham Life Science, Inc., Cleveland, OH, USA).

The sequence variants were confirmed by PCR-based restriction endonuclease assays. The gender of all samples was confirmed by Y-chromosome specific PCR (Research Genetics, Huntsville, AL, USA). For genomic PCRs, primers were designed based on published sequences (Genbank accession number D11139; Hammani et al., 1996). Primer sequences and PCR conditions are available from the corresponding author upon request.

3. Results and discussion

To investigate the possibility that aneurysms are caused by defects in the genes for TIMP1 or TIMP2, the sequences of the coding regions of TIMP1 and TIMP2 were determined in 19 unrelated individuals (12 had AAA, one had AAA and IA, four had IA, and two were clinically unaffected). All except one of the 17 aneurysm patients had a family history for the disease. The type III procollagen cDNA sequences in all of these individuals were normal (Kuivaniemi et al., 1993; Tromp et al., 1993).

The sequence analyses carried out here provided 671 nt of TIMP1 cDNA sequences (all 621 nt of coding sequences) from each individual. Two sequence variations were found. AAA patient JIMM429 had C/T nt 323 (proline codon at amino acid position 87 changes from CCC to CCT). All the other 18 individuals had C at nt 323. Another sequence change was found at nt 434, also reported by others (Tilson et al., 1993). Six individuals had C at this site, 11 had T and two individuals were heterozygous C/T. The sequence change converted the phenylalanine codon TTC at amino acid position 124 to TTT.

For TIMP2, we analyzed 750-nt (all 660 nt of coding sequences), and two sequence variations were identified. AAA patient JIMM398 had C/T at nt 306. Another difference, G to A transition, occurred at nt 573 in three patients (JIMM332, JIMM430 and JIMM257), all of whom were heterozygous for this change. Both variations occurred at the third positions of codons and did not change the amino acids.

The frequencies of these variations were determined among 102 control alleles and 168 alleles from AAA patients (11 of the patients used for DNA sequencing and 73 additional unrelated AAA patients). The TIMP1 gene includes four exons (Genbank accession number: D11139), and the variants are in the second and third exons. Since TIMP1 is on the X chromosome, we report the allele frequencies separately in males and females (Table 1).

Table 1
Frequencies of the polymorphisms in TIMP1 and TIMP2 genes

Location	Chr.[a]	Controls[b]			Unrelated AAA patients[c]			
		Allele frequency[d]			Allele frequency[d]			*P*-value[f]
		M	F	Het.[e]	M	F	Het.[e]	
TIMP1 nt 323	X	C: 0.966 (28) T: 0.034 (1)	1.000 (44) 0.000 (0)	0	0.953 (61) 0.047 (3)	0.950 (38) 0.050 (2)	0.023	M: 1.0000 F: 0.2238
TIMP1 nt 434	X	C: 0.483 (14) T: 0.517 (15)	0.273 (12) 0.727 (32)	0.157	0.578 (37) 0.422 (27)	0.625 (25) 0.375 (15)	0.107	M: 0.5005 F: 0.0019
TIMP2 nt 306	17	C: 0.931 (54) T: 0.069 (4)	0.977 (43) 0.023 (1)	0.157	0.961 (123) 0.039 (5)	0.975 (39) 0.025 (1)	0.071	M: 0.6089 F: 1.0000
TIMP3 nt 573	17	G: 0.793 (46) A: 0.207 (12)	0.886 (39) 0.114 (5)	0.212	0.914 (117) 0.086 (11)	0.950 (38) 0.050 (2)	0.155	M: 0.0374 F: 0.4370

[a] Chromosomal localization of the gene.
[b] Twenty-nine controls were males and 22 were females (all 51 were from the US).
[c] Sixty-four AAA patients were males (21 US, 17 Belgian, 13 Canadian, 7 Swedish, 3 British and 3 Finnish) and 20 were females (11 US, 6 Canadian, 2 Finnish and one Italian).
[d] Numbers in parentheses indicate number of alleles observed. M, males; F, females.
[e] Observed heterozygosity. For TIMP1, calculated using female alleles only.
[f] Comparison of allele frequencies between control and AAA groups using Fischer's exact test.

No significant differences in the frequencies of the nt 323 polymorphism were found between controls and AAA patients. The frequencies between the two female groups were, however, significantly different ($P = 0.0019$) for the nt 434 polymorphism.

The TIMP2 gene contains five exons (Hammani et al., 1996), and the sequence variants are in exons 1 and 3. The TIMP2 sequence variants were polymorphisms with minor allele frequencies of 0.049 and 0.167 for the nt 306 and nt 573, respectively (Table 1), with no significant differences between the controls and the AAA group with nt 306 polymorphism. The frequencies of nt 573 polymorphism were different between the control and AAA groups in males. In further analyses with 30 additional US control individuals, and by dividing the groups according to ethnic origin, the frequencies remained significantly different in males (Table 2). Among the US males, the difference between controls and AAA patients was even more significant (Table 2).

In summary, no mutations were found in the TIMP1 and TIMP2 genes in aneurysm patients. The differences in allele frequencies of nt 573 TIMP2 polymorphism between the control and AAA groups are interesting preliminary findings that need further follow-up with larger groups and carefully selected control groups for each ethnic group. It is possible that a mutation in the TIMP1 or TIMP2 gene contributes to

Table 2
Comparison of the allele frequencies for TIMP2 nt 573 polymorphism

Ethinicity	Controls[a]		Unrelated AAA patients[b]		
	M[c]	F[c]	M[c]	F[c]	*P*-value[d]
US	G: 0.804 (74) A: 0.196 (18)	0.861 (62) 0.139 (10)	0.976 (41) 0.024 (1)	1.000 (22) 0.000 (0)	M: 0.0174 F: 0.1098 M + F: 0.0033
Others	G: A:		0.884 (76) 0.116 (10)	0.889 (16) 0.111 (2)	M: 0.2122[e] F: 1.0000[e]
Total	G: 0.804 (74) A: 0.196 (18)	0.861 (62) 0.139 (10)	0.914 (117) 0.086 (11)	0.950 (38) 0.050 (2)	M: 0.0300 F: 0.2549 M + F: 0.0156

[a] Eighty-two US American control individuals, 36 females and 46 males.
[b] For details, see Table 1.
[c] Numbers in parentheses indicate number of alleles observed. M, males, F, females.
[d] Comparison of allele frequencies between control and AAA groups using Fisher's exact test.
[e] Comparison across populations may not be valid due to allele frequency differences; however, it is evident that adding the non-US patients to the total does not inflate the *P*-values; rather, it is conservative.

the disease process in patients with aneurysms. Such mutations could be either mutations in the promoter sequence or large-scale rearrangements in the genome, not easily detected by direct sequencing of RT-PCR products.

The polymorphisms will also be useful for genetic analyses in other diseases. For example TIMP2 is a candidate gene for autosomal-dominant retinitis pigmentosa (Bardien et al., 1995), and altered activities have been reported in tumor tissues (Bramhall et al., 1997).

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APPENDIX 13

Genome scan for familial abdominal aortic aneurysm using sex and family history as covariates suggests genetic heterogeneity and identifies linkage to chromosome 19q13. **H. Shibamura, J.M. Olson, C. van Vlijmen-van Keulen, S.G. Buxbaum, D.M. Dudek, G. Tromp, T. Ogata, M. Skunca, N. Sakalihasan, G. Pals, R. Limet, G.L. McKean, O. Defawe, A. Verloes, C. Arthur, A.G. Lossing, M. Burnett, T. Sueda, H. Kuivaniemi.** *Circulation 2004;109:2103-21*

Genome Scan for Familial Abdominal Aortic Aneurysm Using Sex and Family History as Covariates Suggests Genetic Heterogeneity and Identifies Linkage to Chromosome 19q13

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Background—Abdominal aortic aneurysm (AAA) is a relatively common disease, with 1% to 2% of the population harboring aneurysms. Genetic risk factors are likely to contribute to the development of AAAs, although no such risk factors have been identified.

Methods and Results—We performed a whole-genome scan of AAA using affected-relative-pair (ARP) linkage analysis that includes covariates to allow for genetic heterogeneity. We found strong evidence of linkage (logarithm of odds [LOD] score=4.64) to a region near marker *D19S433* at 51.88 centimorgans (cM) on chromosome 19 with 36 families (75 ARPs) when including sex and the number of affected first-degree relatives of the proband (N_{aff}) as covariates. We then genotyped 83 additional families for the same markers and typed additional markers for all families and obtained a LOD score of 4.75 (P=0.00014) with sex, N_{aff}, and their interaction as covariates near marker *D19S416* (58.69 cM). We also identified a region on chromosome 4 with a LOD score of 3.73 (P=0.0012) near marker *D4S1644* using the same covariate model as for chromosome 19.

Conclusions—Our results provide evidence for genetic heterogeneity and the presence of susceptibility loci for AAA on chromosomes 19q13 and 4q31. **(*Circulation*. 2004;109:2103-2108.)**

Key Words: aorta ■ aneurysm ■ genetics ■ mapping

Approximately 15% of patients with abdominal aortic aneurysms (AAAs) and without any recognizable connective tissue disorder, such as Ehlers-Danlos syndrome or Marfan syndrome, have a positive family history for AAA.[1] Two segregation studies favored a genetic model in explaining the familial aggregation of AAA and suggested the presence of a major gene effect.[2,3] Finding a susceptibility gene for AAA could lead to a simple DNA test to identify individuals at risk for developing an AAA. Such a test could be extremely useful because surgery for unruptured AAA is highly successful, with low mortality and morbidity.[4] However, diagnosing AAAs is difficult because most AAAs are asymptomatic before their rupture, and population-based ultrasonography screening to detect AAAs is not used routinely.

The aim of the present study was to find susceptibility loci for AAA with the use of linkage analysis with covariates to allow for locus heterogeneity.[5–7] We used affected-relative-pair (ARP) linkage analyses, methods recognized as useful for identifying genes in complex genetic diseases.[8] Additionally, we chose the 2-phase/2-stage design for cost-effectiveness and for minimizing the effort required in genotyping while maintaining statistical power to detect linkage.[9]

Methods

Subjects and Phenotyping

Families with at least 2 members with AAA[10] were identified; details on the family collection have been reported previously[11] and are

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TABLE 1. Characteristics of AAA Families

Category	Group 1	Group 2	Total
No. of families	36	83	119
Average No. of affected individuals per family (range)	3.4(2–7)	3.1(2–7)	3.2(2–7)
Families with at least 4 affected first-degree relatives (%)	9 (25)	29 (35)	38 (32)
No. of affected individuals genotyped (male)	86 (75)	195 (155)	281 (230)
Status of AAA in			
Individuals genotyped (male)			
Elective surgery	67 (61)	131 (104)	198 (165)
Rupture	8 (7)	11 (11)	19 (18)
Detected by ultrasonography	11 (7)	53 (40)	64 (47)
Other affected individuals not genotyped (male)*			
Elective surgery	12 (11)	24 (20)	36 (31)
Rupture	10 (8)	17 (14)	27 (22)
Detected by ultrasonography	2 (1)	10 (6)	12 (7)
Unconfirmed	5 (3)	7 (4)	12 (7)
Unaffected individuals genotyped (male)	52 (20)	67 (26)	119 (46)
ASPs genotyped	62	151	213
Other ARPs genotyped	13	9	22
Total ARPs in study	75	160	235

*There were a total of 87 (29 in group 1 and 58 in group 2) affected first-degree relatives who were known to have AAA but from whom no sample was available because of death before the start of the study (n=73) or unwillingness to provide a sample (n=14). In 5 and 7 such cases in groups 1 and 2, respectively, information about details of AAA was not available.

summarized in Table 1. An accepted definition of arterial aneurysm[10] was used.[12–14] Patients were identified from surgery records at vascular surgery units and were then contacted; consent was obtained, and family histories were collected. A certified vascular surgeon led the effort to identify AAA patients and affected family members. Family histories of any new patients presenting for surgical repair of AAAs at these sites were obtained in interviews conducted by a research nurse specifically trained for this work. In most cases, only a limited amount of information was available from relatives of second degree or greater. A specific questionnaire assessing skin and skeletal manifestations characteristic of Ehlers-Danlos syndrome type IV or Marfan syndrome was used to identify individuals with these disorders. Families with these disorders were excluded from the study. Whenever possible, the AAA diagnosis of a deceased family member was verified by requesting autopsy or medical records. Some family members, if aged ≥50 years, were examined by ultrasonography and were identified as affected if the infrarenal aortic diameter was ≥3.0 cm, a cutoff point used previously.[14] Occurrences of isolated iliac artery or other aneurysms (such as thoracic or thoracoabdominal) were noted but were not included in the study. All families were white: 42 Canadian, 36 Dutch, 23 Belgian, 10 American, 3 British, 3 Finnish, 1 Italian, and 1 Swedish. The study was approved by the institutional review boards of Wayne State University School of Medicine and each patient recruiting center,[11] and the subjects gave informed consent.

Design for DNA Linkage Study

We used an ARP design because the mode of inheritance of AAA is unknown and because an unaffected individual may develop an AAA subsequently or carry the susceptibility gene with incomplete penetrance. A 2-phase/2-stage design for DNA linkage analysis was chosen,[9] in which a 10- to 15-centimorgan (cM) genome scan is performed on a relatively small number of ARPs (stage 1 of phase I), followed by typing of additional markers in regions detected in stage 1 (stage 2 of phase I), and finally followed by additional typing of new ARPs (phase II) in all positive regions obtained in the first phase. For the combined data set of 213 affected sibling pairs (ASPs) and 22 other ARPs from 119 families (groups 1 and 2; Table 1), we had at least 95% power to detect "significant linkage" (logarithm of odds [LOD] score of 3.6)[15] for a locus with a locus-specific relative risk of 2.3 in the absence of locus heterogeneity.

Genotyping

We isolated genomic DNA from peripheral blood using a Puregene kit (Gentra Systems, Inc). A whole-genome scan was performed by the Mammalian Genotyping Service with the use of screening set 10 with 405 highly polymorphic microsatellite markers and an average marker-to-marker distance of 10 cM.[16] Additional microsatellite markers on chromosome 19 were genotyped as described previously.[17] Before genotyping polymerase chain reactions were performed, a whole-genome amplification was carried out to increase the amount of template DNA available for genotyping and to ensure that limited resources were used cost-effectively.[18] Additional genotyping on chromosomes 3, 4, 5, 6, 9, 14, and 21 after the whole-genome scan was performed by deCODE Genetics Inc. A slightly smaller number (116 ARPs) of samples were genotyped in group 2 for these chromosomes compared with the number of samples genotyped for chromosome 19 (157 ARPs) in our own laboratory, where new ARPs were included into the study continuously. In addition, 2 new ASPs and 1 other new ARP were identified in group 1 families while the study was in progress, and they were included in chromosome 19 analyses.

Statistical Analyses

The genotype data were analyzed for genetic linkage with the multipoint model-free ARP LOD score analysis with the use of the computer program LODPAL from S.A.G.E. (version 4.2).[19] To allow for covariate-related locus heterogeneity, we applied a covariate-based ARP LOD score method.[6] The model is a 1-parameter modification of the conditional logistic parameterization of the ASP LOD score introduced by Olson.[6] An optimal mode of inheritance parameter[20] is specified that allows one to fit only a single additional parameter per covariate. The model is parameterized in

TABLE 2. Group 1 and 2 LOD Scores for Baseline and Covariate Models for Regions With Largest LOD Scores for Group 1

Group	Chromosome	Map Position,* cM	Flanking Markers	Multipoint LOD Score	
				Baseline†	+Sex+N_{aff}‡
1	3	94	D3S3644–27666CA8.D§	0.94‖	2.60¶
2	3	98	27486CA1§	0.00	1.17
1	3	178	D3S3523–D3S1574	0.00	3.55¶
2	3	169	D3S3523–D3S1574	0.10	0.42
1	4	28	D4S403–D4S1567	0.10	2.86¶
2	4	16	D4S394	0.00	0.79
1	4	73	D4S3248–D4S2432	0.63‖	3.00¶
2	4	68	D4S3355–D4S2978	0.35	3.20¶
1	4	144	D4S1644–D4S1586	0.54	4.45¶
2	4	132	D4S2959	0.00	3.03¶
1	5	144	D5S1983–D5S2011	0.00	3.30¶
2	5	141	D5S1983	0.72‖	1.14
1	5	183	D5S211–D5S2008	0.01	2.86¶
2	5	190	D5S211–D5S2008	0.00	0.28
1	6	187	D6S1719–D6S1027	0.61‖	4.22¶
2	6	186	D6S1719–D6S1027	0.36	0.43
1	9	121	D9S930–D9S177	0.00	5.83¶
2	9	126	D9S177–D9S2145	0.00	0.84
1	14	72	D14S63–DG14S37§	0.00	2.66¶
2	14	75	DG14S37§–D14S588	0.00	0.68
1	19	50	D19S931–D19SS433	0.00	4.64¶
2	19	59	D19S245–D19S587	0.00	1.22
1	21	21	D21S1257–D21S2052	0.88‖	2.45¶
2	21	21	D21S1257–D21S2052	0.00	0.61

*Indicates position of peak LOD score based on Marshfield genetic map.[16]
Multipoint LOD score analyzed †without covariates or ‡with sex and N_{aff} as covariates.
§These markers are from deCODE marker set.
‖$P<0.05$.
¶$P<0.01$.

terms of offspring recurrence risk ratio (λ_1), conditional on K covariates x_k, as follows

$$\lambda_1(x)=\exp(\beta+\Sigma_{k=1}^{K}\gamma_k x_k), \quad (1)$$

where β is a parameter that measures the "average" linkage in the sample, and the γ_k are covariate-specific parameters that measure the change in linkage as a function of the covariates and in terms of the recurrence risk ratio for monozygotic twins (λ_2), conditional on K covariates x_k, as follows

$$\lambda_2(x)=3.634\lambda_1(x)-2.634. \quad (2)$$

To simplify specification of constraints on parameter estimates, to improve numerical stability, and so that β reflects average allele sharing, all covariates are centered around their sample mean before inclusion. In general, the values of β and γ_k depend on the choice of "coding scheme" for the covariates; a linear transformation of the covariate changes neither the LOD score nor the estimates of covariate-specific recurrence risk ratios. More importantly, conclusions about the existence of locus heterogeneity and the extent or nature of locus heterogeneity do not depend on the estimated value of β (which may equal zero).

Asymptotic distributions of the resulting likelihood ratio tests were used to obtain probability values.[6] We report as LOD scores the likelihood ratio statistics (LRSs) divided by 4.605 (ie, $2\log_e 10$). Critical values for the LRSs were obtained as follows. The distribution of the LRS for the basic 1-parameter model is a 50:50 mixture of a point mass at zero and a χ^2 distribution with 1 *df*. Addition of K covariates gives an LRS with a distribution that is a 50:50 mixture of a χ^2 with K *df* and a χ^2 with $K+1$ *df*. The difference in LRS between nested models that differ by J covariates has a χ^2 distribution with J *df*. One can therefore test both the significance of the contribution of a covariate and the overall evidence for linkage. The overall evidence for linkage includes information about both the "average" linkage for the sample and the change in linkage as a function of the covariate.

Results

A whole-genome scan was performed with 36 AAA families, including 62 ASPs and 13 other ARPs (group 1; Table 1). We performed a model-free multipoint linkage analysis and identified 4 regions, on chromosomes 3, 4, 6, and 21, as significant at the $\alpha=0.05$ level (baseline values in Table 2). We then extended the analyses to include sex and number of affected first-degree relatives of the proband (N_{aff}) as covariates, and a total of 12 regions on chromosomes 3, 4, 5, 6, 9, 14, 19, and 21 were identified with a covariate effect

TABLE 3. Multiple Regression Analysis of Chromosome 4 and 5 Regions

	Group 1 (62 ASPs, 75 Total ARPs)				Group 2 (106 ASPs, 116 Total ARPs)				Total Sample (168 ASPs, 191 Total ARPs)			
			Parameter Estimates				Parameter Estimates				Parameter Estimates	
Model*	LOD Score	P†	β	$\gamma_1, \gamma_2, \ldots$	LOD Score	P†	β	$\gamma_1, \gamma_2, \ldots$	LOD Score	P†	β	$\gamma_1, \gamma_2, \ldots$
Chromosome 4 at 70 cM (*D4S2978*)												
Baseline	0.24	…	0.15	…	0.35	…	0.11	…	0.73	0.0334	0.13	…
+Sex	0.39	…	0.12	−0.31	0.85	…	0.17	0.29	0.96	…	0.16	0.17
+ N_{aff}	0.39	…	0.20	0.11	1.80§	0.0099	0.40	0.42	1.41	0.0249	0.18	0.12
+Sex+N_{aff}	0.82	…	0.35	−1.03, −0.23	3.20‡§	0.0013	0.71	0.21, 0.67	1.65	0.0387	0.21	0.17, 0.12
+Sex+N_{aff}+sex*N_{aff}	3.75‡§	0.0012	0.26	−0.27, 1.24, −0.73	3.74	0.0012	1.43	2.28, −1.39, 1.31	2.41‡§	0.018	1.17	2.64, −1.51, 1.35
Chromosome 4 at 140 cM (*D4S1644*)												
Baseline	0.63	0.0443	0.28	…	0.00	…	0.00	…	0.00	…	0.00	…
+Sex	0.81	…	0.28	−0.49	2.40‡§	0.0024	0.00	−0.40	3.04‡§	0.00055	0.04	−0.41
+N_{aff}	0.81	…	0.35	−0.12	0.01	…	0.00	0.01	0.12	…	0.00	−0.04
+Sex+N_{aff}	1.59‡§	0.0440	0.42	−0.85, −0.17	2.55	0.0056	0.00	−0.42, 0.02	3.35	0.00097	0.05	−0.43, −0.03
+Sex+N_{aff}+sex*N_{aff}	1.69	…	0.33	−0.09, 0.45, −0.35	2.74	0.0094	0.00	−0.46, −0.05, 0.03	3.73	0.0012	0.11	−0.54, −0.20, 0.09
Chromosome 5 at 140cM (*D5S816*)												
Baseline	0.00	…	0.00	…	0.72‡§	0.0343	0.17	…	0.23	…	0.08	…
+Sex	0.09	…	0.00	0.27	1.11	…	0.20	−0.18	0.35	…	0.09	−0.09
+N_{aff}	1.44‡§	0.0232	0.05	0.39	0.76	…	0.18	0.03	0.40	…	0.09	0.05
+Sex+N_{aff}	2.49‡§	0.0063	0.29	0.33, 0.60	1.14	…	0.20	−0.18, 0.03	0.50	…	0.09	−0.08, 0.05
+Sex+N_{aff}+sex*N_{aff}	2.36	0.203	0.20	0.21, 0.62, −0.05	1.45	…	0.23	−0.15, 0.24, −0.10	1.02	…	0.12	−0.06, 0.25, −0.11

*Linkage analysis models: baseline, without covariates; sex, sex as a covariate; N_{aff}, No. of affected first-degree relatives of probands as a covariate; sex+N_{aff}, sex and N_{aff} as a covariate; sex+N_{aff} + sex * N_{aff}, sex and N_{aff} and their interactions as a covariate.

†*P* for overall linkage effect (average and covariate-related), if $P<0.05$.

‡Most parsimonious model.

§Significant covariate effect compared with nearest nested model.

significant at the $\alpha=0.01$ level, suggesting the presence of genetic heterogeneity (Table 2).

Twelve regions that were significant in the whole-genome scan were selected for a follow-up study, and additional microsatellite markers were genotyped in the 36 families and in 83 new AAA families that included 151 ASPs and 9 other ARPs (groups 1 and 2; Table 1). Three loci (68 and 132 cM on chromosome 4, and 141 cM on chromosome 5) showed some evidence of linkage in group 2 (Table 2), and these regions were selected for detailed analyses (Table 3). Table 3 shows the LOD scores and parameter estimates for groups 1 and 2 as well as the total sample at the location that gave the highest LOD score for the total sample. In the combined analysis with groups 1 and 2 together, the locus on chromosome 5 did not appear significant (Table 3). The region at 140 cM on chromosome 4 had a LOD score of 3.73 ($P=0.0012$) (Table 3). The 70-cM region had a peak LOD score of 3.13 ($P=0.0042$), although the parameter estimates were unstable (not shown), and we therefore report the LOD score of 2.41, which was 4 cM away from the peak, to be able to give more accurate parameter estimates (Table 3).

The chromosome 19 region was also analyzed further because (1) it had the second highest LOD score in the original genome scan (Table 2); (2) we have recently identified a putative locus for intracranial aneurysms on chromosome 19[17]; and (3) it contains a large number of biologically

TABLE 4. Multiple Regression Analysis of Chromosome 19 at 58.69 cM (*D19S416*)

	Group 1 (64 ASPs, 78 Total ARPs)*				Group 2 (149 ASPs, 157 Total ARPs)*				Total Sample (213 ASPs, 235 Total ARPs)*			
			Parameter Estimates				Parameter Estimates				Parameter Estimates	
Model	LOD Score	P†	β	$\gamma_1, \gamma_2, \ldots$	LCD Score	P†	β	$\gamma_1, \gamma_2, \ldots$	LCD Score	P†	β	$\gamma_1, \gamma_2, \ldots$
Baseline	0.00	…	0.00	…	0.00	…	0.00	…	0.00	…	0.00	…
+Sex	0.45	…	0.00	−0.36	0.00	…	0.00	0.01	0.07	…	0.00	−0.06
+ N_{aff}	3.30‡§	0.00030	0.00	0.40	0.23	…	0.00	0.07	1.22‡	0.039	0.00	0.11
+Sex+N_{aff}	3.61	0.00054	0.00	0.08, 0.48	0.46	…	0.00	−0.18, 0.12	1.74	0.032	0.00	−0.18, 0.14
+Sex+N_{aff}+sex*N_{aff}	4.38	0.00031	0.06	0.41, −0.30, 0.46	4.12‡§	0.00054	0.00	0.14, −0.35, 0.30	4.75‡§	0.00014	0.00	0.14, −0.30, 0.29

*No. of ARPs genotyped for chromosome 19 was larger than that analyzed for the other chromosomes (for details, see Methods). For definition of other footnotes, see Table 3.

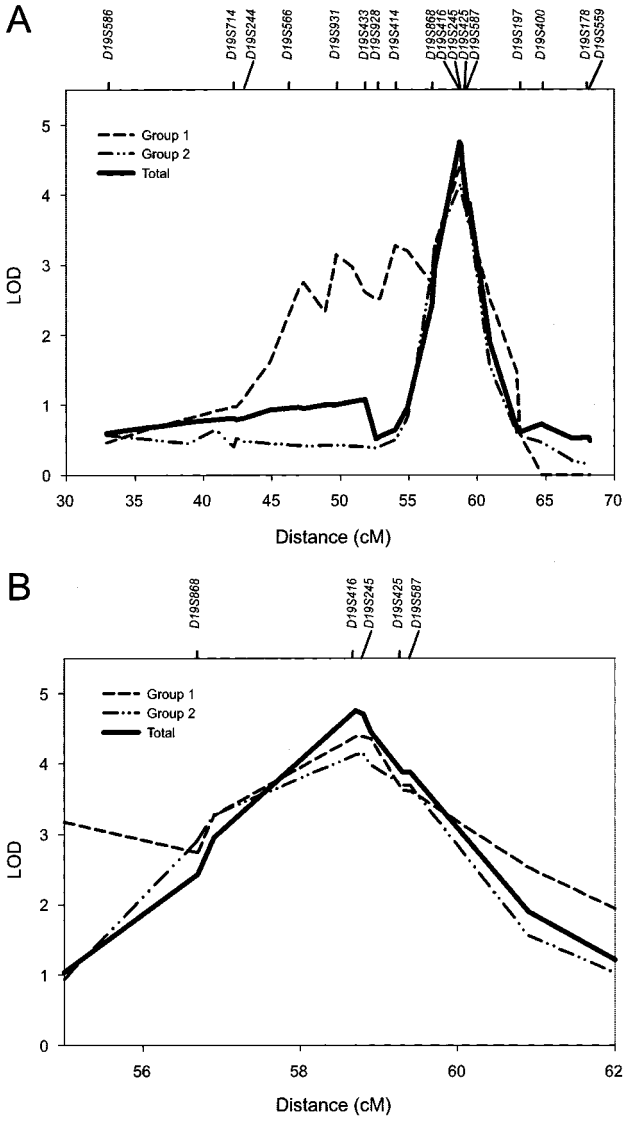
plausible candidate genes.[21] The highest LOD score on chromosome 19 for group 2 was 4.12 (P=0.00054) near *D19S416* and 58.69 cM from the p-terminus when sex, N_{aff}, and their interaction were used as covariates (Table 4). In the combined analysis with groups 1 and 2, including 213 ASPs and 22 other ARPs, the maximum LOD score was 4.75 (P=0.00014) at 58 cM, just proximal to *D19S416*, with sex, N_{aff}, and their interaction as covariates (Table 4). The interaction term (sex*N_{aff}) was significant in the total sample (P=0.00317) as well as in the 2 subsamples. These results suggested that female-female pairs from families with larger numbers of affected persons are most at risk from this locus, although this locus also gives substantial risk to male-male pairs from families with fewer affected persons. Both groups 1 and 2 had the peak LOD score at same location on chromosome 19 (Figure). The best, most parsimonious model was the one with N_{aff} as a covariate in group 1 and a model using sex, N_{aff}, and their interaction as covariates in group 2 (Table 4).

Discussion

We found no evidence of linkage unless sex and number of affected persons were included as covariates in the linkage model. How then should our results be interpreted? As Dizier and coworkers[22] have shown, absence of a linkage signal can be due to a factor on which the siblings differ, such as a characteristic of the disease (eg, severity), or an environmental factor. For common diseases that are genetically complex, such situations may be the rule rather than the exception.[5–7] By allowing for heterogeneity in the analysis by including covariates chosen a priori, we avoid these concerns and are able to detect linkage signals obscured by the presence of heterogeneity.

No prior DNA linkage studies with AAA exist, although 3 studies investigated familial thoracic aortic aneurysms and dissections (TAAD) and identified linked loci on 5q,[23] 11q,[24] and 3p24–25.[25] Because our collection of AAA families excluded patients with TAAD[11] and the AAA loci do not overlap with the TAAD loci, different genetic risk factors are probably involved in the development of TAAD and AAA.

There are several plausible candidate genes in the 2 regions with the highest LOD scores, such as *IL15* (interleukin 15; a plausible candidate gene with respect to inflammation in AAA), *GAB1* (GRB2-associated binding protein 1; an important mediator of branching tubulogenesis and a central protein in cellular growth response, transformation, and apoptosis), and *EDNRA* (endothelin receptor type A; an endothelin-1 receptor expressed in many human tissues with the highest level in the aorta) around 140 cM on chromosome 4, as well as *LRP3* (LDL receptor–related protein 3), *HPN* (transmembrane protease, serine 1; a serine-type peptidase involved in cell growth and maintenance), *PDCD5* (programmed cell death 5; a protein expressed in tumor cells during apoptosis independent of the apoptosis-inducing stimuli), and *PEPD* (peptidase D; an Xaa-Pro dipeptidase important in collagen catabolism) on chromosome 19.[21,26] *LRP3* is particularly interesting because conditional knockout mice for *LRP1*, another member of the gene family, developed arterial aneurysms and atherosclerosis.[27]



A, Multipoint LOD score plot for AAA on chromosome 19. Sex and N_{aff} were used as covariates. The *x* axis shows distance in centimorgans on chromosome 19; *y* axis, LOD score. B, A higher-resolution plot for the region between 55 and 62 cM on chromosome 19, illustrating that several closely spaced markers support the peak shown in A.

It is likely that additional AAA loci will be identified by testing other possible covariates, such as smoking, hypertension, and coronary artery disease, which was not possible in this study because these risk factors are so common both in the general population and in patients with AAA that the relatively small number of families in this study did not provide enough power to study them.

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THESES ANNEXES

1. *Fibromuscular dysplasia of the internal carotid artery.*

2. *Effect of cardiac resynchronization therapy on functional mitral regurgitation in heart failure.*

3. *Isolated aneurysms of the iliac arteries.*