A particular phenotype of ascending aorta aneurysms as precursor of type A aortic dissection†

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Received 7 May 2012; received in revised form 17 June 2012; accepted 25 June 2012

Abstract

OBJECTIVES: We aimed to identify a phenotype of ascending thoracic aortic aneurysm (TAA), which, more than others, evolves into type A dissection (TAD).

METHODS: Aortic specimens were obtained from patients undergoing surgical repair of TAA and TAD (108 and 26, respectively). Histopathological and immunohistochemical analyses were performed by using adequate tissue specimens, appropriate techniques and criteria.

RESULTS: We identified the three following TAA phenotypes: phenotype I (cystic medial degeneration balanced by a substitutive fibrosis, in absence of medial apoptosis and with a faint collagenase concentration), phenotype II (cystic medial degeneration of higher grade, respectively, than substitutive fibrosis, with focal medial apoptosis and moderate collagenase concentration), and phenotype III (elevated cystic medial degeneration without substitutive fibrosis, with plurifocal medial apoptosis and severe collagenase concentration). The same medial degenerative lesions of TAA phenotype III were observed in TAD tissue samples.

CONCLUSIONS: The morphological identity of medial lesions observed in both the TAA phenotype III and in TAD aortas might be assumed to be the precursor—and consequently the optimal biomarker—of dissection, independently of aneurysm diameter or valvular disorder. Identification of genetic risk factors, useful both in diagnostics and in developing more targeted treatment for individual patients, might also be needed.

Keywords: Aneurysm • Dissection • Thoracic aortic aneurysm phenotype III

INTRODUCTION

Type A aortic dissection (TAD) represents the most common aortic emergency. Population-based studies suggest that the incidence of TAD ranges from 2 to 3.5 cases per 100,000 person-years, with approximately two thirds of dissections involving the ascending thoracic aorta and one third involving the descending thoracic aorta [1–4]. It is associated with a mortality rate of up to 80% if left untreated.

Despite the lethality of these disease processes, their underlying mechanisms remain poorly understood. It is remarkable that this condition occurs in aortic walls having both enlarged aneurysmatic or/and normal diameters.

The medial layer of the aorta is composed of smooth muscle cells and extracellular matrix proteins, primarily elastin and collagen. Maintaining a balanced composition of vascular smooth muscle cells and extracellular matrix proteins appears to be critical for preserving the important functional properties of the thoracic aorta, especially its mechanical compliance with pulsatile blood flow. Disturbances in the metabolic balance, resulting in excessive extracellular matrix degradation, might lead to progressive aortic wall deterioration, expansion, and rupture [5, 6].

This study is a comparative histopathologic study of medial changes between thoracic ascending aneurysms (TAAs) and TAD. In particular, our principal aim was to identify a phenotype of ascending TAAs that was able, more than the others, to evolve in the dissection complication, independently of aneurysm diameter or valvular disorder.

MATERIALS AND METHODS

Our study received approval from the local ethic committees and all participants gave their informed consent. Data were encoded to ensure the anonymity of patients in the study and...
control groups. All measurements were performed without knowledge of the nature of the study.

Clinical data

Our study included 134 individuals [97 men (72%) and 37 (28%) women; mean age: 62.95 ± 11.44] from Western Sicily. They were enrolled from January 2004 to July 2008 at time of their admission to the cardiac surgery unit of Palermo University Hospital (Table 1).

Of these patients, 108 (80.6%) were operated on because of aneurysm and 26 (19%) because of dissection of the ascending aorta. None were affected by classic Marfan syndrome (according to Ghent criteria), nor by Ehler's Danlos syndrome [4, 7-9]. In addition, the specimens obtained from thoracic aorta also had no histological signs of aortitis. This opens up the possibility that the cases of aneurysm had a dilatative pathology of unknown etiology and, in some cases, were complicated atherosclerotic lesions [10, 11].

For each patient, assessment of the diameter of the ascending aorta was made both in the operating room and preoperatively, by transthoracic echocardiography (TTE) and transoesophageal echocardiography (TEE) and carried out by (i) estimating the dimensions of the aortic annulus, sinuses of Valsalva and proximal ascending aorta (2.5 cm above the sinotubular junction) in the parasternal long-axis view and (ii) evaluating the dimensions of the aortic arch from the suprasternal view. Echocardiography-derived sizes were reported as internal diameter size [12]. Colour Doppler was used to assess the presence and severity of aortic regurgitation and stenosis. Additionally, aortic root and ascending aorta diameters were measured using a helical computed tomography (CT) image analysis technique. The mean size of photographed aneurysmatic ascending aorta specimens (see below) after 24 h storage was 53.3 ± 8 (Table 1).

Relevant medical histories of aortic disease were obtained from patients’ medical records. Thus, demographic and clinical features, comorbidity conditions and pharmacological treatments were collected. Our patients had commonly suffered from hypertension for several years. In all cases hypertension was controlled by beta-blockers (Table 1).

In order to detect histopathological abnormalities, control ascending aortas were obtained from 30 individuals (20 men and 10 women; mean age 55 ± 11.57 years), who died from causes unrelated to aortic disease and no sepsis at the time of death, as confirmed by autopsy.

Aortic specimens, histopathological assays

Full aortic segments were collected from the resected aortic walls of 134 patients at the time of surgery and fixed in 10% neutral buffered formalin for 24 h and then processed for routine paraffin embedding. Surgical specimens were subsequently photographed and the maximum transverse diameter was measured. Multiple histological sections from each sample were prepared and stained with haematoxylin-eosin, Weigert-van Gieson and Alcian-PAS for microscopic examination. Histopathological abnormalities of aortic wall media were graded and defined according to the definitions and grading systems used by Bechel et al. [13].

Serial sections of these tissues were also used for immunohistochemical staining. Aortic wall was mainly evaluated for the following histological features: fragmentation of elastic and collagen fibre network, presence of necrosis, apoptosis, amounts of MMP-9, and inflammatory cell infiltration.

Immunohistochemical assays

Immunohistochemical analyses were performed on 5µm thick paraffin-embedded sections. The deparaffinized sections were treated for 20 min in a microwave oven in 10 mM citrate buffer PH6, or TRIS/EDTA PH9. Sections were then incubated for 1 h with specific monoclonal antibodies against MMP-9 (Clone 15W2, NCL-MMP9 439 1:50; Novocastra Laboratories Ltd, UK), or isotype-matched controls at appropriate dilutions. After washing in TBS 1X (Tris-buffered solution), staining was performed by biotinylated link antibody and peroxidise-labelled streptovidin kits.
(3-amino-9-ethylcarbazole) substrate chromogen. After that, the counterstaining of cells and tissue sections was performed using aqueous haematoxylin (Novocastra Laboratories Ltd, UK).

**TUNEL testing**

We performed TdT (terminal deoxynucleotidyl transferase)-mediated X-dUTP (deoxyuridine triphosphate nucleotides) nick end-labelling (TUNEL) reaction (In Situ Cell Death Detection kit; Roche Diagnostics SpA, Milan, Italy) on full-thickness aortic wall paraffin sections (5 μm). Tissues were deparaffinized and then permeabilized with PBS 0.1% sodium citrate/0.1% Triton X-100. Specimens were then incubated with TdT and fluorescein-labeled dUTP in a humidified atmosphere for 1 h at 37°C. In situ apoptosis staining was revealed by using an AP converter. DNA strand breaks were detected by using the 5-bromo-4-chloro-3-indolyl-phosphate (BCIP/NBT; Dako, Italy) substrate and revealed by using an AP converter. Tissues were subsequently counterstained with eosin under light microscopy.

**Semi-quantitative evaluation of MMP-9 by immunohistochemical assays**

A semi-quantitative evaluation of MMP-9 in the aortic specimens of 134 patients was performed in course of immunohistochemical assays. Staining was classified as faint, moderate or severe.

**Statistical analysis**

All analyses were performed with R (R Foundation) and Excel (Microsoft) software. Fisher's test was conducted to compare, according to gender, all demographic and clinical features, comorbidity conditions and pharmacological treatments, and also to verify the hypothesis of association between elementary lesions and valvular dysfunction. To verify the hypothesis of a relationship between quantitative variables and histopathological classifications, non-parametric Kruskal-Wallis tests were executed; this was due to the strong asymmetry of the distributions. A log-linear (Poisson) model was adapted to analyse the association between apoptosis, phlogosis, and collagenases for each level of histopathological classification.

**RESULTS**

**Clinical data**

Table 1 reports demographic and clinical features, comorbidity conditions and pharmacological treatments of all patients.

<table>
<thead>
<tr>
<th>ADA (%)</th>
<th>NADA (%)</th>
<th>TAD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 35)</td>
<td>(n = 73)</td>
<td>(n = 26)</td>
</tr>
<tr>
<td><strong>Fibrosis: no. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>4 (11)</td>
<td>16 (22)</td>
</tr>
<tr>
<td>Grade I</td>
<td>4 (11)</td>
<td>49 (67)</td>
</tr>
<tr>
<td>Grade II</td>
<td>19 (55)</td>
<td>8 (11)</td>
</tr>
<tr>
<td>Grade III</td>
<td>8 (23)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Elastic fragmentation: no. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>18 (51)</td>
<td>17 (23)</td>
</tr>
<tr>
<td>Grade II</td>
<td>11 (32)</td>
<td>41 (56)</td>
</tr>
<tr>
<td>Grade III</td>
<td>6 (17)</td>
<td>45 (23)</td>
</tr>
<tr>
<td><strong>Medionecrosis: no. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>6 (28)</td>
<td>7 (10)</td>
</tr>
<tr>
<td>Grade II</td>
<td>23 (66)</td>
<td>42 (57)</td>
</tr>
<tr>
<td>Grade III</td>
<td>5 (6)</td>
<td>24 (33)</td>
</tr>
<tr>
<td><strong>Cystic necrosis: no. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>14 (40)</td>
<td>9 (12)</td>
</tr>
<tr>
<td>Grade II</td>
<td>16 (46)</td>
<td>36 (49)</td>
</tr>
<tr>
<td>Grade III</td>
<td>5 (14)</td>
<td>28 (39)</td>
</tr>
<tr>
<td><strong>Apoptosis: no. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0 (0)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Focal</td>
<td>19 (54)</td>
<td>14 (19)</td>
</tr>
<tr>
<td>Plurifocal</td>
<td>16 (46)</td>
<td>55 (76)</td>
</tr>
<tr>
<td><strong>Metalloproteinas: no. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Faint</td>
<td>19 (54)</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Moderate</td>
<td>7 (20)</td>
<td>30 (41)</td>
</tr>
<tr>
<td>Severe</td>
<td>8 (23)</td>
<td>38 (52)</td>
</tr>
</tbody>
</table>

**Histological and immunohistochemical observations**

In the control group, all subjects presented normal aortic wall without media-degenerative lesions.

Among 108 degenerative TAAs that we studied, 35 (32%) were atherosclerotic aneurysms (ADAs) and 73 (68%) were non-atherosclerotic aneurysms (NADAs).

(a) ADAs: histologically, media-degenerative lesions were present in all cases of ADAs. Table 2 reports the severity of elastic fragmentation, medionecrosis, cystic necrosis and medial fibrosis in ADAs. No significant associations were observed between aortic valve dysfunction, with or without cuspid pathological lesions, or aneurysm diameter and medial change severity (P-value >0.05 by Fisher's test; data not shown). Apoptosis was plurifocal in 16 cases (54%) and focal in 19 cases (46%). Evaluation of MMP-9 in the aortic media revealed the absence of these collagenases in 1 case (3%) and their presence in 34 cases (97%) in differing concentrations [faint: 19 cases (54%); moderate: 7 cases (20%); severe: 8 cases (23%)].

(b) NADAs: histologically, media-degenerative lesions were present in all cases of NADAs. Table 2 reports the severity of elastic fragmentation, medionecrosis, cystic necrosis and medial fibrosis in NADAs. No significant associations were observed between aortic valve dysfunction, with or without cuspid pathological lesions, or aneurysm diameter and medial change severity (P-value >0.05 by Fisher's test; data not shown). Apoptosis of media smooth muscle cells was absent in 5 cases (5%), plurifocal in 55 cases (76%) and focal in 14 cases (19%). Evaluation of collagenases in aortic media revealed the presence of MMP-9 in all cases in differing conditions.
Identification of three phenotypes in non-atherosclerotic degenerative aneurysms cases

In the context of NADAs, we identified three phenotypes characterized by a different quantitative relationship between cystic medial degeneration, fibrosis and apoptosis:

(i) phenotype I (13 cases): cystic medial degeneration balanced by a substitutive fibrosis, in absence of medial apoptosis, and with a faint collagenases concentration.

(ii) phenotype II (22 cases): cystic medial degeneration higher than substitutive fibrosis, with focal medial apoptosis and moderate collagenases concentration.

(iii) phenotype III (38 cases): elevated cystic medial degeneration, without substitutive fibrosis, with plurifocal medial apoptosis and severe collagenases concentration.

Demographic and clinical features, comorbidity conditions and pharmacological treatments were compared between the three NADA phenotypes. No significant differences were detected (data not shown). In contrast, significant statistical differences were observed by comparing abnormalities of extracellular matrix components among three NADA phenotypes (fibrosis \( P < 0.005 \); elastic fragmentation \( P = 0.002 \); medionecrosis \( P = 0.004 \); cystic necrosis \( P = 0.07 \); apoptosis \( P < 0.0001 \); MMP-9 amount \( P = 0.004 \), by \( \chi^2 \) test and appropriate tables). In particular, NADA phenotype III was characterized by grade II and III elevated elastic fragmentation, increased medionecrosis and grade II and III cystic necrosis (grade III absent in the other two NADA phenotypes), plurifocal apoptosis, moderate and severe amounts of MMP-9 in respect of NADA phenotypes I and II. (Table 3 and Fig. 1)

DISCUSSION

Acute ascending aorta dissection is relatively rare compared to other causes of cardiovascular disease and continues to defy our attempts to predict or prevent it. Identification of patients at risk for aortic dissection is difficult. Established clinical risk factors are systemic hypertension (widespread in the general population) and aortic dilation or aneurysm, which can only be found with diagnostic imaging. Even patients with Marfan syndrome, Ehlers-Danlos syndrome, familial aortic aneurysm, or congenital bicuspid aortic valve, who are known to be at increased risk for dissection, are frequently unrecognized until an acute aortic syndrome occurs.

The mainstay of prevention of aortic dissection, aside from treatment of hypertension, is elective aortic surgery in patients with dilated ascending aortas. Guidelines for timing of aortic root repair are based on clinical observations by experienced clinicians and surgeons, and a consensus based on clinical series and patient characteristics. Taking into account the consensus, surgery to prevent rupture or dissection of the ascending thoracic aortic aneurysm should be recommended when the ascending aortic diameter reaches 5.5 cm for non-Marfan patients and 4.5 cm in Marfan patients.

However, many dissection patients do not seem to have markedly dilated aortas at the time of presentation [14, 15].

In an autopsy study of patients with type A aortic dissection Neri et al. examined 220 patients, including 94 patients with a connective tissue disorder including BAV disease (mean aortic diameter, 41.8 mm), and 126 without a connective tissue disorder (mean aortic diameter, 41.3 mm). The authors demonstrated that aortic dissection occurred in one third of patients with a normal aortic diameter. Fifty-seven percent of patients had an aortic diameter >40 mm and only 10% of patients had a true aneurysm. The authors concluded that dissection, superimposing on near-normal aortic size, might be an expression of functional wall changes other than dilatation [16].

Accordingly, in our study, we observed that the severity of aortic media degeneration in TAD and in TAAs is not related to the diameter of the aneurysm. We also tested that TAD is often associated with a non-atherosclerotic pathogenesis. In fact, in the ADA aortas, the grade of medial degenerative lesions...
was balanced to the grade of substitutive medial fibrosis. In contrast, in NADA and TAD, medial fibrosis was absent or of grade I (Figs. 2 and 3).

The relative absence of reparative fibrosis should predispose patients to aortic rupture. In particular, our study showed that TAD has the same histological and immunohistochemical features as phenotype III of NADA: elevated cystic medial degeneration, without substitutive fibrosis, with plurifocal medial apoptosis and severe collagenases concentration. Our results agree with recent studies that showed that an up-regulation of metalloproteinases, related to inflammatory processes or genetic aspects, might affect the formation of TAA and TADs. Jones et al. demonstrated that the loss of specific micro-RNA expression may allow for the elaboration of MMPs capable of driving aortic remodelling during TAA development [17]. Zhang et al. concluded that interleukin-1β and interferon-γ might cause the formation of TAD and TAA, possibly through the up-regulation of matrix metalloproteinase-9 and the apoptosis of media cells in humans [18]. Levula et al. concluded that the involvement of MMP-8 and MMP-15, together with inflammation consisting of B-cells may indicate active remodelling of the aortic wall, leading to TAD [19].
In contrast with the data showed by Robert et al., our study confirmed that, in most cases of TAD, elastic fragmentation was moderate and severe [20]. The loss of elastic fibre integrity might be one of the most important elements that affects smooth muscle cells apoptosis.

CONCLUSION

The morphological identity of medial lesions observed in the phenotype III of NADA and in the aortic dissection leads us to suppose that this phenotype might represent the precursor of dissection, independently of aneurysm diameter and/or valvular dysfunction. Finally, the high quantity of metalloproteinases in NADA phenotype III, without phlogosis reaction, suggests that research into a particular genetic polymorphism be carried out on these patients and their families.

Study limitations

The principle limitation of this study is the limited sample size and the rarity of BAV pathology. Also, surgical recommendations require genetic and clinical data as main support.

FUNDING

This work was supported by grants from the Italian Ministry of Education, University and Research to Giuseppina Candore and Giovanni Ruvolo.
ACKNOWLEDGMENTS

The authors gratefully acknowledge Federico Torretta, who is a doctor of statistics and who contributed to the statistical analysis of the study data.

Conflict of interest: none declared.

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