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Morphological Patterns Identification in PET+ Aneurysmatic Lesions

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Abstract

The size of abdominal aortic aneurysm (AAA) is a well-known risk factor, predictive for rupture. The employment of Positron Emission Tomography (PET) has recently proven to be helpful in determining which AAA are at imminent risk of rupture. We applied our macroscopic sampling and histological examination protocol to 14 AAA (2 of which PET positive), in order to determine the correlations between metabolic activation of the aneurysmal wall and histomorphological findings. Our aim is to obtain a better understanding of the pathogenesis of AAA rupture and to provide a further contribute in their diagnostic evaluation. We applied our histochemical protocol (Hematoxylin-Eosin (HE), Alcian Blue (pH-1), PAS, Masson Thrichromic, and Weigert) on aortic aneurysm specimen and evaluated the proliferative index by immunohistochemistry (Ki-67/MIB-1) in order to detect any relationship between PET positivity, metabolic activity and histological patterns. Both PET positive and PET negative aortic specimen showed follicular lymphocytic aggregates, especially located within the adventitia. Nevertheless, we noticed a more elevated proliferative index (Ki-67) of the flogistic aggregates in our two groups (PET positive and PET negative). We believe that further IHC investigations with enzymes that are well-known implied in AAA pathogenesis (i.e. MMP-8 and MMP-9) should provide more accurate information in predicting the risk of rupture.

Introduction

The AAA evolutive history is characterized by a progressive expansion, slowly at first, and then, even after several years, a quicker expansion phase preluding towards its most dramatic complication: the wall rupture with haemorrage and hemodynamic shock. Moreover, the incidence of abdominal aortic aneurysms (AAAs) has increased in the last two decades, due in part to an ageing population, with increasing number of smokers and also improved detection. Ruptured AAAs cause 1.3% of all deaths among males between the ages of 65-85 years. One third of untreated AAAs will rupture, with an associated mortality rate of 65-85%, (half of these deaths occurring prior to arriving to surgery) [1, 3]. However, elective repair of these aneurysms has a reported mean 30-day mortality of 2-6% [4]. A lot of investigations have been applied on the attempt to clarify every pathogenetic aspect of its origin and evolution. Understanding these complex process could be crucial to identify the most important pathogenetic factors associated with higher risk of rupture. The most reliable risk factor for predicting rupture is the diameter of the aneurysmatic abdominal aorta (>5 cm) [5]. The conventional diagnostic procedures (Doppler-US and CT) manage to determine this parameter and to follow it through time (fig. 1). Even so, the rupture remains a quite unpredictable event. In some cases it occurs for aneurysmatic walls <5 cm in diameter. In other cases, the patients'health conditions advice against major surgery and, taking into account that the evidence of a dilation >5 cm is not necessarily associated with an imminent rupture, the choice could prove to be very difficult in such circumstances. A new important contribution in the diagnostic of AAA assessment was provided by the introduction of PET [6]. Positron Emission Tomography imaging applications are classically associated with important neurophysiological studies and, more recently, largely employed in oncological diagnosis. PET imaging was developed in the mid 1970s; like any nuclear medicine imaging technique, it is based on the detection of photons emitted by the patient after administration of a radiolabeled tracer. In routine clinical practice, the vast majority of PET studies are performed using 18F-fluoro-deoxy-glucose (18F-FDG), which reflects glucose metabolism.

We hope that the correlation between these three markers (PET positivity, histologic morphological analysis and enzymatic activity) could provide us a clear description and sequence of these events. Also we expect to add new evaluation elements to the study of AAA formation and progression that could prove to be helpful in deciding the most appropriate therapeutic choice (follow up or surgery).

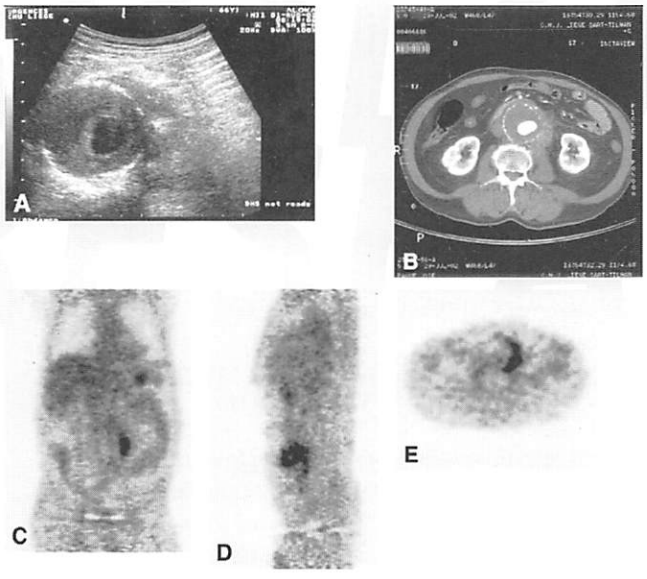

Fig. 1 - (A) Ultrasonography. (B) Abdominal CT scan. (CDE) Pet examinations in coronal, sagittal and transaxial sections. All images show AAA lesions

Materials and methods

In our Pathologic Anatomy Department we have studied aortic specimen for several years, mostly affected by aneurysmatic pathology, following an accurate and strict operative protocol (fig. 2).
Aortic specimens are fixed in formaldehyde (10% buffered) for a minimum of 18-24 hours; we measure length and calibre, wall thickness, appearance, consistence and intimal and adventitial colours. Each lesion is accurately described and documented photographically.

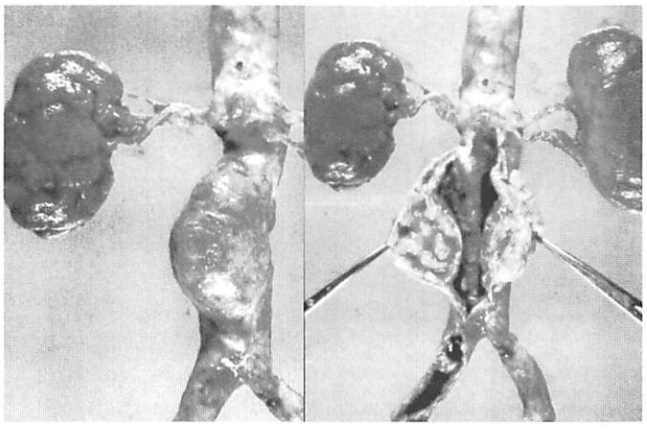

Fig. 2 - Macroscopic aspect of an AAA.

Then, the vessel fragments are serially sectioned and sampled. The material is, at this point, processed and embedded in paraffin. Finally it is cut in microsections and coloured with histochemical procedures. In this study we applied the following panel of colourations: Hematoxylin-Eosin (for a general morphological valuation) Alcian Blue at pH1 (for detecting acid mucopolysaccharides) PAS (neuter mucopolysaccharides) Masson Thrichromic (collagen) Weigert (elastic fibers) (fig.3). We also employed an immunohistochemical evaluation of MIB1 (Ki-67) in order to obtain a proliferation index assessment.

Results

We investigated the metabolic activity of the aneurysmal wall by whole-body positron emission tomography (PET) in 14 patients with a documented AAA: 2 were PET positive (group A) and 12 PET negative (group B). We selected, cut and treated different samples following our diagnostic protocol as previously described (fig. 4). We noticed a remarkable increase of inflammatory cells number (especially lymphocytes and plasmacells) in intima, media and vasa vasorum, with a neural hyperplastic reactivity (fig. 5). Such infiltration had an increased expression in PET positive's group.

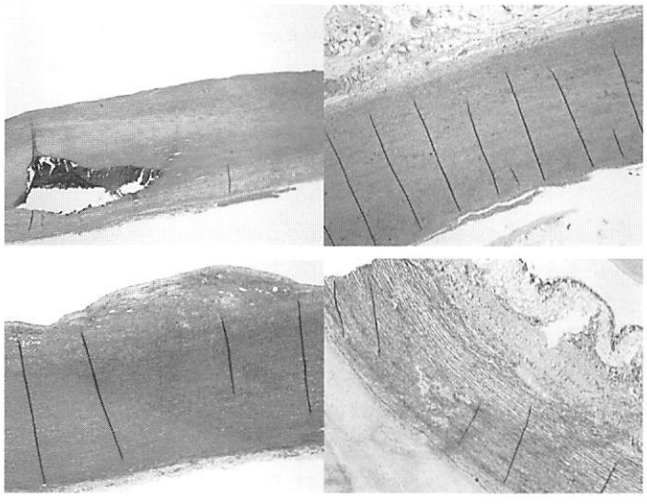

Fig. 3 - Microscopic aspect of an AAA. A) Alcian Blue at pH1. B) PAS. C) Masson Thrichomic. D) Weigert

To investigate deeper this observation, we also applied immunohistochemical evaluation of Ki-67, a protein expressed during all active phases of the cellular cycle. This marker demonstrates an active replication inside accumulations of inflammatory cells histologically detected previously.
All group A showed a marked and conspicuous positivity, while in group B (PET negative) six were Ki-67 positive and six were Ki-67 negative.

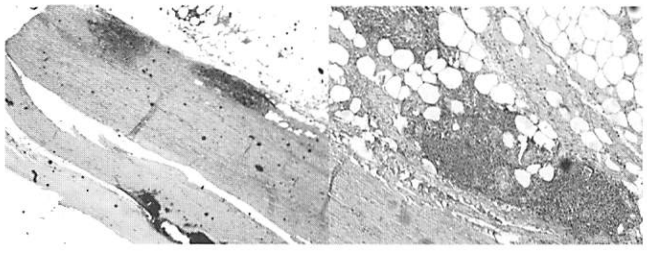

Fig. 4 - Histological features of the inflammatory atherosclerotic aneurysm

Discussion

The aorta dilation is the result of many pathogenetic mechanisms: different causes (atherosclerosis, hypertension, syphilis, vasculitis, traumatic events among others) weaken wall media (with a support function) in different tracts. A progressive wall leakage slowly produce expansion and greater weakness, according to Laplace's law. [7-11] We could investigating it, with diagnostic imaging, like an abdominal mass,

bigger compared to the normal aorta. This tissue appears frail so its evolution is erosion, fissuration and rupture. The process of expansion preceding fissuration and rupture seem to depend upon the release of matrix metalloproteinases (MMP), produced and/or activated by inflammatory cells, causing degradation of elastin and collagen in the aneurysmal walls [8]. Elastin degradation and collagen remodelling depend on the activity of a variety of enzymes, including some elastases, plasminogen activators and MMPs. A positive correlation between plasma MMPs 8 and 9 in this process has been reported. In our patients, using hematoxylineosin coloration, we observed a great accumulation of inflammatory cells in follicular aggregation, like lymph node germinative centre. It corresponds to a clinical entity named inflammatory atherosclerotic aneurysm. The inflammatory atherosclerotic aneurysm is a variant of the common atherosclerotic aneurysm. Although both inflammatory and atherosclerotic AAA most commonly affect the infrarenal portion of the abdominal aorta, patients with the inflammatory variant are younger, smokers and usually symptomatic, chiefly from back or abdominal pain. The preferential localization is abdominal aorta, but not the only one.

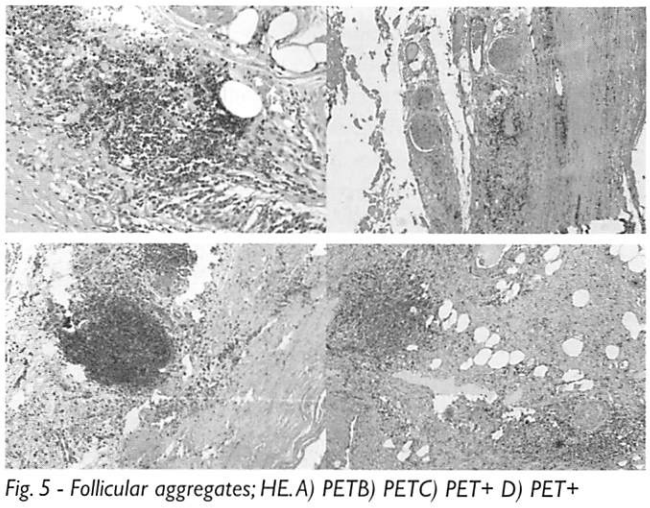

Fig. 5 - Follicular aggregates; HE. A) PETB) PETC) PET+ D) PET+

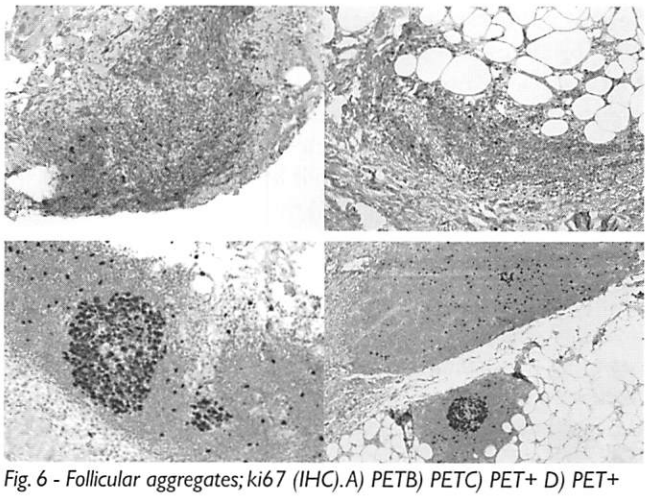

Fig. 6 - Follicular aggregates; ki67 (IHC). A) PETB) PETC) PET+ D) PET+

It's characterized by chronic periaortitis with a great number of lymphocytes and plasmacells; its evolution is a considerable fibrosis and adhesion with nearby structures. The consequences are:

- aortic wall thickening and its adhesion with other anatomical structures
- retroperitoneal fibrosis involving both ureters and causing obstructive uropathy.

Besides, after our microscopical observation and PET information, we believed to find a Ki-67 positivity only in group A (PET +) as marker of metabolic activity of the aneurysmal wall, but we found some positivity in PET too (in 6 cases of 12) (fig. 6).

In conclusion, we observed that accumulation of inflammatory cells and Ki-67 evaluation aren't accurate enough in singling out patients at immediate risk, even in presence of PET result, for we found them in both PET + and in PET-AAA.

Nonetheless, we noticed a different expression of Ki-67 infiltration pattern in two groups: in group A it was remarkable, higher quantitatively, while in group B it has a lower positiveness.

We think it could be useful to investigate the role of the potential collagenolytic MMPs as sensitive predictor of the risk for AAA rupture because it could help us to proceed with surgery or favouring a surveillance strategy.

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