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Telocytes in human isolated atrial amyloidosis: ultrastructural remodelling

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Abstract

The human heart can be frequently affected by an organ-limited amyloidosis called isolated atrial amyloidosis (IAA). IAA is a frequent histopathological finding in patients with long-standing atrial fibrillation (AF). The aim of this paper was to investigate the ultrastructure of cardiomyocytes and telocytes in patients with AF and IAA. Human atrial biopsies were obtained from 37 patients undergoing cardiac surgery, 23 having AF (62%). Small fragments were harvested from the left and right atrial appendages and from the atrial sleeves of pulmonary veins and processed for electron microscopy (EM). Additional fragments were paraffin embedded for Congo-red staining. The EM examination certified that 17 patients had IAA and 82% of them had AF. EM showed that amyloid deposits, composed of characteristic 10-nm-thick filaments were strictly extra-cellular. Although, under light microscope some amyloid deposits seemed to be located within the cardiomyocyte cytoplasm, EM showed that these deposits are actually located in interstitial recesses. Moreover, EM revealed that telopodes, the long and slender processes of telocytes, usually surround the amyloid deposits limiting their spreading into the interstitium. Our results come to endorse the presumptive association of AF and IAA, and show the exclusive, extracellular localization of amyloid fibrils. The particular connection of telopodes with amyloid deposits suggests their involvement in isolated atrial amyloidosis and AF pathogenesis.

Keywords: atrial fibrillation • isolated atrial amyloidosis • atrial natriuretic peptide • cardiomyocytes • telocytes • telopodes • interstitial Cajal-like cells

Introduction

Amyloidosis comprises a family of conditions characterized by the formation of specific protein deposits in tissues. Typically, each amyloid deposit has a single culprit protein that acquires an abnormal β-sheet configuration determining the formation of about 10-nm-thick, insoluble filaments. To date, some 27 different forms of amyloid [1] were described but its light or electron microscopic (EM) appearance is identical among forms [2]. In addition to these systemic forms [3], there are several localized variants of amyloidosis in the heart and the great vessels.

The heart can be affected by a strictly localized variant of amyloidosis called isolated atrial amyloidosis (IAA) [3]. The incidence of IAA increases with age, up to 90% of all patients in the ninth decade [4]. The fibril protein deposited in a certain percentage of cases is the α-atrial natriuretic peptide (ANP), a hormone synthesized and secreted predominantly by atrial cardiomyocytes [5].

Thus, IAA appears as a frequent histological finding in patients with long-standing atrial fibrillation (AF). Amyloid deposition is more frequent in the left atrial appendage and seems to correlate with AF duration and the female status. These facts endorse the hypothesis that amyloidosis has a pivotal role in the atrial remodelling which characterizes long-standing AF and underline the multi-factorial origin of the so-called 'atrial myopathy' of AF [6].

A study carried out on autopsy heart samples from 100 elderly patients showed that left atrial deposits are more frequent than right atrial deposits, and the distribution of IAA in the left atrium was more pronounced in the anterior wall than in both the posterior wall and the left appendage [7]. The same study reported that patients with chronic AF have heavier IAA deposits than those with sinus rhythm.

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Once the amyloid is deposited, it becomes a permanent structural alteration of atrial cardiomyocytes. ANP fibrils seem to induce apoptosis whereas amyloid deposits in the heart disturb myocyte contractility and conduction [4]. Although IAA is far more common than AL (light chains) amyloidosis or senile cardiovascular amyloidosis [4], little is known about its fine ultrastructural features, organ distribution and alleged role in the pathogenesis of cardiac arrhythmias.

Telocytes [8] are a distinct type of stromal cells described in the cardiac interstitium [9–15]. They are characterized by telopodes, very long (tens of μm) and very thin (usually less than 0.5 μm) cell processes [8]. These cells previously have been identified and described as 'interstitial Cajal-like cells' [16–20] and recently termed 'telocytes' because of their long, slender processes (telopodes) embracing the myocardial cells [8].

The aim of this paper was to investigate the ultrastructural features of cardiomyocytes and interstitial cells, in particular the telocytes, in patients with AF and IAA.

Material and methods

Patients and clinical data

Human cardiac biopsy tissue was obtained from 37 patients undergoing coronary artery bypass grafting or valvular surgery. Tissue samples were collected from 17 patients from 'C.C. Iliescu' Institute for Cardiovascular Diseases, Bucharest, Romania, and 20 samples were cardiac biopsies collected from the Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany. The study was performed with the approval of Ethics Committee of 'Victor Babeş' Institute of Pathology. Tissue samples were collected from patients who had given informed consent before surgery.

From these 37 patients, 11 were women and 26 men and 23 of them had AF. The age of the patients: 5 were between 40 and 50 year old, 11 between 51 and 60, 20 between 61 and 70 and only 1 over 70 years. None of the patients had been formerly diagnosed with systemic amyloidosis. The basic cardiac condition of these 37 patients was the following: 16 had coronary diseases, 14 suffered of valvular heart diseases and 7 had both, coronary and valvular diseases.

Sampling

Atrial biopsies collected from all patients enrolled in this study were processed for EM. For 10 patients, additional tissue samples were fixed in formaldehyde and embedded in paraffin. For 24 patients, the surgical units provided tissue samples only from the left atrial appendages. For the other 13 patients, three different cardiac samples were harvested: left and right atrial appendages, and from the atrial sleeves of the pulmonary vein.

Light microscopy (LM)

Atrial samples taken from 10 patients were fixed in 7% buffered formaldehyde and paraffin embedded. Serial sections at 3–5 μm were stained with haematoxylin and eosin, van Gieson and Congo-red.

LM was also performed on semithin sections (~1 μm thick) of Epon embedded tissue and stained with 1% toluidine blue. Digital images were recorded using a CCD Axiocam HRc Zeiss camera with AxioVision software (Carl Zeiss Imaging solution GmbH, Germany), on a Nikon Eclipse E600 microscope (Nikon Instruments Inc., Japan).

Electron microscopy

Small fragments of myocardium with epicardium were processed for transmission EM according to routine procedures, as previously described [18]. Ultrathin sections of about 70 nm were double stained with uranyl acetate and lead citrate, and examined using a Morgagni 286 transmission electron microscope (FEI Company, the Netherlands) at 60 kV. Digital electron micrographs were recorded with a MegaView III CCD performed with iTEM-SIS software (Olympus, Soft Imaging System GmbH, Germany).

Results

LM with or without polarizing filters failed to identify amyloid deposits on Congo-red stained paraffin sections. The 1-μm-thick Epon sections showed some more details: the interstitial area was slightly enlarged, the atrial cardiomyocytes profiles were smaller, with irregular contours and a dispersed appearance (Fig. 1A). In a few cases, we found cardiomyocytes with stellate profiles due to small interstitial recesses (Fig. 1B).

EM examination of cellular organelles revealed that the myofibrils were more or less centrally gathered, with the mitochondria and the glycogen peripherally placed (Fig. 1C and D). The first ultrastructural examination showed several remodelling features of the working cardiomyocytes. The routine EM examination revealed the amyloid deposits in 5 out of the 37 patients (13.5%). This unexpected finding determined us to perform a second examination of all the biopsies, with special attention for amyloid deposits. As concerning the patients from which three samples were collected, none exhibited amyloid deposits in all three fragments; usually only one, rarely two samples showed such deposits. Thus, we decided to re-analyse all the biopsies available.

The second examination revealed a higher number of cases with deposits of amyloid filaments. Thus, among the 24 patients with only one atrial sample, we found eight patients displaying amyloid deposition (33.3%), whereas in the second group (13 patients with three atrial samples) nine were positive (69.2%). The left atrial appendage was positive in all patients, whereas the right appendage in only three cases. Concerning other areas, such as atrial left and right walls and pulmonary vein sleeves we found positive only one sample each. The highest frequency of amyloid fibrils was found in the left appendage (100%).

Our EM investigation showed that from the total of 37 patients, 17 developed IAA (46%) (Fig. 2A) and 14 of them had AF and IAA; 60.89% of the patients with AF presented IAA, and 82.35% of those with IAA had AF also (Fig. 2B). On the other hand, 23 out of 37 patients were previously diagnosed with AF (62%).

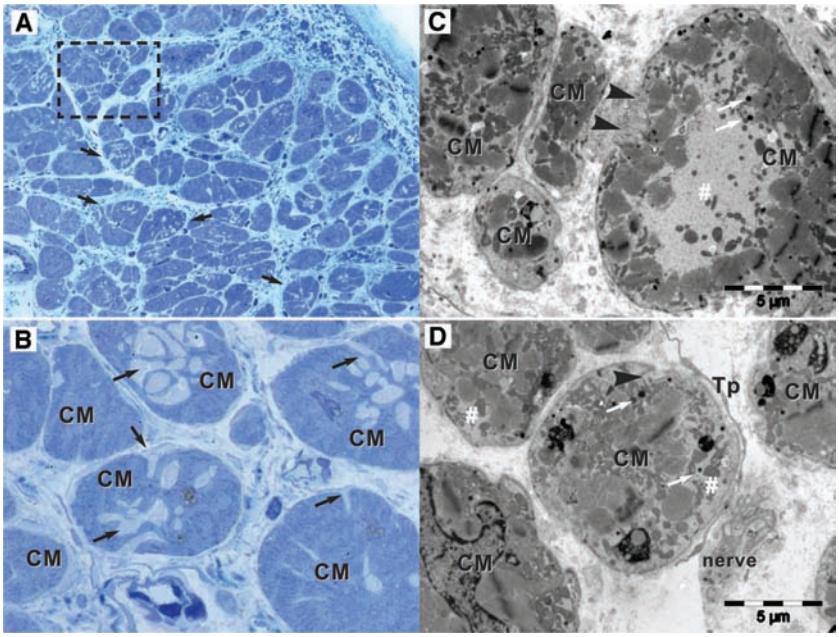

Fig. 1 (**A** and **B**) Light microscopy on toluidine blue stained semithin sections. (**A**) Cross-sectioned atrial cardiomyocytes showing smaller profiles and irregular contours. The interstitial area is enlarged, clearly isolating the cardiomyocytes. The cardiomyocytes show small interstitial recesses (arrows). Ob. 20×. (**B**) Higher magnification of the area enclosed by rectangle in Figure 1A. Several atrial cardiomyocytes (my) with stellate profiles show many small interstitial recesses (invaginations) marked by arrows. Ob. 100×. (**C** and **D**) Electron microscopy details of rectangular marked area in Figure 1A. Atrial cardiomyocytes (CM) showing cellular oedema (**C**) or centrally gathered myofibrils and peripherally placed mitochondria (#), glycogen granules and lipofuscin (**D**). Both images show small recesses (arrowheads) in the periphery of cardiomyocytes. Atrial natriuretic peptide (ANP) granules (white arrows). A telopode (Tp) surrounding a cardiomyocyte is close to a nerve fibre.

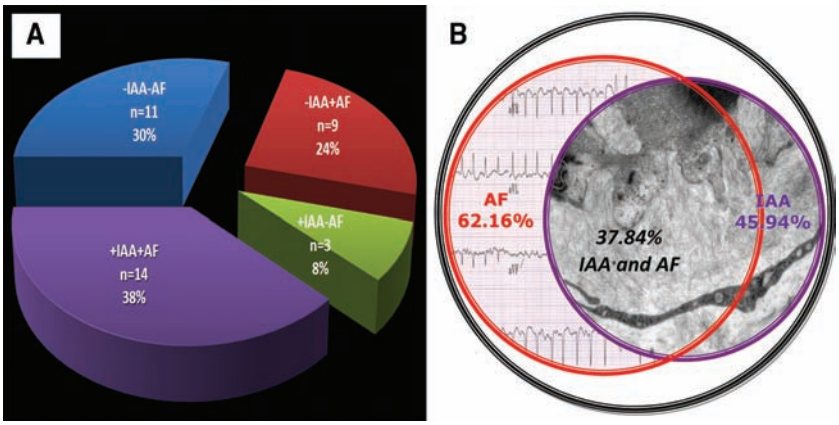

Fig. 2 (**A**) Chart shows the relationship of isolated atrial amyloidosis (IAA) with atrial fibillation (AF). Total number of patients: 37. *n*, number of patients. −IAA-AF: patients without IAA or AF. −IAA+AF: patients with AF and IAA negative. +IAA-AF: patients with IAA and no AF. +IAA+AF: patients having both IAA and AF. (**B**) Schematic figure highlighting the association IAA and AF. From a total of 37 patients (black circle), 17 developed IAA (45.94%-lilac circle) and 23 suffered of AF (62.16%-red circle). Fourteen patients had both IAA and AF (37.84% from all investigated cases).

The amyloid fibrillar deposits were located in the interstitial areas, in close contact with either atrial cardiomyocytes or small blood vessels in some cases. EM showed that all these deposits had the same characteristic feature: about 8–12 nm thick, randomly arranged, non-branching filaments (Figs 3–12). The amyloid fibrils were in close contact with the peripheral lamina of atrial working cardiomyocytes (Fig. 3). They occurred exclusively in the extracellular space. The fibrils were concentrated in small sarcolemmal recesses (Fig. 4). When these invaginations were deeper, they could have been transversely sectioned, giving the false impression of intracellular inclusions (Fig. 5). These invaginations were sometimes placed near the intercellular junctions (Fig. 6). Inside these pockets, the amyloid fibrils displayed a random distribution. As a rule, all these invaginations, either in longitudinal or in cross section, were always bordered by the myocyte plasma membrane associated with caveolae and external lamina (Fig. 7). On the interstitial side, the peri-myocyte fibrillar deposits seemed to have a special association with the slender processes of telocytes, the telopodes, which have the tendency to wrap the fibrils (Fig. 8). This kind of fibril wrapping was either between telopodes and cardiomyocytes, or vessels (Fig. 9), or by a telopode only (Fig. 10). When the telopodes surrounded a mass of fibrils, the amyloid filaments were gathered as small bunches (Fig. 11). Some telopodes involved in this process displayed a honeycomb aspect with bunches of fibrils in their small meshes (Fig. 12).

Discussion

IAA is a common post-mortem finding in the elderly patients, and has been reported to occur in as many as 90% in those over

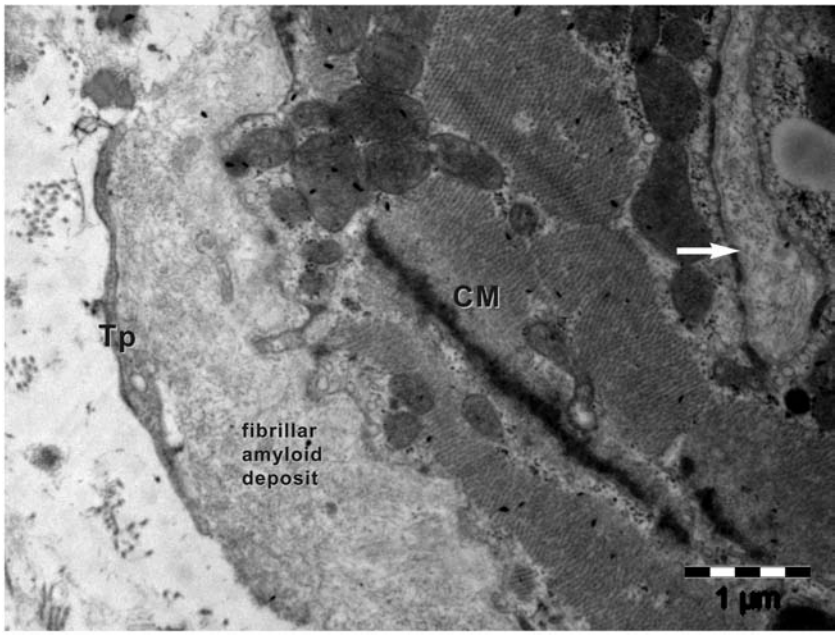

Fig. 3 Electron microscopy image showing interstitial amyloid deposit in between an atrial cardiomyocyte (CM) and a telopode (Tp). Note the cardiomyocyte recess filled with amyloid fibrils (white arrow).

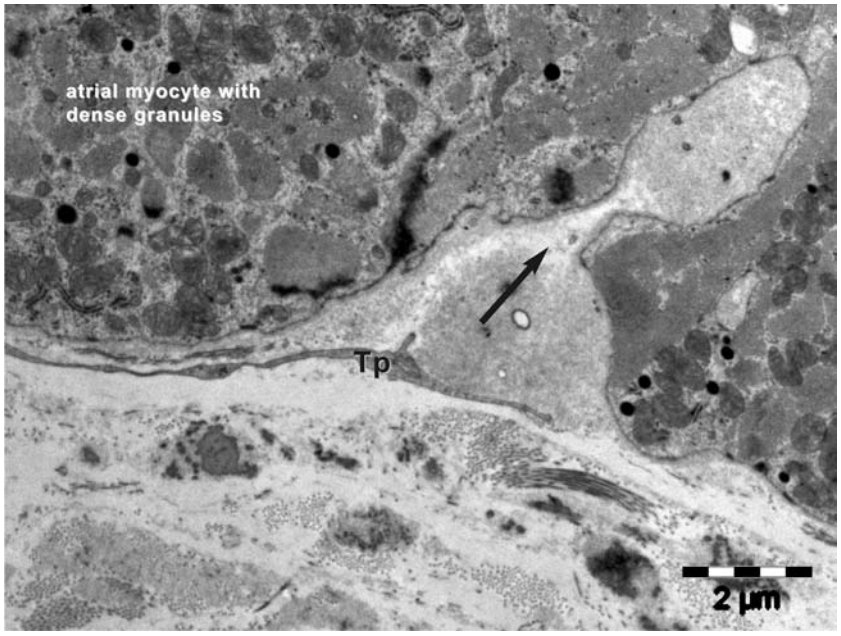

Fig. 4 Electron microscopy showing sarcolemmal small recess of an atrial cardiomyocyte loaded with amyloid fibrils (arrow). A telopode (Tp) borders the interstitial face of amyloid deposit.

90 year of age [2, 7, 21]. IAA first appears in the third decade and its prevalence increases linearly by 15–20% with each subsequent decade [22, 23]. IAA was found to be more common in the chronic rheumatic heart and in mitral valve diseases [4].

None of our patients was known to have amyloidosis prior to this investigation. All of them but one were under 70 year old; the majority (53%) being in the 7th decade of life [23]. Left atrial deposits are more widespread than those in the right atrium. These deposits are usually found in appendages [21], although other studies mention the highest frequency in the left anterior wall [7, 2]. In our series the results showed a prevalence of amyloid deposits in the left appendage (73%) and equally distributed in the atrial walls, right appendage and pulmonary vein sleeves.

Contrary to some author's opinion [21], our results clearly demonstrated the extracellular location of amyloid deposits, even for those appearing as intracellular in LM. The deposits were

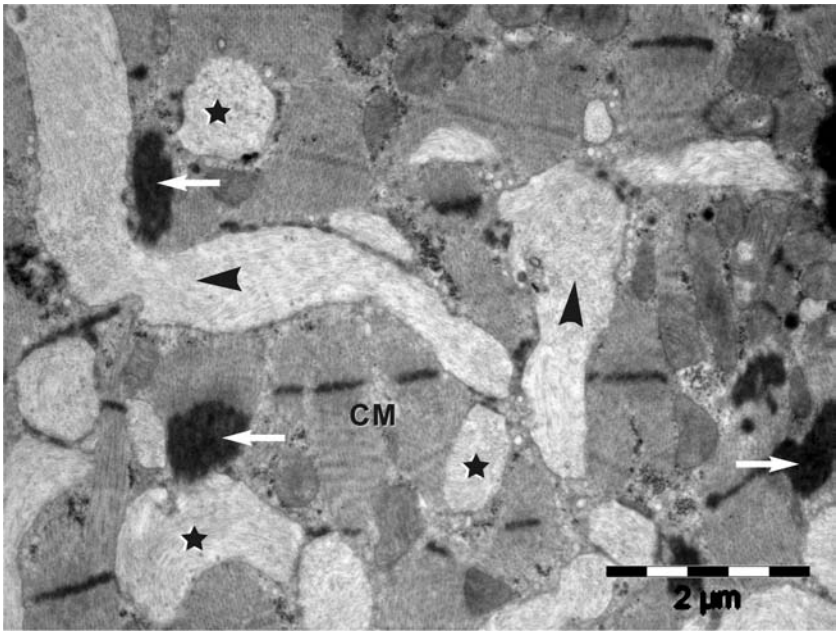

Fig. 5 Electron micrograph showing longitudinally (black arrowheads) and transversally (stars) sectioned deep cardiomyocytic invaginations. Z line streaming (white arrows) next to the plasma membrane of a cardiomyocyte (CM).

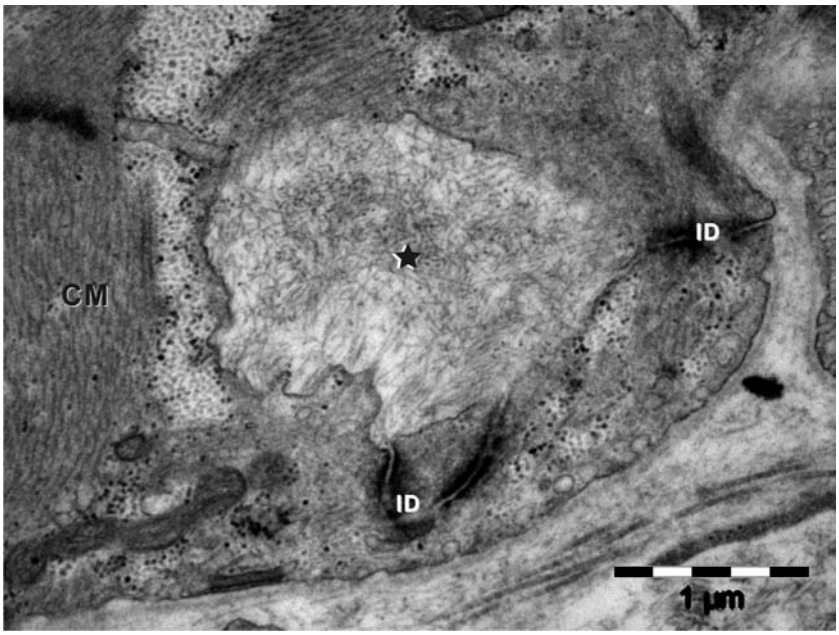

Fig. 6 Electron micrograph showing randomly arranged, unbranched amyloid fibrils (star) loaded in a recess of the cardiomyocyte (CM) close to an intercalated disc (ID).

placed in cellular invaginations, always surrounded by sarcolemma of atrial cardiomyocytes. This feature, of seemingly intracellular amyloid filaments, was carefully investigated, because this confusion is frequently mentioned for amyloidosis [21, 25, 26]. The extracellular location of IAA can be accurately demonstrated only by EM. Thus, the circuit of the amyloid material seems to be from these invaginations towards the interstitial area, because the atrial cardiomyocytes are clearly involved in the synthesis of amyloidogenic peptide [2, 27]. Thus, it is reasonable to assume that these intra-myocytic invaginations start on the cell periphery and progress towards its central area as the aggregation process advances.

As already known, the distribution of amyloid deposits is uneven in the atrial cardiomyocytes [21]. For that reason, several samples were harvested from different areas for increasing the chances of an accurate diagnosis. In our series, the percentage of IAA increased from 13.5% in the single sample patients, to 69.2% in those with triple samples. It is obvious that this

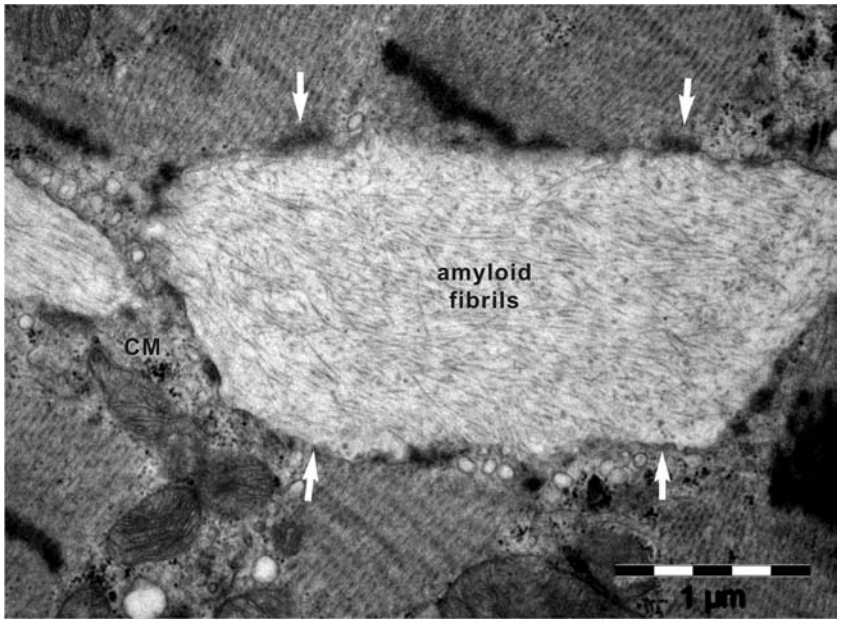

Fig. 7 Cardiomyocyte (CM) recess with unbranched amyloid fibrils (8–12 nm diameter) surrounded by plasma membrane (arrows).

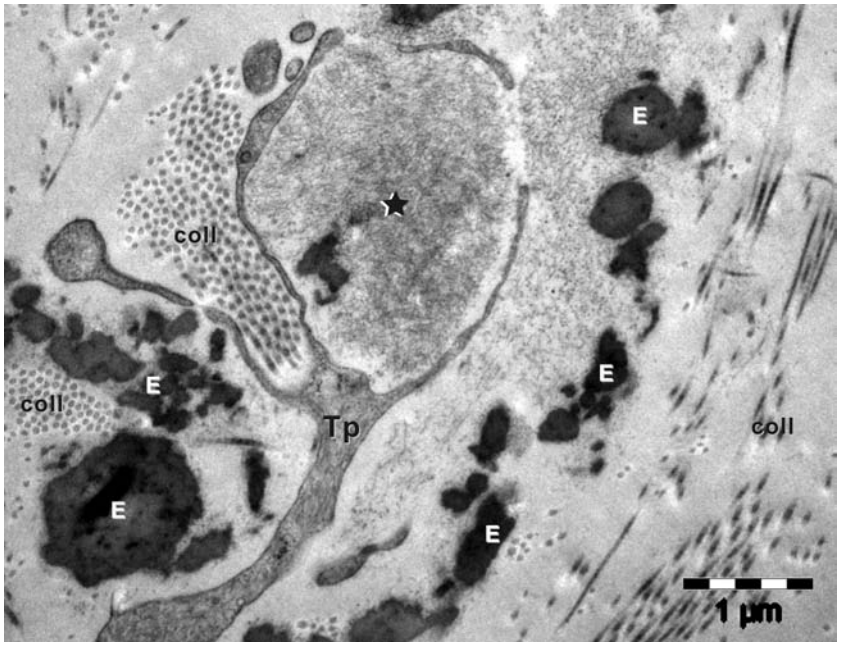

Fig. 8 Electron microscopy of atrial interstitial area. A telopode (Tp) surrounds a bunch of amyloid fibrils (star). Coll, collagen fibres; E, elastin.

percentage would increase even further if more samples should be studied.

Not even one of our amyloid positive patients was diagnosed on Congo red stained sections. It is widely recognized that Congo red is not the proper method for IAA diagnosis, Sirius Red giving better results [7]. We consider that EM is the most sensitive method currently available for the identification of this type of amyloid deposits. It is advisable to proceed with this technique (EM) after an LM evaluation of thick sections, looking for cross-sectioned cardiomyocytes with irregular contours and surrounded by enlarged interstitial areas.

During the last few years, researchers provided evidence that longstanding AF associates with IAA [4, 6, 28]. Our results are in agreement with the possible association of IAA and the AF [29]. In our patients group 62.16% suffered of AF, and 45.94% developed IAA. Thus, the 14 patients having both IAA and AF represent 60.89% of the AF cases, and 82.35% of those with IAA. Keeping in mind the difficulty of finding the amyloid deposits, we consider these figures

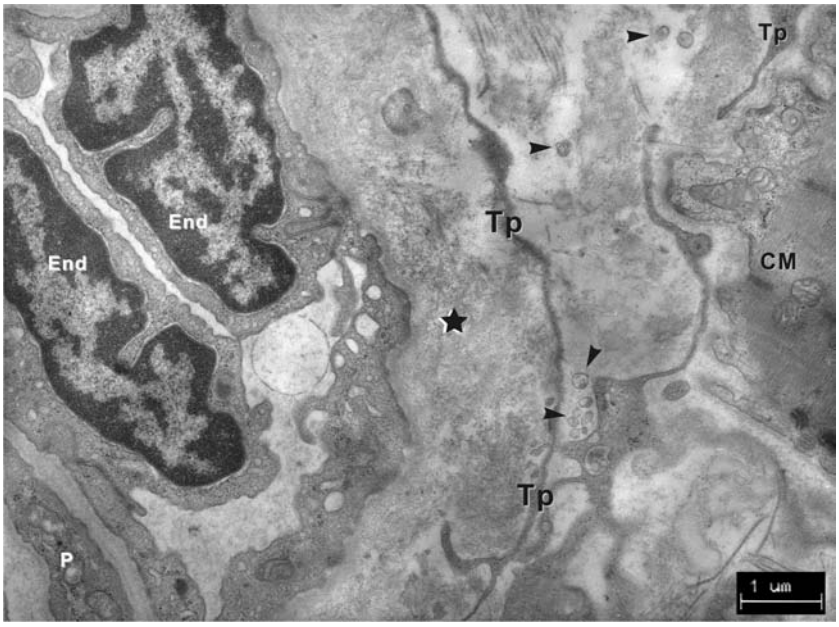

Fig. 9 Telopodes (Tp) surrounding an amyloid deposit (stars) in the periphery of a blood vessel. Small shed vesicles (arrowheads) can be seen in the vicinity of telopodes. CM, cardiomyocyte; End, endothelium; P, pericyte.

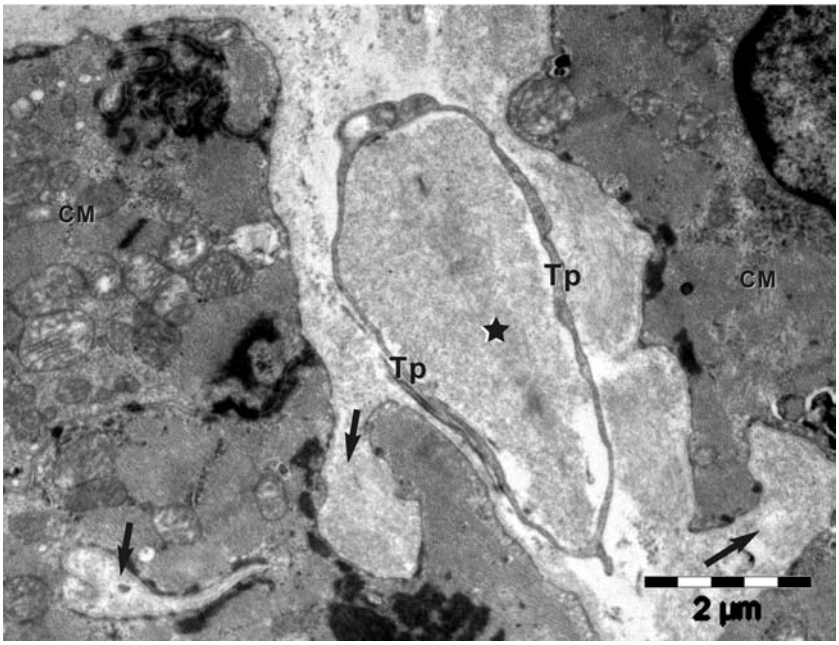

Fig. 10 Electron microscopy image showing how interstitial amyloid fibrils (star) are wrapped by a telopode (Tp). Cardiomyocytes (CM) recesses (arrows) containing amyloid fibrils close to intercalated disks.

quite significant. Thus, IAA could be considered as a further negative consequence of the neurohormonal disturbances [30].

An important finding of our study is the special ultrastructural relationship between cardiac telocytes [8–20] and amyloid deposits. The long and slender processes of telocytes, the telopodes, develop a wrapping activity gathering masses of amyloid fibrils, partially or totally surrounding them. These cellular 'bags' made by telopodes have sometimes inner cytoplasmic processes with honeycomb-like appearance which fragments in bunches the amyloid fibrils. Therefore, we consider that telocytes may play an important role in amyloid deposits formation. Telopodes usually surround the amyloid deposits, suggesting that telocytes are involved not only in filaments gathering but also play an important role in amyloidogenesis itself.

No matter if IAA is the cause or the consequence of AF [30], any future therapeutic strategies for AF must take into consideration a possible IAA coexistence and the telocytes involvement in their pathogenesis.

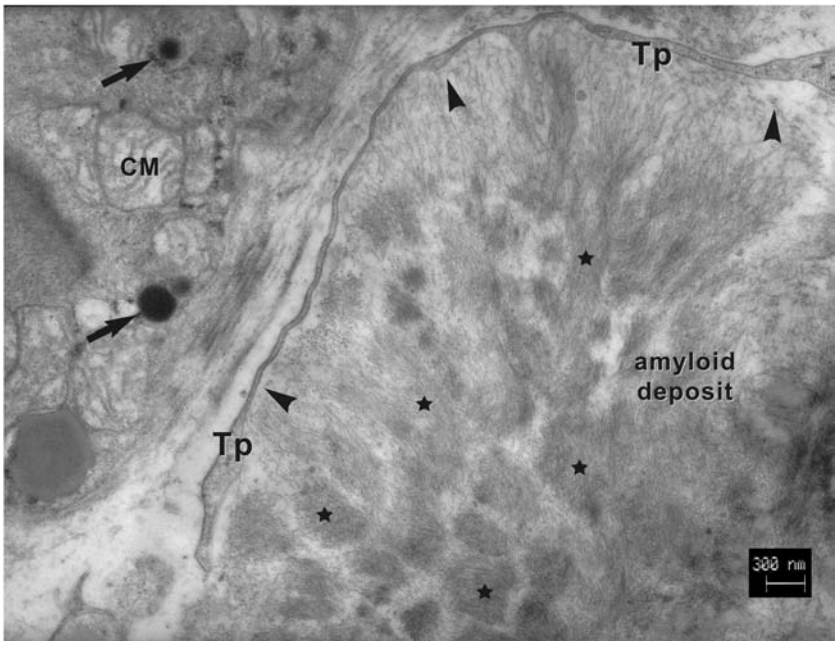

Fig. 11 Telopode (Tp) surrounding (arrowheads) an amyloid deposit fragmented in small bunches (stars). The atrial myocyte (CM) contains atrial natriuretic peptide granule (arrows) next to the plasma membrane.

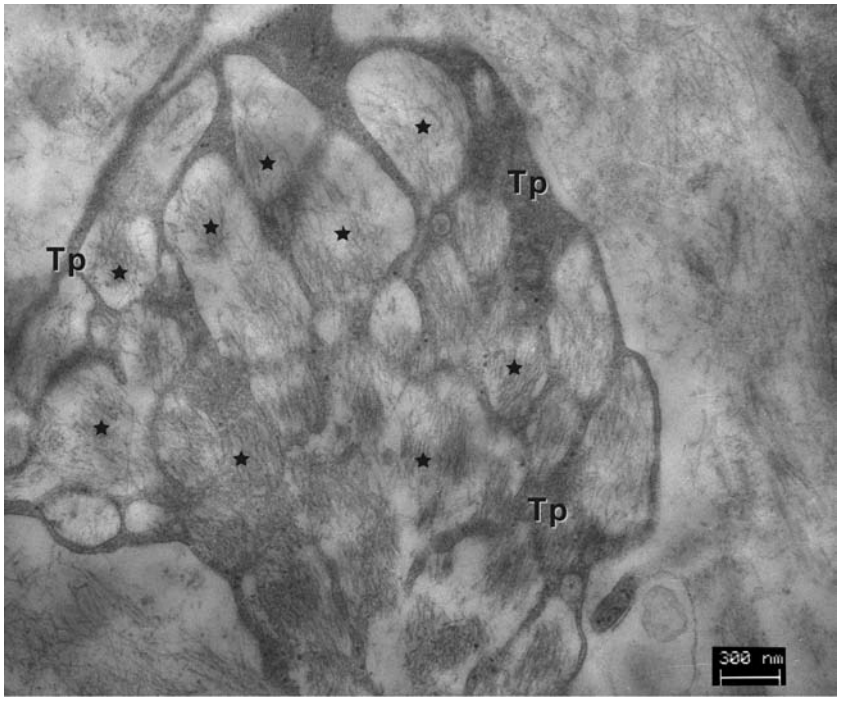

Fig. 12 Electron micrograph showing the honeycomb-like structure of a telopode (Tp) containing small bunches of amyloid filaments (stars).

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