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Cardiovascular Pathology 18 (2009) 205–216

CARDIOVASCULAR PATHOLOGY

Original Article

Classification of amyloid deposits in diagnostic cardiac specimens by immunofluorescence

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Received 24 January 2008; received in revised form 25 April 2008; accepted 29 May 2008

Abstract

Background: At least 12 distinct forms of amyloidosis are known to involve the heart or great vessels. Patient treatment regimens require proper subtyping of amyloid deposits in small diagnostic cardiac specimens. A growing lack of confidence in immunohistochemical staining for subtyping amyloid has arisen primarily as a result of studies utilizing immunoperoxidase staining of formalin-fixed paraffin-embedded tissue. Immunofluorescence staining on fresh frozen tissue is generally considered superior to immunoperoxidase staining for subtyping amyloid; however, this technique has not previously been reported in a series of cardiac specimens. **Methods:** Amyloid deposits were subtyped in 17 cardiac specimens and 23 renal specimens using an immunofluorescence panel. **Results:** Amyloid deposits were successfully subtyped as AL, AH, or AA amyloid by immunofluorescence in 82% of cardiac specimens and 87% of renal specimens. In all cases, the amyloid classification was in good agreement with available clinical and laboratory assessments. A cross-study analysis of 163 cases of AL amyloidosis reveals probable systemic misdiagnosis of cardiac AL amyloidosis by the immunoperoxidase technique, but not by the immunofluorescence technique. **Conclusions:** Amyloid deposits can be reliably subtyped in small diagnostic cardiac specimens using immunofluorescence. The practical aspects of implementing an immunofluorescence approach are compared with those of other approaches for subtyping amyloid in the clinical setting.

Keywords: Amyloid; Amyloidosis; AH Amyloid; AL Amyloid; AA Amyloid; Immunofluorescence; Misdiagnosis; Cardiac; Heart

1. Introduction

1.1. Types of amyloid involving the heart

The amyloidoses comprise a family of diseases characterized by the formation of a specific type of protein deposit in tissues [1]. In these amyloid deposits, there is typically a single culprit protein that adopts an abnormal extended β-sheet conformation, which facilitates the formation of large insoluble fibrils. Amyloid deposits are classified based on the specific protein forming these amyloid fibrils. Currently, there are at least 27 distinct forms of amyloid, which differ in their distribution (systemic vs. localized), mode of acquisition (hereditary vs. acquired), and clinical relevance [2]. It is the extended β-sheet structure of amyloid fibrils that allows Congo red dye to bind the deposits in an orderly fashion so as to display green birefringence upon the application of plane-polarized light. In addition to the specific culprit protein, most if not all amyloid deposits contain nonspecific proteins including serum amyloid P, apolipoprotein E, and heparan sulfate proteoglycans [1–4]. The proteoglycans in amyloid deposits allow these deposits to stain with sulfated alcian blue. Serum amyloid P is often exploited as a general immunohistochemical marker for all amyloid deposits in tissue and as a radio-labeled nuclear medicine probe to assess for whole-body amyloid involvement [5].

Of the 27 currently known types of amyloid, 12 types have been reported to involve the heart or great vessels (Table 1). In the setting of a plasma cell dyscrasia or other immunoglobulin-producing lymphoproliferative disorders,

This work was supported by the Department of Pathology, Massachusetts General Hospital.

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1054-8807/08/$ – see front matter
doi:10.1016/j.carpath.2008.05.004

Table 1
Types of amyloid that involve the heart and great vessels

Amyloid protein	Precursor protein	Systemic vs. localized	Associations or tissue localization	Reference
AL	Immunoglobulin light chain	Systemic	Plasma cell dyscrasia	[6–8]
AH	Immunoglobulin heavy chain	Systemic	Plasma cell dyscrasia	[9]
ATTR	Transthyretin	Systemic	Senile systemic	[14,15]
			Hereditary	[16–18]
AA	Serum amyloid A	Systemic	Chronic inflammation	[21]
$A\beta_2M$	β_2-Microglobulin	Systemic	Hemodialysis	[22]
AApoAI	Apolipoprotein A-I	Systemic	Hereditary	[23]
		Localized	Atherosclerotic plaques	[33]
AApoAII	Apolipoprotein A-II	Systemic	Hereditary	[24]
AApoAIV	Apolipoprotein A-IV	Systemic	Senile systemic	[19]
AGel	Gelsolin	Systemic	Hereditary	[25]
Abri	ABriPP	Systemic	Hereditary	[26]
AANF	Atrial natriuretic factor	Localized	Atria	[27–30]
AMed	Lactadherin	Localized	Arterial media	[31,32]
–	Unknown	Localized	Cardiac valves	[34]
			Mural thrombi	[35]

cardiac amyloid may form as a result of the overproduction of a monoclonal amyloidogenic immunoglobulin [6–8]. Immunoglobulin-related amyloid is most often due to the deposition of the immunoglobulin light chain, AL amyloid, but occasionally results from the deposition of the immunoglobulin heavy chain, AH amyloid [9–12]. Transthyretin may form amyloid deposits in the heart either in patients with a normal wild-type gene sequence as a manifestation of senile systemic amyloidosis [13–15], or as part of a hereditary disease in patients carrying an amyloidogenic mutation in one of their transthyretin genes [16,17]. Over 80 such transthyretin mutations are currently known, some of which cause a late-onset clinical presentation, with patients presenting with cardiac amyloidosis in the seventh decade of life or later [18]. Senile systemic amyloidosis involving the heart may also result from the deposition of apolipoprotein A-IV [19]. Systemic amyloidosis due to serum amyloid A occurs in the setting of chronic inflammatory conditions. Such AA amyloidosis typically manifests with more involvement of the kidneys, liver, and spleen than the heart, but the heart may be involved in severe cases [20,21]. Chronic hemodialysis is associated with the systemic deposition of β2-microglobulin containing amyloid, which may involve the heart [22]. In addition to transthyretin, mutations of a growing list of other genes are now known to result in systemic familial amyloidosis that involves the heart, specifically apolipoprotein A-I, apolipoprotein A-II, gelsolin, and Abri precursor protein [23–26].

In addition to these systemic amyloidoses, several localized forms of amyloid are encountered in the heart and great vessels. Atrial amyloid is a form of amyloid due to atrial natriuretic peptide, which is present in a majority of older adults, but its distribution is limited to the cardiac atria [27,28]. It has been suggested that the deposition of atrial amyloid may be related to the presence and/or severity of atrial fibrillation, but this association is not certain [29,30]. Small amyloid deposits derived from a fragment of lactadherin are frequently observed in the media of the aorta and other large- and medium-sized arteries [31,32]. This arterial medial amyloid is associated with aging, but otherwise its clinical significance is unclear. Amyloid due to deposition of wild-type apolipoprotein A-I has been observed in atherosclerotic plaques [33]. Similar "dystrophic" amyloid has been observed in a high proportion of cardiac valves resected for calcific stenosis or rheumatic disease as well as in mural thrombi [34,35]. In these latter two cases, the nature of the amyloid is unclear; however, awareness of these localized forms of cardiovascular amyloid is necessary to avoid inappropriately attributing such amyloid to a clinically significant systemic process.

1.2. Necessity for the classification of amyloid in cardiac surgical specimens

Each case of cardiac amyloidosis is in effect any one of at least 12 distinct diseases, each with its distinct prognosis and clinical management. In the clinical management and treatment of patients with cardiac amyloidosis, a critical step is to identify the type of amyloid present in the tissue [36–39]. Thus, successful and proper classification of the amyloid deposits in diagnostic cardiovascular specimens has an important impact on patient care. Much of the recent outcry for proper classification of amyloid deposits is derived from the current treatment for AL amyloidosis [40–43]. Not uncommonly, this disorder is now treated with melphalan-based chemotherapy with autologous peripheral stem cell rescue. This treatment may be instituted in the setting of a normal bone marrow biopsy based primarily on the classification of the amyloid in the cardiac biopsy. The treatment-related mortality for this approach may reach as high as 10–25% [37]; thus misclassification of senile amyloid or hereditary amyloid as AL amyloid must be avoided. In addition, in some medical centers, chemotherapy and stem cell rescue for AL amyloidosis are preceded by cardiac transplantation, again emphasizing the necessity for accurate subtyping of the amyloid [44–46].