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Original contribution

An evaluation of Congo red fluorescence for the diagnosis of amyloidosis☆,☆☆

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Summary Congo red stain apple-green birefringence under polarized light is the most popular method for detecting amyloid; however, it has limitations. The goal of this study was to evaluate if examination of Congo red stain by fluorescent microscopy (FM) significantly enhances the diagnostic yield. Congo red–stained tissue sections were retrospectively and prospectively examined by light microscopy (LM) with and without polarizer and by FM using the Texas red filter and results by each method compared. Congo red–stained amyloid recognized by LM was unequivocally and easily identified by FM in each of 48 cases. In 22 of them, FM either confirmed the presence of a small amount of amyloid or lead to a definitive diagnosis, which was otherwise missed. Eight cases with Congo red–negative by LM were also negative by FM. In 8 cases with a false-positive Congo red stain, FM still detected the signal in 5, but it was absent in 3 cases. In conclusion, Congo red fluorescence improves the diagnostic yield of LM for both positive and negative cases.

1. Introduction

Amyloidosis comprises a spectrum of diseases characterized by the deposition of amyloid in a variety of organs. These deposits can be local or generalized, the latter causing a progressive disease with poor prognoses.

The diagnosis of amyloidosis relies on identification of amyloid deposits on tissue sections. Traditionally, material positive by Congo red stain, which also shows apple-green birefringence under polarized light, is considered the criterion standard for diagnosing amyloid. Indeed, the presence of apple-green birefringence is even mandatory for this diagnosis in some studies [1-4]. However, this method has its limitations. It has been shown to be unreliable in some cases, in particular, with a minute amount of amyloid [2,5,6]. Moreover, the Congo red dye bound to amyloid needs tissue sections to be of a required thickness (>8 μm) to show birefringence [7]. Therefore, if birefringence is solely relied on for the diagnosis of amyloidosis, some cases would be missed. More than 4 decades ago, Klatskin [8] questioned the specificity of the Congo red polarization method; under appropriate processing conditions, he found foci of green birefringence in connective tissues of liver and several normal organs both in man and in the rat. And even the "apple-green birefringence in polarized light," words traditionally use to describe the properties of Congo red–stained amyloid, has been questioned more recently by Howie et al [9], who observed that more than one color may be seen, and in some cases, green is not even present.

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To increase the detection of amyloid, alternative methods have been used, including immunohistochemical staining using antibodies against variable amyloid components or examination of Congo red–stained tissue sections by fluorescent microscopy (FM). Although combined Congo red stain and immunohistochemistry has been reported to be more sensitive than Congo red stain alone [10], limitations of the immunohistochemical method may include availability of antibodies and the possibility of negative results in cases with very small amounts of amyloid [6]. There is growing evidence supporting the high sensitivity of Congo red fluorescence to detect amyloid [1,5,6,11,12]. The goal of this study was to evaluate the possibility that examination of Congo red–stained tissue sections by FM significantly enhances the diagnostic yield in histologic sections from a variety of tissue types.

2. Materials and methods

Sixty-four specimens were studied in both retrospective and prospective manner. This study was reviewed and approved by the Institutional Review Board of The Houston Methodist Hospital, Research Institute, Houston, TX. These cases were categorized into 3 groups.

Group 1 included 26 cases with abundant amyloid deposition, in which the identification of amyloid was straightforward, even by routine hematoxylin and eosin (H&E) stain; this group included 6 heart specimens (3 explants and 3 biopsies); 5 liver specimens (4 biopsies and 1 explant); 3 kidney biopsies; 3 corneal excisions; 2 small bowel biopsies; 2 pancreatectomy specimens; and 5 excisional biopsies of thyroid, urinary bladder, lymph node, vocal cord, and lung (one of each).

Group 2 consisted of 22 cases in which amyloid deposition was scant or minuscule; in some of these cases, the detection of amyloid was initially problematic but finally confirmed. These cases included 9 gastrointestinal tract (esophagus, stomach, duodenum, and colorectal) biopsies; 4 liver specimens (3 biopsies and 1 excision); 3 heart biopsies; 3 fine needle biopsies of abdominal fat pad; and biopsies (one of each) of kidney, tongue, and liver. In each case of this group, tissue was submitted with the clinical suspicion for amyloidosis. Tissue was stained with Congo red stain for each of them. In several cases, amyloid was present in small amount but was still easily recognizable by light microscopy (LM). In other cases, the amyloid was so scanty that it was initially missed by LM (examined by the attending pathologists). These cases were then consulted with the authors, who examined by the same tissue sections by FM. This demonstrated illumination typical for amyloidosis. Examination by LM again focusing on the areas of illumination now convincingly showed amyloid, albeit in very scant amount. The diagnosis of amyloid in each of these cases was also supported by the presence of amyloid in other organs and/or laboratory finding including the presence of circulating monoclonal light chains.

Group 3 included 16 cases with tissue types that may simulate amyloidosis in routine H&E stain, some of which are also well known for a false-positive Congo red staining. They included elastic tissue, poorly cellular hyalinized fibrous tissue, colloid of thyroid gland, aggregated red blood cells in vascular lumen, uromodulin deposition in kidney tissue, or fibrin deposition. The correct nature of these tissue types in most cases were readily recognized by LM morphology. In others, additional stains such as Verhoff's stain for elastic tissue, Masson trichrome stain for fibrous tissue and fibrin, or periodic acid-Schiff stain for uromodulin helped identify the tissue types.

In each case, tissue sections of 4- to 10-μm thick were previously or prospectively stained for Congo red. Congo red staining was performed using the alkaline Congo red method for amyloid, as described by Puchtler et al [13]. H&E-stained and Congo red–stained sections of each case were examined by routine LM, with or without a polarizer and by FM (Olympus microscope; Olympus America, Center Valley, PA) under a fluorescein isothiocyanate filter (FITC) with an excitation peak at 490 nm and a fluorescence emission maximum of 520 nm and a Texas red filter with an excitation peak of 596 nm and emission maximum of 620 nm. In some selected cases, unstained slides consecutive to those used for Congo red stain were also examined in the same way. The results by each method were compared.

3. Results

The Congo red–stained amyloid deposits showed the typical pinkish to pink-reddish color in bright light. When Texas red filter was used with ultraviolet (UV) light, the Congo red–stained amyloid showed a bright red color against a dark background. The unstained, H&E-stained, and the Congo red–stained slides, regardless of the presence or absence of amyloid, showed no specific staining pattern under FITC filter.

Amyloid was strongly Congo red–positive by LM examination in all 26 cases of group 1. The "apple-green" birefringence expected by examination with polarizer was very focal or even absent in some cases (Fig. 1A and B), or it was focally identified in areas without congophilia, as seen in Fig. 1D and E. Examination using the fluorescent Texas red filter showed strong signal in all Congo red–positive amyloid cases (Fig. 1C, F, H, and J). Structures other than amyloid deposition did not show this intense type of illumination (Fig. 1D-F).

For the 22 cases of group 2, similar observations as in group 1 were made. In each case, the scant amount of Congo red–stained amyloid was easily and instantaneously recognized under fluorescent Texas red filter examination. Furthermore, there were 2 cases (myocardial needle biopsy