Forensic Science International 289 (2018) 150–153

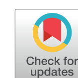
Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Technical Note

Lethal cardiac amyloidosis: Modification of the Congo Red technique on a forensic case



A. Rancati[a], S. Andreola[a], P. Bailo[b], M. Boracchi[a], P. Fociani[c], G. Gentile[a], R. Zoja[a,*]

[a] Forensic Pathology Laboratory, Dept. of Biomedical Sciences for Health, University of Milan, Via Luigi Mangiagalli, 37, 20133 Milano, Italy
[b] Forensic Genetics Laboratory, Dept. of Biomedical Sciences for Health, University of Milan, Via Luigi Mangiagalli, 37, 20133 Milano, Italy
[c] University of Milan, Italy

ARTICLE INFO

Article history:
Received 23 April 2018
Received in revised form 16 May 2018
Accepted 17 May 2018
Available online 26 May 2018

Keywords:
Autopsy
Cardiac amyloidosis
Congo Red staining
Forensic pathology

ABSTRACT

Congo Red staining is usually used in diagnosing amyloidosis, a pathology characterized by the storage of abnormal proteins in several human organs. When assessed on samples fixated in formalin and embended in paraffin, this staining can undergo several artefacts, causing diagnostic and interpretative difficulties due to its weak stainability and a consequent reduced visibility of the amyloid. These complications, in time, requested several variations of this staining technique, especially in clinical practice, while in the forensic field no protocols has ever been adapted to cadaveric samples, a material that is already characteristically burdened by a peculiar stainability.

In our work, studying a sudden death caused by cardiac amyloidosis and diagnosed only with post-mortem exams, we present a modified Congo Red staining used with the purpose to demonstrate amyloid in cadaveric material after the unsuccessfully use of all standard protocols.

© 2018 Published by Elsevier B.V.

1. Introduction

Congo Red staining is usually used as a standard histological dying in microscopic diagnosis of amyloidosis. This pathology is characterized by an atypical folding of a usually soluble protein [1] that collects in several tissues [2] organizing in fibrils and becoming dysfunctional. Protein, defined as “amyloid”, accumulates mainly in the extracellular matrix damaging the tissues and, if affecting vital organs, can lead to death [3]. The Congo Red staining is probably based on hydrogen-bindings that are created between the protein-based or polysaccharide-based component of the amyloid proteins and the dye but this reaction has not yet been clarified.

Sensitivity and specificity of the Congo Red staining are quite important for diagnostic purposes [4] and this technique is used as a “gold standard” in order to evaluate the presence of amyloid; this procedure takes advantage of the capability of the dying ($C_{32}H_{22}N_6Na_2O_6S_2$ – CAS Number 573-58-0) [5] to stain the amyloid fibrils, coloring them in orange-red. When observed with a polarized light microscope, fibrils have a characteristic anisotropy with an apple-green birefringence, more or less intense, with a different reaction compared to other tissues, as collagen-rich tissues, that are stained as well but with a less intense coloring. If assessed on tissues treated with formalin and embended in paraffin, this technique can reveal some technical difficulties related to a more troublesome staining and detectability of the amyloid proteins. These aspects have led many laboratories to create their own unique staining protocols [6–9] in order to detect particularly complex cases of amyloidosis and with the purpose of avoiding false positive cases of this pathology (“*phantom amyloidosis*”) [4].

Vice versa, in the forensic field, because of the peculiar characteristics of the samples, often putrefied and therefore with altered staining properties, the diagnostic suspect must be investigated with immunohistochemical techniques. In this work we are proposing a modified specific Congo Red staining for evaluating the presence of amyloid on cadaveric samples, used in a case of cardiac lethal pathology that resulted to be negative at standard Congo Red dying.

2. Material and methods

A 95-year-old woman suffering from Alzheimer’s disease and neurosensory hearing loss, cared for by a foreign caregiver, was found semi-conscious in her own bed by the caregiver late in the evening. Although the rescue team had been promptly alerted, the elderly lady arrived dead to the A&E of a well-known hospital in

* Corresponding author at: Sezione di Medicina Legale, Università degli Studi, Via Luigi Mangiagalli, 37, 20133 Milano, Italy.
E-mail address: riccardo.zoia@unimi.it (R. Zoja).

https://doi.org/10.1016/j.forsciint.2018.05.029
0379-0738/© 2018 Published by Elsevier B.V.

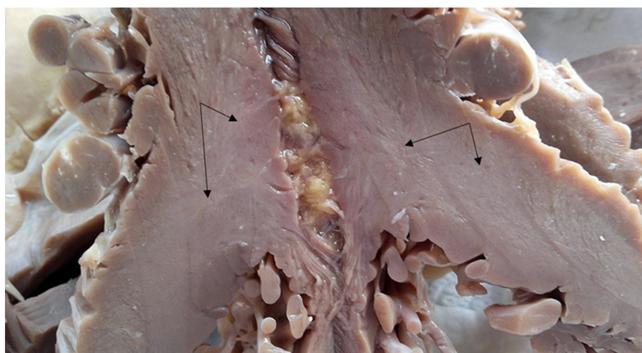


Fig. 1. Macroscopic view of the heart showing in increasing dimension of the cardiac chambers, bi-ventricular and septal hypertrophy, increasing of consistency and multiple lighter-colored areas (arrows) in the cardiac muscle.

Milan. The investigating magistrate ordered the judicial autopsy three days after her death because suspecting a violent cause of death. The cause of death was not explained by the external macroscopic autopsy examination and the examination of the internal organs. Only the liver, spleen and heart had a volumetric increase, but this was not enough to determine the cause of death. The investigating magistrate authorized further laboratory investigations and the collection of biological fluids (blood, urine and bile) and fragments of organs fixed in buffered formalin (brain, lungs, whole heart, spleen, pancreas, kidneys and liver).

After standard fixation, the histological reduction of the biological fragments of the different organs and of the whole heart was performed. This procedure confirmed the dimensional increase of the heart, accompanied by a "rubbery" consistency with the presence of multiple-focal lighter-colored areas in the myocardial background (Fig. 1).

After a period of 20 days of formaldehyde (10% neutral buffered formalin in phosphate buffer 0.05 M-Bio-Optica®, Milan) immersion, the obtained samples were subjected to routine histological examination techniques including increasing scale of ethanol (Merck®, Milan) dehydration, the clarification with xylene-substitute (Merck®, Milan) and the embending in high melting point paraffin (60 °C) (Merck®, Milan). With a microtome, 2-μ-thick laminar slides were obtained from all samples, were stained with H&E (Merck®, Milan) and were observed with Leica DMR optical microscope, acquiring the most significant images with Leica DC300F digital camera.

3. Results

In all the cardiac slides, stained with H&E, there are numerous irregular interstitial areas of hyaline material and fibrillary appearance. These findings may be suggestive of cardiac amyloidosis. Then further cardiac slides of different thickness, between 2 and 10 μ, were cut out. These samples were subjected to different staining protocols with Congo Red [3,10–12] (Merck®, Milan), but resulted to be negative for amyloidosis (Table 1, Fig. 2).

Following the negative results of the sample stained with Congo Red and observed with polarized light, we wondered if we had made some technical errors of preparation/staining. We cut out more cardiac slides, this time 6 μ thick, and stained these samples with a Congo Red staining of our experimentation to see if it was a technical error. In this technique, we used the same concentration of the Congo Red dye (0.5% in 50% alcohol solution) (Merck®, Milan) of techniques 3 and 4, but increasing the staining time up to 10 min. In addition, we also modified the differentiation in 0.2% potassium hydroxide in 80% alcohol solution (Merck®, Milan), by performing a rapid immersion and removing the unalloyed excess in water. Then we stained the nuclei with a mixture of hematoxylin of Harris and Mayer (1:1) (Merck®, Milan) for only 30 s, differentiating in running water for 2 min. The subsequent dehydration in increasing scale of ethanol (96%, absolute alcohol 1 and absolute alcohol 2) and clarification were quickly performed for just 1 min.

Procedure:

- dehydrate and bring the sections to water;
- immerse the slides in distilled water;
- stain with 0.5% Congo Red in 50% alcohol solution for 10 min;
- differentiate in 0.2% potassium hydroxide in 80% alcohol solution with rapid immersion;
- wash in running water for 1 min;
- stain the nuclei with a mixture of Mayer's and Harris Hematoxylin solution 1:1 for 30 s;
- differentiate in running water for 2 min;
- dehydrate, clarify and mount.

Results:

- orange–red positive amyloid;
- apple green birefringence present (Fig. 3).

4. Discussion

In the usual staining preparation of samples for microscopic observation [13], conventional histological examination continues to be founded on the five classical phases, including: fixation, dehydration, inclusion, cutting and staining [14]. This happen despite important innovative techniques (molecular biology assays and immunological techniques) that are increasingly involved in laboratory procedures. Over time, the methods of conventional histological staining on well-preserved biological samples have undergone significant improvement: both in terms of efficacy and specificity [15] and they are now considered consolidated and befitting for everyday practice [16].

Vice versa, the forensic field does not have its own specific protocols aimed to the analysis of a specific type of substrate. For example the cadaveric material, being in a more or less advanced phase of putrefaction or sampled from particularly destructive lesions, presents itself to the microscopic observation with numerous problems related to the overlap between "real pathology and the artefactual one" [17], with substantial structural

Table 1
Schematic view of different Congo Red staining used.

	Stain used	Congo Red	Time	Differenziante	Time	Colorante nucleare	Time
1 2	AFIP McGRAW-HILL 1968 AFIP 1992	1% Congo Red acqueous solution	1 h	Alkaline alcohol solution (1 ml NaOH 1% in 99.0 ml Ethyl alcohol solution 50%)	3–5 s Quick dive	Mayer's Hematoxylin solution	5 min
3 4	BANCROFT GAMBLE 2008 SUVARNA, LAYTON, BANCROFT 2013	0,5% Congo Red in 50% alcohol solution	5 min	0,2% potassium hydroxide in 80% alcohol solution	3–10 s	Mayer's Hematoxylin solution	Not specified

Download English Version:

https://daneshyari.com/en/article/6550863

Download Persian Version:

https://daneshyari.com/article/6550863

Daneshyari.com