

ORIGINAL PAPER

Comparative study of Congo red fluorescence and immunohistochemistry in cutaneous amyloidosis

A. FERNANDEZ-FLORES

Service of Cellular Pathology, Clinica Ponferrada,
Ponferrada, Spain

Abstract

Background: Detection of amyloid can be done by several techniques either histochemical or immunohistochemical. Among them, one of the less mentioned in texts of reference and in reports on amyloidosis, is the examination with ultraviolet light of the stain of Congo red. We intend to examine cases of amyloidosis stained with Congo red with ultraviolet light and to see if such method offers advantages with respect to Congo red only and to immunohistochemistry. **Material and Methods:** We examined 12 cases of cutaneous amyloidosis Hematoxylin-Eosin, Congo red stains (with and without permanganate treatment), Thioflavin T and immunohistochemical stains. We also evaluated Congo red fluorescence (CRF). **Results:** 66.66% were women and 33.34% were men. The traditional methods for the detection of amyloid (Congo red and Thioflavin T) were positive in 87.50% while immunohistochemistry was positive in 100% of the cases. In one case, immunohistochemistry detected Congo red negative deposits of amyloid. In another case, immunohistochemistry was strong while CRF was weak. CRF was always weak in all the cases in which it was seen. **Conclusions:** Immunohistochemistry is superior in the detection of cutaneous amyloid over the other techniques tested. CRF did not result, in our experience, to be so useful.

Keywords: immunohistochemistry, amyloid, Congo red fluorescence, cutaneous amyloidosis.

□ Introduction

Detection of amyloid can be done by several techniques either histochemical or immunohistochemical. Among them, one of the less mentioned in texts of reference and in reports on amyloidosis, is the examination with ultraviolet light the stain of Congo red. Some publications have remarked the use of such a method in renal pathology [1], outstanding how the technique can be performed in frozen tissue [1]. Nevertheless, the reports on Congo red fluorescence (CRF) on dermatopathology are not so frequent. Linke RP, for instance, published a series in which he included two cutaneous amyloidosis [2].

Some studies have compared the technique of CRF with other methods of detection of amyloid, such as Congo red staining on light microscopy, Congo red staining on polarized microscopy and immunohistochemistry [3]. The authors concluded that the best method for the quantification of amyloid was the CRF [3].

The goal of this study is a qualitative one instead of a quantitative one. We intend to examine cases of amyloidosis stained with Congo red with ultraviolet light, and to see if such a method offers advantages with respect to Congo red only and to immunohistochemistry.

□ Material and Methods

We have examined 12 cases of cutaneous amyloidosis, including cases of macular amyloidosis and systemic amyloidosis involving the skin. The deposits were quantitatively varied, including cases with minimal dermal deposits.

In all the cases, we examined Hematoxylin-Eosin slides, Congo red stains (with and without permanganate treatment), Thioflavin T and immunohistochemical stains. The antibodies used were AE1/AE3 (Dako, Monoclonal Mouse Anti-Human, clone AE1/AE3, code M3515), kappa light chain immunoglobulin (Dako, polyclonal rabbit anti-human, code A0191), lambda light chain immunoglobulin (Dako, polyclonal rabbit anti-human, code A0193) and AA amyloid (Dako, monoclonal mouse anti-human, clone mcl, code M0759).

Congo red stains were also examined with ultraviolet light in a dark room, evaluating the fluorescence of the deposits.

□ Results

The results are summarized in Table 1.

Table 1 – Results of the cases studied. CRF: Congo red fluorescence

Case No.	Age [years]	Gender	Type of amyloidosis	Congo red	Congo red (Permanganate pre-treatment)	CRF	Thioflavin T	Immunohistochemistry
1.	45	F	macular	-	-	-	-	+ AE1/AE3
2.	56	F	macular	+	-	+	+	+ AE1/AE3
3.	40	F	systemic	++	++	+	+	Kappa+, lambda -

Case No.	Age [years]	Gender	Type of amyloidosis	Congo red	Congo red (Permanganate pre-treatment)	CRF	Thioflavin T	Immunohistochemistry
4.	43	M	macular	+	-	+	+	+ AE1/AE3
5.	40	F	systemic	+	+	+ weak	+	Kappa+, lambda -
6.	35	F	lichen amyloidosus	+	-	+	+	+ AE1/AE3
7.	49	F	macular	+	-	+	+	+ AE1/AE3
8.	55	F	macular	+	-	+	+	+ AE1/AE3
9.	39	F	macular	+	-	+	+	+ AE1/AE3
10.	70	M	macular	+	-	+	+	+ AA
11.	79	F	macular	+	-	+	+	+ AE1/AE3
12.	64	F	macular	-	-	-	-	+ AE1/AE3

8/12 patients were women (66.66%) and 4/16 were men (33.34%). The mean age was 38.44 years (range: 35–79 years). Ten cases corresponded to macular amyloidosis (62%), two cases were systemic amyloidosis with cutaneous involvement (12.5%) and the other

was a lichen amyloidosus (6.25%). Amyloid deposits varied in quantity from subtle dermal deposits (Figure 1) to massive diffuse deposits (Figure 2).

The intensity of CRF of our cases was not as intense as the one described in literature (Figure 3).

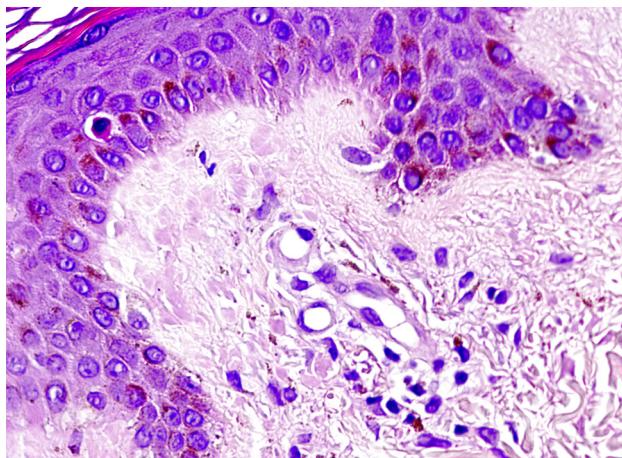


Figure 1 – Subtle deposits of amyloid in a case of macular amyloidosis.

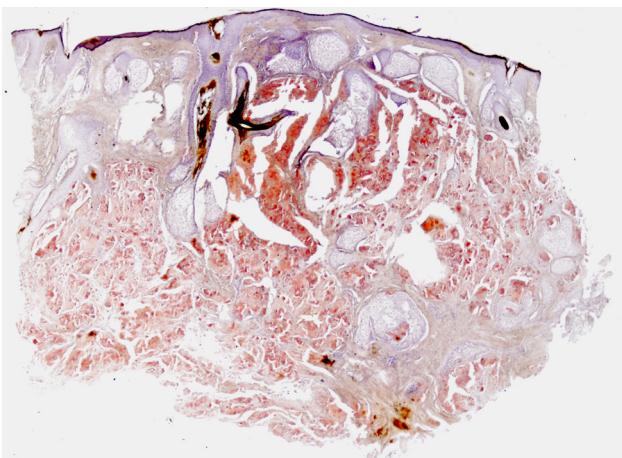


Figure 2 – Congo red stain in a case of cutaneous involvement by systemic amyloidosis.

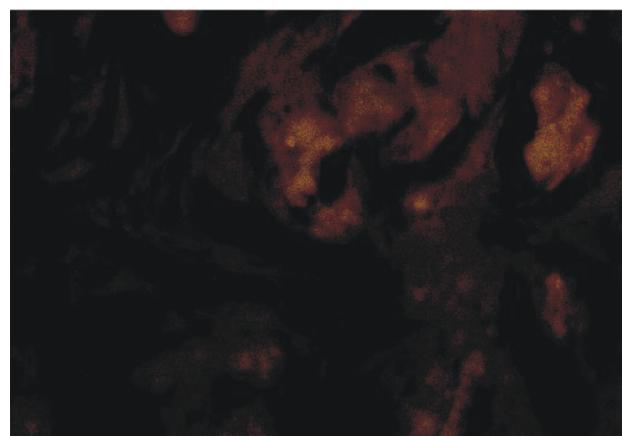
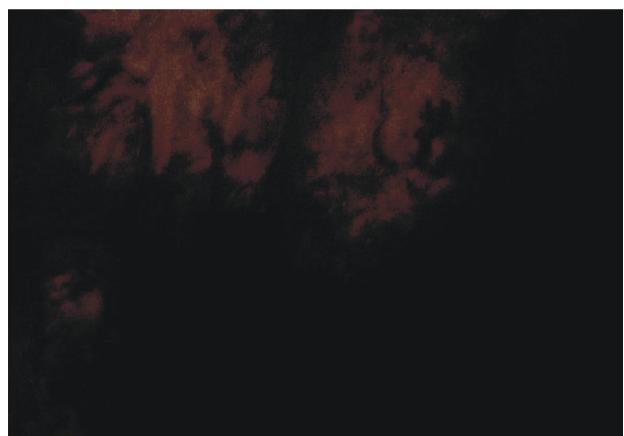


Figure 3 – Weak Congo red fluorescence was the rule in our cases.



The traditional methods for the detection of amyloid (Congo red and Thioflavin T) were positive in 14/16 cases (87.50%). This contrasts with the immunohistochemistry, that was positive in 100% of the cases. In case No. 1, the immunohistochemistry allowed us to see the deposits of amyloid easily that, although small in quantity, were found in the papillary dermis even though such deposits had been Congo red negative (Figure 4).

We were able to observe CRF only in those cases that were Congo red positive, in other words, positivity for Congo red is “*sine qua non*” condition to see CRF. Case No. 5 was another example of the superiority of the immunohistochemical technique in the detection of amyloid: while CRF was weak, the immunohistochemistry was intense with antibodies for kappa light change (Figure 5).

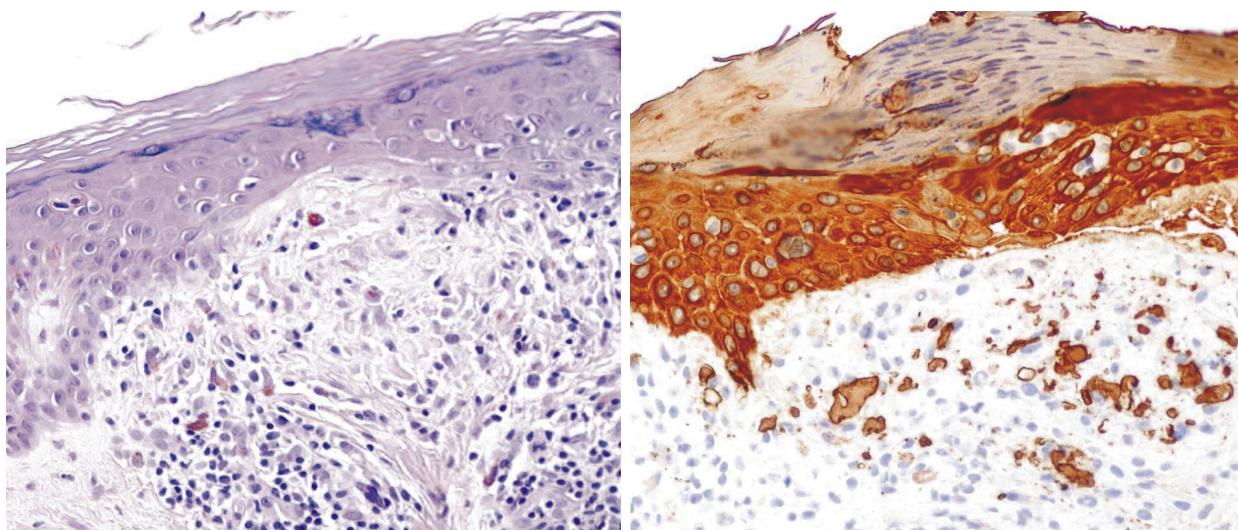


Figure 4 – A case of macular amyloidosis that was positive with immunohistochemistry for keratins, while Congo red did not stain it.

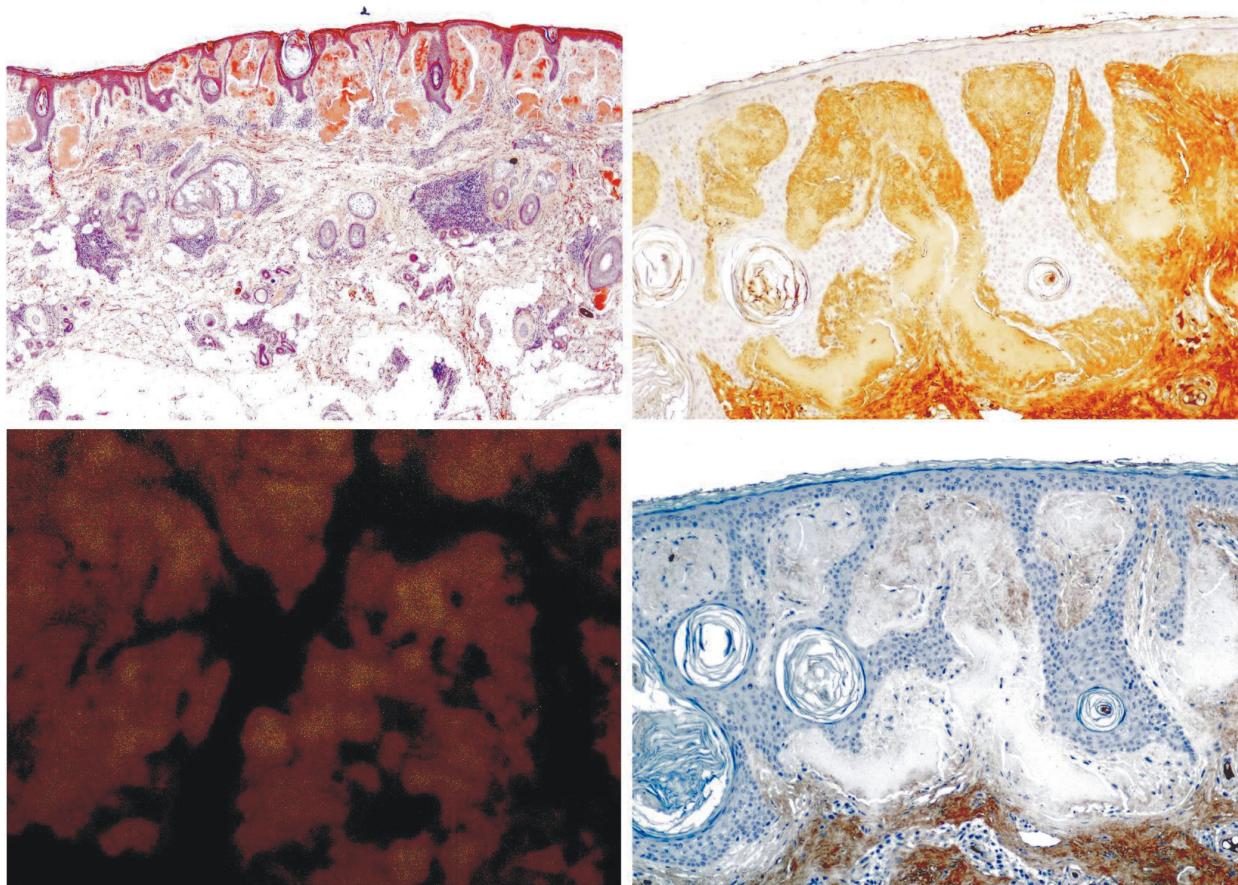


Figure 5 – Cutaneous involvement by systemic amyloidosis with deposits of kappa light chains. Congo red was positive (top left) but Congo red fluorescence was not as strong as expected (bottom left). Nevertheless, the immunostaining for kappa chains (top right) was intense and contrasted with the negativity for lambda chains (bottom right).

Discussion

Several outstanding reports and books on amyloidosis refer to several techniques to diagnose amyloidosis, including Congo red [4, 5], Thioflavin T, metachromasia after staining with crystal violet or methyl violet, and the classic apple green birefringence when observed under polarized light. Nevertheless, Congo red fluorescence (CRF) is hardly ever mentioned,

in spite of being one of the most useful techniques. Some authors have compared several techniques in the quantification of amyloid, such as CRF, Congo red staining on light microscopy, Congo red staining on polarized microscopy and immunohistochemistry [3]. They concluded that CRF is the best method for such purpose [3]. Others have studied CRF in the diagnosis of renal amyloidosis and have remarked the advantage of using it with frozen tissue [1].

CRF was reported nearly fifty years ago [5]. To be precise, Puchtler H and Sweat F described how the “amyloid was colored bright red, and other tissue structures were pale greenish gray”, when slides of amyloidosis stained with Congo red were examined under ultraviolet light [5]. Such authors remarked the use of the method to detect small deposits of amyloid: “*the high color contrast between bright red amyloid and grayish autofluorescence of other tissue structures aided the detection of traces of amyloid which were not clearly visible in normal microscopy*” [5].

CRF has been used in dermatopathology. Linke RP studied two cases of cutaneous amyloidosis with CRF less frequently [2]. One of them was a case of lichen amyloidosus and the other was a case of notalgia paresthetica (both cases therefore corresponded to amyloid keratin). In both cases, the authors described how while the amyloid stained either weakly or very weakly with Congo red, whilst CRF was strong or intensive [2]. This contrasts with what we observed in our study, where only when there was positivity with Congo red, we saw CRF. Moreover, the latter was not as intense as it is described in literature.

Our study also demonstrated how in several cases, the immunohistochemistry was superior to Congo red or to CRF in cases in which the deposits of amyloid were scanty.

□ Conclusions

Immunohistochemistry is superior in the detection of cutaneous amyloid over the other techniques tested.

In our experience, CRF did not result to be so useful, since we only saw positivity when there was already positivity for Congo red. Moreover, in some cases, the fluorescence was weak while immunohistochemistry was strongly positive.

References

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Corresponding author

Angel Fernandez-Flores, MD, PhD, S. Patología Celular, Clinica Ponferrada, Avenida Galicia 1, 24400 Ponferrada, Spain; Phone (00 34) 987 42 37 32, Fax (00 34) 987 42 91 02, e-mail: gpyauflowerlion@terra.es

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