

# Fat tissue analysis by Congo red method or by amyloid A protein quantification in clinical AA amyloidosis

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## INTRODUCTION

Quantification of amyloid A protein can be used to detect AA amyloid in fat tissue. The method confirms the AA-type of amyloid and can be automated to become observer-independent.

## OBJECTIVE

To study the sensitivity of amyloid A protein quantification and Congo red method in fat tissue of patients with clinical AA amyloidosis.

Fat tissue available	
For Congo red:	For amyloid A analysis:
Score 0: 15	Sufficient in 12 (80%)
Score 1: 60	Sufficient in 54 (90%)
Score 2: 55	Sufficient in 49 (89%)
Score 3: 45	Sufficient in 41 (91%)
<b>Total 175</b>	<b>Sufficient in 156 (89%)</b>

## PATIENTS AND METHODS

Abdominal subcutaneous fat tissue of patients was analysed at the start of the phase II/III clinical trial with Fibrillex™, a GAG-mimetic drug. All patients had AA amyloidosis proven by a biopsy positively stained with Congo red as well as positive anti-AA immunohistology. All patients had renal symptoms, i.e. proteinuria of  $\geq 1$  g/day or diminished creatinine clearance between 20 and 60 ml/min.

AA protein in fat tissue was quantified by ELISA using murine monoclonal anti-SAA antibodies (Normal range  $<12.6$  ng/mg fat tissue).

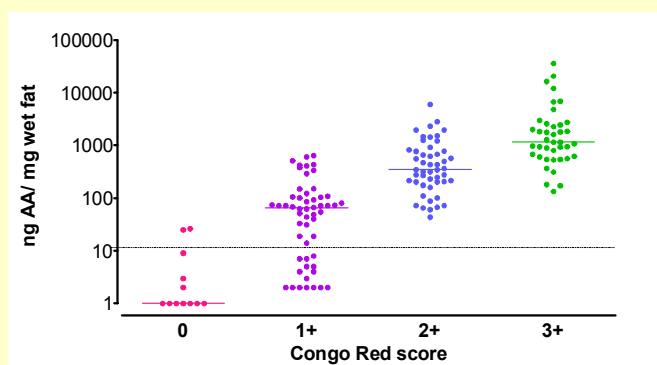
Congo red stained slides (CR) were scored semi-quantitatively from 0 to 3+.

## RESULTS

Fat tissue was available of 175 patients for analysis. Ample fat tissue to be analysed with both methods (at least about 60 mg) was available in 156 patients (see table).

Congo red slides were positive in 144 (92%) and AA protein concentration  $>12.6$  ng/mg fat tissue in 131 (84%) specimens.

Both methods were concordant: median AA concentration rose from 1.0 ng/mg fat tissue for CR-0, to 64.5 ng/mg for CR-1+, to 345 ng/mg for CR-2+, and to 1146 ng/mg for CR-3+ score (see figure).



**Figure.** Detection of amyloid in fat tissue of 156 patients with clinical AA amyloidosis by Congo red stain (score 0-3+) and amyloid A protein quantification (ng/mg fat). The dotted line is the upper limit of controls without AA amyloidosis (12.6 ng/mg fat).

## CONCLUSIONS

- Compared to quantification of amyloid A protein the Congo red method is more sensitive (92% vs. 84%) to detect amyloid in fat tissue of patients with clinical AA amyloidosis.
- However, good equipment and experience remain prerequisites for reliable results of the Congo red stain, especially when deposits are minute