

# Diagnostic performance of new capture ELISAs for free light chain quantification in fat tissue of patients with AL amyloidosis

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## INTRODUCTION

Fast and reliable typing of amyloid is essential for prognosis and treatment of patients after amyloid detection. Guanidine extracts from fat tissue enables immunochemical detection and quantification of the amyloid protein.

## OBJECTIVE

Development of capture-ELISAs for lambda and kappa free light chain concentrations in aspirated fat tissue:

To study their diagnostic performance for detecting and typing AL amyloid.

To relate the quantitative results to semi-quantitative grading of amyloid in Congo red-stained fat smears.

## PATIENTS AND METHODS

Fat tissue aspirates of 175 AL-lambda and 63 AL-kappa amyloidosis patients were studied at diagnosis. Controls were from 27 AA, 21 ATTR, 2 AApoAI, 1 Aβ2M, 29 localized amyloidosis and 129 non-amyloidosis disease patients.

The amount of amyloid in fat tissue was semi-quantitatively graded in Congo red-stained specimens from 0 to 4+.

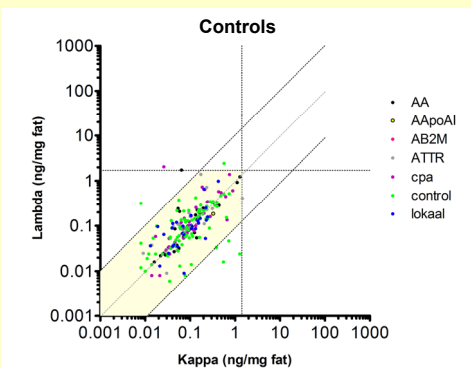
Free lambda and kappa free light chain concentrations were measured using two newly developed ELISAs for lambda and kappa each, using specific antibodies raised against hidden epitopes. The 99% reference limits of controls were calculated for kappa, lambda and the ratio of both free light chains.

## RESULTS

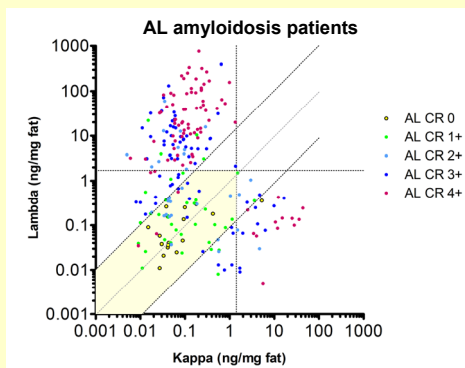
A reference area was defined by the upper 99% reference limits of 1.41 ng/mg fat for the concentration of kappa and 1.71 ng/mg fat for lambda and a kappa/lambda ratio between 0.09 and 10.2. Nine controls had values outside this reference area, resulting in a specificity of 96% (figure 1). Sensitivity was 6% (1 of 17) for the 0 graded group of AL patients, 33% (12 of 36) for 1+, 59% (19 of 32) for 2+, 91% (63 of 69) for 3+, and 96% (81 of 84) for 4+. In abundant amyloid (3+ and 4+) sensitivity was 94% (figure 2).

During follow-up of 8 AL patients with complete response, the kappa/lambda ratio normalised in all patients after treatment (figure 3).

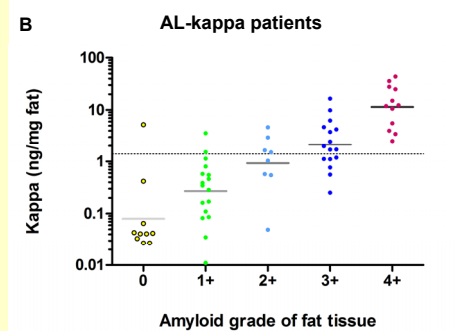
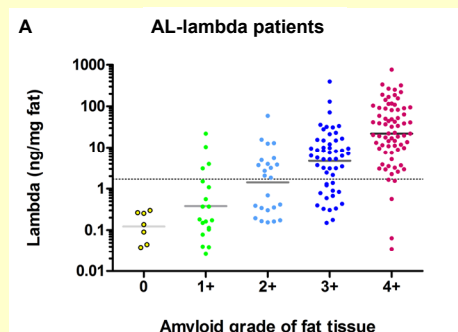
A linear trend was present ( $P < 0.0001$ ) between the mean concentrations of both kappa ( $R^2$  0.62) and lambda ( $R^2$  0.25) and the grade of amyloid (figure 4).



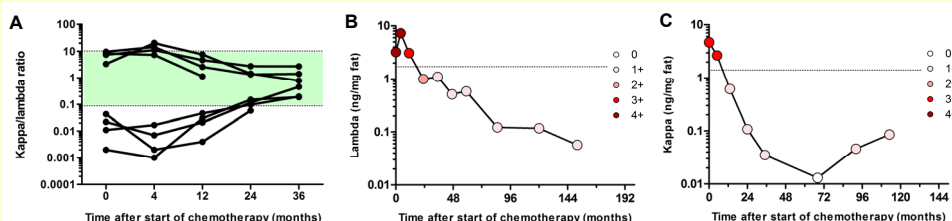
**Figure 1.** Control upper 99% reference limits of concentrations of lambda and kappa, and lower and upper 99% limits for kappa/lambda ratio in fat aspirates.



**Figure 2.** Fat aspirate concentrations of kappa and lambda free light chain and their ratio in 175 AL-lambda and 63 AL-kappa patients.



**Figure 4.** Concordance between lambda (A) and kappa (B) free light chain concentrations and amyloid grade in fat tissue of patients with AL amyloidosis. The dotted lines mark the 99% upper reference limits.



**Figure 3.** Kappa/lambda ratio in fat tissue aspirates of 8 AL amyloidosis patients with complete response during follow-up after start of chemotherapy (A) One individual AL-lambda (B) and one AL-kappa (C) amyloidosis patient are visualised. The dotted lines mark the 99% upper reference limits.

## CONCLUSIONS

- Immunochemical measurement by ELISA of lambda and kappa free light chains in fat tissue is a fast and useful method for typing amyloid in patients with systemic AL amyloidosis.
- The specificity is high and the sensitivity is determined by the grade of amyloid: reliably high in abundant amyloid (graded 3+ and 4+), moderate in little amyloid (2+) and low in minute amyloid (1+).

