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Diagnostic accuracy of new indirect ELISAs for free light-chain quantification in fat tissue of patients with AL amyloidosis

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Background

Fast and reliable typing of amyloid is essential for prognosis and treatment of a patient after amyloid has confirmed to be present. Immunohistochemistry is helpful for correct typing of amyloid in biopsies, but frequently fails to distinguish unambiguously between AL and ATTR type. Guanidine extraction of amyloid from fat tissue enables immunochemical detection and quantification of the characterizing protein. This approach has been successful in the typing of AA and ATTR amyloid [1,2]. Apart from typing, the quantitative results may reflect the severity of amyloid deposition in tissue during the course of the disease. Therefore, two indirect ELISAs were developed for lambda and for kappa free light chain, their diagnostic performances studied for typing of AL amyloid, and the quantitative results related to the semi-quantitative grading of amyloid in Congo red-stained fat aspirates.

Materials and methods

The first subcutaneous abdominal fat tissue aspirates of 197 consecutive patients with AL amyloidosis were studied. The AL subtype was lambda in 145 patients and kappa in 52 patients. The results were compared with those of 209 controls (27 AA, 34 ATTR, 2 AApoAI, 1 Aβ2M, 29 localized amyloidosis and 116 non-amyloidosis controls). The amount of amyloid in fat tissue was graded semi-quantitatively in Congo red-stained specimens: 0 (no amyloid), 1+ (<1% of inspected area), 2+ (1–10%), 3+ (10–60%), 4+ (>60%). A minimum of 30 mg of fat tissue per patient was aimed to be used for extraction of the amyloid in 1 ml of a 6 M guanidine solution [3]. Free lambda and kappa light-chain concentrations were measured using two newly developed ELISAs for lambda and kappa each. Microtiter plates (Corning) were coated overnight with samples, diluted 1:200–1:25 000. Rabbit anti-human lambda or kappa free light chains (Dako,

Figure 1. Figure representing the concordance between kappa (left part) and lambda (right part) free light-chain concentrations and amyloid grade in fat tissue of patients with AL-kappa (left) and AL-lambda (right) amyloidosis. The dotted lines mark the one-sided 99% upper reference limits.

1:500) were used in combination with goat anti-rabbit Ig-HRP (Dako, 1:1000), followed by a color reaction with TMB. The controls were used to calculate the 99% upper reference limits for kappa and for lambda, and the two reference limits for the ratio of both light chains.

Results

The intra-assay variability coefficients for lambda and kappa were 7.5% and 8.6% and the inter-assay variability coefficients were 9.7% and 9.4%, respectively. The limit of detection was 4.66 ng/ml for lambda and 3.34 ng/ml for kappa and the lower limit of quantification (LLOQ) was 22.2 ng/ml for lambda and 13.8 ng/ml for kappa. In case of 30 mg fat tissue, the LLOQ was 0.74 ng/mg fat for lambda and 0.46 ng/mg fat for kappa. Upper 99% reference limits were 1.71 ng/mg fat for lambda and 1.41 ng/mg fat for kappa. The lower and upper 99% reference limits for the kappa/lambda ratio were 0.09 and 10.2. An area defined by the upper limit of 1.41 ng/mg fat for kappa and the upper limit of 1.71 ng/mg fat for lambda and a kappa/lambda ratio between 0.09 and 10.2 was chosen as a reference area. Nine controls had values outside this reference area, resulting in a specificity of 96% (95% CI is 92–98%). Sensitivity was 7% (1 of 14) for the 0-graded group of AL patients, 33% (9 of 27) for the 1+ group, 63% (15 of 24) for the 2+ group, 95% (56 of 59) for the 3+ group, and 99% (72 of 73) for the 4+ group. In abundant amyloid (3+ and 4+) sensitivity is 97% (95% CI is 92–99%). A linear trend was present ($p < 0.0001$) between the mean

values of both kappa ($R^2 = 0.61$) and lambda ($R^2 = 0.30$) and the grade of amyloid (Figure 1).

Discussion and conclusions

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

Declaration of interest

The authors have no conflict of interest to report.

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