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SERUM 14-3-3 ETA: A NOVEL BIOMARKER OF RHEUMATOID ARTHRITIS

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Background: 14-3-3 proteins represent a family of ubiquitously expressed intracellular chaperonins consisting of seven different highly conserved isoforms. Kilani *et al.* previously demonstrated by immunoblot analysis that the η isoform of 14-3-3 was abundantly expressed extracellularly, in the synovial fluid and serum of patients with arthritis as compared to presumed healthy subjects.

Objectives: This study aimed to quantify the serum expression levels of 14-3-3 η in 265 subjects and investigate the biomarker's diagnostic potential in RA using a quantitative immunoassay.

Methods: Serum concentrations of 14-3-3 η were measured in 135 RA patients and 130 age- and gender-matched controls using the Augurex 14-3-3 η investigational grade-ELISA to determine the biomarker's differential expression in RA. Two-tailed t-tests and Mann-Whitney u-tests were run to compare group-differences in serum concentrations. RA patients had a rheumatologist-confirmed diagnosis, were on standard DMARDs but naïve of biological therapy. Controls were 55 presumed healthy individuals, 65 with other autoimmune diseases, 5 with osteoporosis, and 5 with gout. "Autoimmune controls" included 10 each of psoriasis, ulcerative colitis, type 1 diabetes, SLE, Crohn's and 5 each of Sjogren's, scleroderma, and multiple sclerosis. A ROC curve was generated providing an area under the curve (AUC) and corresponding likelihood ratios (LR) for various 14-3-3 η serum concentration cut-offs. Pearson correlations (r) for 14-3-3 η with age and RF titres were run to determine existing correlations between these variables.

Results: Mean and median 14-3-3 η serum concentrations in RA subjects were 4.58 and 1.12ng/ml, respectively compared to mean and median in controls of 0.25 and 0.12ng/ml ($p < 0.0001$). The area under the ROC curve was 0.847 (95%CI 0.799 to 0.895; $p < 0.0001$). A best cut-off level of 0.19ng/ml provides a positive likelihood ratio (LR+) of 5 and a negative likelihood ratio (LR-) of 0.27 for RA versus all controls. With 14-3-3 η levels above 1.30 and 2.18, LR+ was 10 and 50, and LR- was 0.55 and 0.62, respectively. 67% of RA patients were Rheumatoid factor (RF) positive while 78% were 14-3-3 η positive when 0.19ng/ml was used as a cut-off. Nevertheless, 14-3-3 η and RF titres correlated poorly (correlation coefficient: 0.036). In a logistic regression analysis, RF (OR (95%CI) = 21 (6.7 to 65)) and 14-3-3 η (OR = 8.4 (3.3 to 22)) contributed independently in distinguishing between RA and controls (R-square: 0.53).

Conclusions: 14-3-3 η is a novel biomarker that may help in diagnosing RA. Based upon its differential expression in rheumatoid arthritis in comparison to healthy and disease controls, 14-3-3 η may have diagnostic utility and may be used in combination with other serological markers to identify patients with RA.

References: Kilani RT *et al.* Detection of high levels of 2 specific isoforms of 14-3-3 proteins in synovial fluid from patients with joint inflammation. *J Rheumatol.* 2007 Aug;34(8):1650-7.