Serum Proteomics of Methotrexate Response in Patients with Rheumatoid Arthritis

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Background:

Methotrexate (MTX) is the recommended first-line disease-modifying anti-rheumatic drug (DMARD) for rheumatoid arthritis (RA) in the absence of contraindication by the American College of Rheumatology. Despite its widespread use, only one third of patients with RA achieve low disease activity or remission on MTX monotherapy. There are currently no validated biomarkers or clinical predictors of MTX response in RA. The purposes of this project are to evaluate serum protein expression changes associated with MTX treatment as well as investigate proteomic predictors of MTX response in RA from baseline serum samples.

Methods:

Plasma samples were collected from RA patients before MTX initiation (n = 20, 15 mg/week) and after 16 weeks of treatment. Serum proteomics were performed using the Olink Reveal platform. Normalized protein expression (NPX) values for 1037 proteins before and after MTX initiation were evaluated by paired Wilcoxon signed-rank tests using significance cutoffs of false discovery rate (FDR)-adjusted p-value < 0.1 (accounting for limited sample size) and absolute log2 fold change (FC) ≥ 0.5. Prediction of MTX response was assessed by logistic regression models including clinical factors (age, sex, baseline DAS28-ESR) alone and with baseline NPX of proteins found to be differentially expressed following treatment. MTX response was defined as a reduction in disease activity score in 28 joints with erythrocyte sedimentation rate (DAS28-ESR) of greater than 1.2.

Results:

Three proteins were differentially expressed following MTX exposure. These included an increase in thymidylate synthase (FDR-adjusted-p = 0.002, log2FC = 1.58) after MTX exposure, and decreases in CCL7 (FDR-adjusted-p = 0.08, log2FC = -1.02) and CASP4 (FDR-adjusted-p = 0.04, log2FC = -0.67). A combined model incorporating the 3 differentially expressed proteins with clinical factors (age, sex, DAS28-ESR) demonstrated an improved ability to identify MTX response over clinical factors alone (AUC 0.823 vs. 0.744 after internal validation, p = 0.01).

Conclusions:

Serum proteomic analysis of RA patients initiating MTX therapy revealed multiple proteins impacted by MTX. These proteins improved prediction of MTX response beyond clinical factors, suggesting a potential role as biomarkers. However, further investigation will be necessary to build on these findings using larger sample sizes.