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AB0235

POLYMORPHISMS OF HLA-DRB1 AND TNF-308 G/A ARE ASSOCIATED WITH RADIOGRAPHIC JOINT DESTRUCTION IN PATIENTS WITH VERY EARLY RHEUMATOID ARTHRITIS

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Objectives: To clarify the association between HLA-DRB1 and TNF-308 alleles and joint destruction/further progression during 12 months of the follow-up period (FUP) in patients with early (≤6 months), active, predominately ACPA and RF-positive RA treated according to "Treat to target" strategy.

Methods: The study included 85 patients with early RA and duration of symptoms ≤6 months. RA diagnosis was established according to ACR/2010 criteria. All patients were initially assigned to subcutaneous methotrexate (MTX) with rapid dose escalation to 20-25 mg/week. Combination MTX+biological therapy, target therapy Ultra Performance LC system. Measurement of PBMC-MTX-PGn (n=5) was performed in peripheral blood mononuclear cells (PBMCs) based on LC-MS/MS technology. FPGS activity and MTX-PGn in PBMCs based on LC-MS/MS technology.

Results: The study included 85 patients with early RA and duration of symptoms ≤6 months. RA diagnosis was established according to ACR/2010 criteria. All patients were initially assigned to subcutaneous methotrexate (MTX) with rapid dose escalation to 20-25 mg/week. Combination MTX+biological therapy, target therapy Ultra Performance LC system. Measurement of PBMC-MTX-PGn (n=5) was performed in peripheral blood mononuclear cells (PBMCs) based on LC-MS/MS technology. FPGS activity and MTX-PGn in PBMCs based on LC-MS/MS technology.

Background: methotrexate (MTX) is a widely applied anti-rheumatic and anti-leukemic drug. For its intracellular retention and pharmacologic activity, MTX relies on the enzymatic activity of polyglutamate synthetase (FPGS) to convert MTX into its polyglutamate forms (MTX-PGn). Loss of FPGS activity is associated with reduced MTX activity and although red blood cell (RBC) MTX-PG levels correlate with disease activity in RA patients, 1 it is anticipated to be more relevant to measure MTX-PG in peripheral blood mononuclear cells (PBMCs). Thus, the aim of our study was to develop a LC-MS/MS method to 1) measure FPGS activity replacing laborious radioactive assays, and 2) to measure MTX-PG in PBMCs.

Objectives: To validate a rapid, sensitive and non-radioactive assay to measure FPGS activity and MTX-PG in PBMCs based on LC-MS/MS technology.

Methods: Protein extracts (n=5) of PBMCs of MTX-treated RA patients were incubated for 2 hours at 37°C in FPGS assay buffer (ph8.8) containing 250 μM MTX and 4 mM L-glutamic acid as substrates. Next, MTX-PG formation was analysed with AB Sciex 4000 Q Trap tandem mass spectrometer coupled to an Acquity Ultra Performance LC system. Measurement of PBMC-MTX-PGn (n=5) was performed by extraction of MTX-PG from PBMCs by perchoric acid precipitation. Quantification was performed with 15N-labelled MTX-PGn, internal standards. In FPGS activity and MTX-PG validation studies, human CCRF-CEM leukaemia cells, CEM/R30dm (a FPGS-deficient, MTX-resistant subline of CCRF-CEM), and human acute lymphoblastic leukemia (ALL) cells served as reference.

Results: In CCRF-CEM, the FPGS enzymatic assay showed linearity with protein input (10–250 μg) and incubation time (0.5–3 hours). Substrate affinity parameters (Km) for MTX (65 μM) and L-glutamic acid (2.2 mM) were consistent with earlier reports.2 FPGS activity in CEM/R30dm was <1% of CCRF-CEM. FPGS activity in ALL blasts was similar to CCRF-CEM while FPGS activity in RA patient PBMCs was 1%–5% of CCRF-CEM, and was not-detectable in RBCs. Average individual fractions of total MTX-PGn in RA patient PBMCs were 22.1% (range: 8.2%–36.2%) for MTX-PG1, 32.8% (27.1%–43.6%) for MTX-PG2, 34.4% (30.4%–41.3%) for MTX-PG3, and 10.6% (0.0%–28.4%) for MTX-PG4. Average total MTX-PG levels per number of RA patient PBMCs were 30–50 fold higher than matched numbers of erythrocytes, and 6–9 fold lower than ALL blasts incubated for 24 hours with 1 μM MTX.

Conclusions: A sensitive LC-MS/MS based method was developed for the measurement of FPGS activity and MTX-PGn levels in PBMCs of RA patients. This method holds promise to guide future MTX-therapy response evaluations.

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AB0236

DEVELOPMENT AND VALIDATION OF A SENSITIVE LC-MS/MS-BASED METHOD FOR ANALYSIS OF ENZYMIC ACTIVITY OF POLYGLUTAMATE SYNTHETASE AND METHOTREXATE POLYGLUTAMATES IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF RHEUMATOID ARTHRITIS PATIENTS


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