ordinal scale. Overall subjective satisfaction, radiological improvements, AOFAS forefoot score, and subjective Foot function index were also assessed. Correlation between overall satisfaction after surgery and above other factors was performed. Statistical analysis was done using SPSS and Spearman's rho rank correlation test was performed to analyse the correlation between factors.

Results: Mean follow-up was 38 months (range; 24~54). For preoperative expectations, 30 (75%) patients considered improvement in pain as the most important parameter and improvements in shoe wearable and gross appearance were expected in 7 (17.5%) and 3 (7.5%) patients, respectively. Fulfilment of expectations assessed showed very satisfied in 14 (35%), satisfied in 20 (50%), average in 5 (12.5%) and disappointed in 1 (2.5%) for great toe, and those regarding lesser toe showed very satisfied in 15 (37.5%), satisfied in 18 (45%), average in 6 (15%) and disappointed in 1 (2.5%), respectively. For overall postoperative satisfaction assessment, 16 patients (40%) were excellent while 16 (40%) were good, 6 (15%) fair, and 2 (5%) poor. AOFAS hallux and lesser toe score were 69.7 ±11.71 and 70.7±12.26, respectively. Foot function index was 20.5±12.07 at final follow-up. Preoperative and postoperative hallux valgus angle were 45.6° ±10.55 and 17.4°±5.13, respectively. Those of 1–2 intermetatarsal angle were 14.2°±3.84 and 8.4°±3.72, respectively. Correlation analysis showed that significant factors that affected on overall satisfaction were fulfilment of expectation on the great toe (Spearman's rho=0.842, p<0.001)and that of lesser toe, and AOFAS score. Radiological degree and improvement in HVA and IMA were not significantly associated with patient's satisfaction.

Conclusions: Expectations from surgery on rheumatoid forefoot deformity were improvement in pain as the most common parameter, improvements in shoe wearable and gross appearance. Fulfilment of patients' expectations on the great toe as well as on the lesser toe significantly affected on postoperative satisfaction. Careful counselling for patients' expectations and corresponding fulfilment should be performed before performing reconstructive surgery for rheumatoid forefoot deformity.

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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 TNFα (−308G>A) genes polymorphism and joint destruction/further progression during 12 months of the follow-up period (FUP) in patients with early (<6 months), active, predominantly 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/$^{EULAR\ 2010}$ criteria. All patients were initially assigned to subcutaneous methotrexate (MTX) with rapid dose escalation to 20–25 mg/week. Combination MTX+biological therapy, mainly adalimumab, was used when MTX was ineffective. Joint destruction was assessed by Sharp-Van der Heijde modification scoring method at baseline and after 12 months FUP. Real time polymerase chain reaction (PCR-RT) was used for TNFα gene polymorphism (−308G>A) genotyping. Low resolution PCR-RT with subsequent sequence-based typing of *04 were performed to study HLA-DRB1 gene polymorphism. The HLA-DRB1*01, *04:01, *04:04, *04:05, *04:08, *10 alleles were categorised as SE+ (Shared Epitope) alleles.

Results: It was revealed that the number of erosions and joint space narrowings as well as the total Sharpe score were not associated with the presence and the dose of the SE alleles, either at baseline or after 12 months FUP. However, the progression of joint destruction, assessed as the change (Δ) in the number of erosions, joint space narrowings and the total score, was statistically significantly associated with HLA-DRB1*(SE) genotypes: the carriers of SE (SE+/SE+) double-dose had more advanced progression as compared to (SE+/SE-)/(SE-/SE-) carriers (p<0,028, p<0,019, p<0,035, respectively). As for TNFα gene polymorphism, it was demonstrated that the number of narrowings and total Sharp score values were almost twice as high at baseline in GG genotype carriers as compared to GA genotype carriers ($69,0^{49,5;\ 98,0}$ and $37,0^{29,0;\ 63,0}$ respectively, p<0,005) and ($72,0^{49,5;\ 98,5}$ and $37,0^{29,0;\ 63,0}$ respectively, p<0,004). Similar association was found after 12mo FUP, however, it should be mentioned that further progression of joint space narrowing and total Sharpe score was minimal (p>0,05).

Conclusions: Our data suggest that HLA-DRB1 (SE+) gene polymorphism is associated with the progression of radiographic joint destruction at 12mo FUP in treated pts. Meanwhile TNFα (−308G>A) polymorphism is associated with more pronounced joint destruction at baseline in terms of joint space narrowing and total Sharp score, but without further progression of joint destruction at 12mo in early and active RA pts managed according to "Treat to target" strategy.

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AB0236 DEVELOPMENT AND VALIDATION OF A SENSITIVE LC-MS/MS-BASED METHOD FOR ANALYSIS OF ENZYMATIC ACTIVITY OF FOLYLPOLYGLUTAMATE SYNTHETASE AND METHOTREXATE POLYGLUTAMATES IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF RHEUMATOID ARTHRITIS PATIENTS

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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 folylpolyglutamate synthetase (FPGS) to convert MTX into its polyglutamate forms (MTX-$PG_{2–6}$). Loss of FPGS activity is associated with reduced MTX activity and although red blood cell (RBC) MTX-PG_n levels correlate with disease activity in RA patients,[1] it is anticipated to be more relevant to measure MTX-PG_n 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_n in PBMCs.[2]

Objectives: To validate a rapid, sensitive and non-radioactive assay to measure FPGS activity and MTX-PG_n 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_2 formation was analysed with AB Sciex 4000 Q Trap tandem mass spectrometer coupled to an Acquity Ultra Performance LC system. Measurement of PBMC-MTX-PG_n (n=5) was performed by extraction of MTX-PG_n from PBMCs by perchloric acid precipitation. Quantification was performed with $^{13}C_5{}^{15}N$-labelled MTX-$PG_{1–5}$ 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 leukemic (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.[3] 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 non-detectable in RBCs. Average individual fractions of total MTX-PG_n in RA patient PBMCs were 22,1% (range: 8.2%–36.2%) for MTX-PG_2, 32.8% (27.1%–43.6%) for MTX-PG_3, 34.4% (30.4%–41.3%) for MTX-PG_4 and 10.6% (0.0%–28.4%) for MTX-PG_5. Average total MTX-PG_n 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-PG_n levels in PBMCs of RA patients. This method holds promise to guide future MTX-therapy response evaluations.

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