# Differing contribution of methotrexate polyglutamates to adalimumab blood levels as compared with etanercept

Methotrexate (MTX) is known to improve blood levels and clinical outcome to anti-tumour necrosis factor (anti-TNF) therapy by reducing the formation of antidrug antibodies.[1] Recent findings indicate that MTX prevents immunisation against TNF inhibitors through de novo purine biosynthesis inhibition and generation of immunosuppressive adenosine.[2] This effect is mediated by activation of MTX prodrug to MTX polyglutamates (MTXPG), and we previously reported that MTXPG levels were associated with improved steady state levels of infliximab.[3] In this study, we extended these observations and evaluated the impact of MTXPG on adalimumab and etanercept levels.

The study was cross-sectional by design, multicentred (three sites) and enrolled 169 consecutive consented adult rheumatoid arthritis subjects under MTX in combination with adalimumab (83 subjects) or etanercept (86 subjects). All patients enrolled were inadequate responders to MTX prior to starting anti-TNF therapy and had received MTX with anti-TNF therapy for least 3 months. At the time of a single visit, anticoagulated blood was collected randomly with respect to the last subcutaneous injection of adalimumab or etanercept. Adalimumab and etanercept levels were determined using a TNF reporter gene assay (expressed as µg/mL plasma).[3] Red blood cells (RBC) MTXPG levels (up to 5 glutamic residues) were determined using liquid-chromatography (expressed as nmol/L) as described.[3] C reactive protein (CRP) levels were determined using immunoturbidimetry. All subjects provided informed consent and the protocol was approved by internal review boards at each of the participating institution. Statistical analysis consisted of multivariate linear regression with anti-TNF levels as the dependent variable and RBC $MTXPG_3$ (long-chain, the preponderant MTXPG in circulating RBCs) as the independent predictor, adjusting estimates for TNF dosage, obesity status (body mass index >30 $kg/m^2$), CRP levels, duration of disease and previous anti-TNF usage. Estimates are reported as mean with SEs).

All 169 subjects enrolled in this study (59±1 years; 82% females) were under MTX therapy (102±5 months) and were prescribed anti-TNF therapy for 59±3 months. Mean MTX dosage was 16±0.4 mg/week, and mean RBC $MTXPG_3$ levels were 38±2 nmol/L. CRP levels were 8.0±0.5 mg/L with 39% subjects obese. Demographics by anti-TNF groups are presented in online supplementary table 1. Mean steady state adalimumab and etanercept levels were 11.8±0.8 µg/mL and 3.2±0.2 µg/mL, respectively, while mean RBC $MTXPG_3$ was 38±2 nmol/L and 39±2 for adalimumab and etanercept, respectively.

Heightened long-chain RBC $MTXPG_3$ levels was associated with higher steady state adalimumab (p=0.01) blood levels, while there was no impact on etanercept blood levels (p=0.44) (table 1). These observations remained significant after adjusting for anti-TNF dosage, obesity status (which had a negative impact on adalimumab levels), CRP levels (which tended to associate with lower levels for both monoclonal antibodies), previous use of TNF and disease duration. Very-long chain $MTXPG_{4-5}$ also associated with elevated adalimumab levels (online supplementary table 1).

These data are consistent with the notion that MTX polyglutamation may impact infliximab and adalimumab pharmacokinetics that are immunogenic and prone to anti-idiotype antibody formation.[4] Because etanercept is a fusion TNF receptor construct with little incidence of antidrug antibodies, the impact of MTX metabolism on its exposure is negligible. We acknowledge that our study was cross sectional and that the findings from this pilot study are limited steady state levels. However, our findings suggest that the optimisation of background MTX therapy and achievement of adequate MTXPG levels may potentiate the efficacy of infliximab and adalimumab in addition to facilitating dosage reduction strategies in therapeutic drug monitoring interventions.[5 6]

**Table 1** Multivariate analysis of anti-TNF steady state blood levels in relation to MTXPG levels after adjusting for dosage, obesity status, CRP levels, disease duration and previous anti-TNF

| | Random adalimumab levels µg/mL (n=83) | Random etanercept levels µg/mL (n=86) |
|---|---|---|
| Total $R^2$ | 37.0% | 8.8% |
| Intercept | 3.88±2.59 | 1.33±1.57 |
| RBC $MTXPG_3$ (nmol/L) | 0.07±0.03 (p=0.01)* | −0.01±0.01 (p=0.44) |
| Anti-TNF dose (mg/week) | 0.47±0.10 (p<0.01) | 0.06±0.03 (p=0.07) |
| BMI >30 ($kg/m^2$) | −4.26±1.33 (p<0.01) | −0.18±0.49 (p=0.72) |
| CRP (mg/L) | −0.46±0.16 (p<0.01) | −0.05±0.03 (p=0.12) |
| Disease duration (years) | 0.05±0.07 (p=0.50) | −0.03±0.02 (p=0.23) |
| Previous anti-TNF | −2.54±1.66 (p=0.13) | −0.06±0.77 (p=0.94) |

*Each unit RBC $MTXPG_3$ (expressed as nmol/L) was associated with 0.09 units higher adalimumab levels (expressed as µg/mL). Estimates are given for each of the anti-TNFs as dependent variables with RBC MTXPG3, anti-TNF dose, obese status, CRP, disease duration and previous anti-TNFs as independent predictors.
BMI, body mass index; CRP, C reactive protein; RBC MTXPG, red blood cells methotrexate polyglutamate; TNF, tumour necrosis factor.

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