Multi Drug Resistance (MDR) Protein Activity of T Lymphocytes Assessed by Flow Cytometry is a Predictor of Biological Treatment Response in Rheumatoid Arthritis

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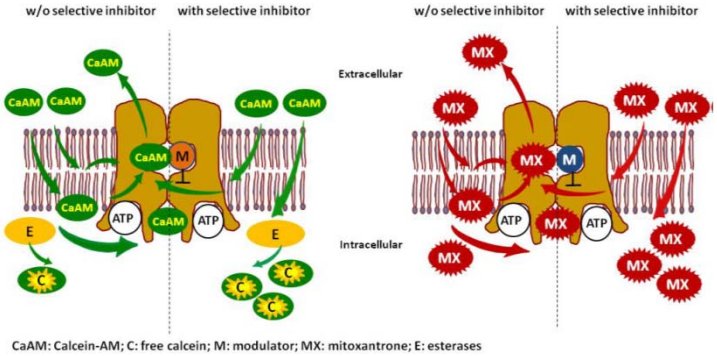
Introduction

Multi-Drug Resistance (MDR) protein function is an independent prognostic marker in certain hematological malignancies. Immune activation is also linked to the expression of MDR proteins in autoimmune disorders. Furthermore, MDR protein function may predict patient response to traditional DMARD, as well as biological treatment in rheumatoid arthritis (RA) helping the physician to tailor the therapy. Switching the patient to biologicals is often challenging due to unpredictable drug susceptibility and high cost, especially in patients with mildly elevated DAS28 scores. However, MDR protein activity values can objectively reflect the actual disease activity of RA patients. In this observational cohort trial, we wished to assess lymphocyte MDR protein activity in RA patients on biological therapy in comparison to healthy controls in order to predict response to treatment.

Methods

We measured the activity of three clinically relevant MDR proteins (MRP1/ABCC1, MDR/ABCB1, BCRP/ABCG2) expressed in MDR activity factor (MAF) values in 23 responder and 8 primary non-responder RA patients before (at 0 week) as well as at 2, 6 and 12 weeks after patients were switched to biological therapy. For this purpose, a novel flow cytometry based, CE-IVD certified diagnostic tool, the **Solvo MDQ Kit™** was applied. In this assay, fluorescent reporter substrates are trapped in the cytoplasm and pumped out by MDR proteins depending on the presence of specific inhibitors, allowing for quantitative, standardized assessment. PBMCs were loaded with fluorescent MDR activity reporter substrates (calcein-AM, em: ~515 nm and mitoxantrone, em: ~684 nm, respectively) and treated with MDR protein specific inhibitors (verapamil, indomethacin and KO134, respectively) to obtain MDR activity factor (MAF) values. Cell surface staining was applied to differentiate CD3+, CD4+ and CD19+ cells. DAS28 scores, CRP, IL-6, aCCP and RF values were also recorded. 35 age-matched, healthy controls were included.



CaAM: Calcein-AM; C: free calcein; M: modulator; MX: mitoxantrone; E: esterases

Activities of multidrug transporters are reflected by the difference between the amount of calcein/mitoxantrone accumulated in the presence or absence of the selective inhibitor(s). When calculating the MAF values this accumulation difference is normalized to the dye uptake measured in the presence of the inhibitor. **Thus, the result of the test becomes independent from factors influencing the cellular accumulation of calcein other than the activity of the multidrug transporters.**

$$\mathbf{MAF_C} = 100\times(F_{Max}-F_0)/F_{Max}$$

$$\mathbf{MAF_{MRP}} = 100\times(F_{MRP}-F_0)/F_{Max}$$

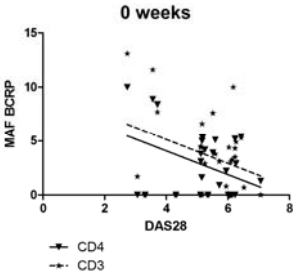
$$\mathbf{MAF_{MDR}} = MAF_C-MAF_{MRP}$$

$$\mathbf{MAF_{BCRP}} = 100\times(F_{MX}-F_B)/F_{MX}$$

F_{Max} – MDR+MRP inhibitor, F_0 – basal, F_{MRP} – MRP inhibitor, F_{MX} – BCRP inhibitor, F_B – basal

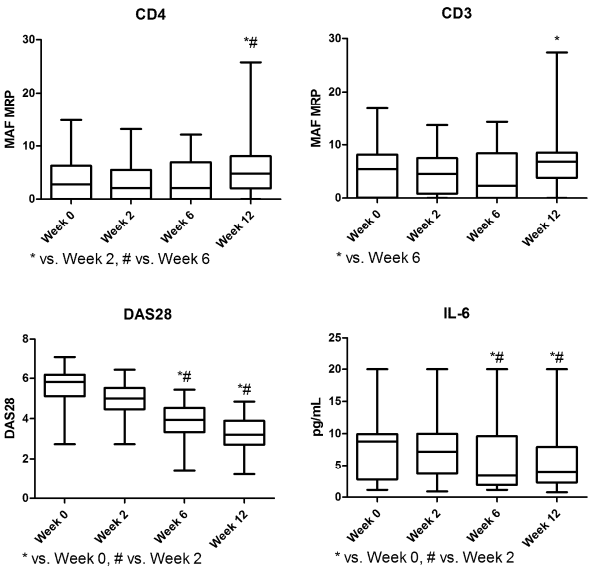
Results 1.

There was an inverse correlation between MAF of BCRP/ABCG2 in CD3 cells ($p = 0.04$, $r = -0.34$) and CD4 cells ($p = 0.03$, $r = -0.40$) and DAS28 at 0 week in RA patients.



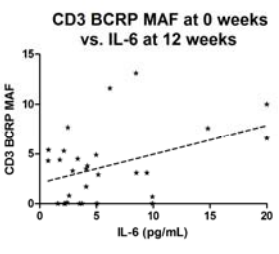
Results 2.

While DAS28 and IL-6 values decreased as the treatment progressed, MAF of MRP1/ABCC1 on CD3 and CD4 cells was increased at 12 weeks in RA patients.



Results 3.

A positive correlation was noted between MAF of BCRP/ABCG2 on CD3 cells at 0 week and IL-6 at 12 weeks ($p = 0.03$, $r = 0.41$) in RA patients.



Conclusion

Our results suggest that low BCRP/ABCG2 and MRP1/ABCC1 MAF activities on CD3 cells may predict the need to start biological therapy in RA patients whose symptoms do not improve on classical DMARD treatment. Further decrease of CD3 BCRP/ABCG2 and increase in CD3 MRP1/ABCC1 MAF upon follow-up may indicate a favorable therapeutic response to biological therapy.

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