PREDICTION OF RESPONSE TO METHOTREXATE TREATMENT IN RA USING MRNA AND MIRNA BIOMARKERS

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Background and objective:
This study aimed at defining predictive mRNA and MicroRNA (miRNA) biomarkers for the response to a future treatment with methotrexate (MTX) in whole blood samples of patients with rheumatoid arthritis (RA).

Materials and Methods:
For this purpose PAX gene whole blood samples from RA patients (n=52) before treatment were collected. Extraction of intracellular RNA including miRNA was performed according to PAXgene Blood miRNA Kit protocol. For identification of predictive mRNA candidates RNA samples were amplified employing the GeneChip 3'IVT Kit and hybridized onto HgU133 Plus 2.0 Arrays. For determination of predictive miRNA candidates, samples were processed and labelling was carried out with the GeneChip Hybridization, Wash & Stain Kit in the Fluidics Station. Signals were normalized by MAS5.0 algorithm and BioRetis online database.

Results:
Classification of RA patients into good, moderate and non-responders was performed according to the DAS28 and EULAR response criteria after 12 weeks of MTX treatment. Differential mRNA and miRNA expression before treatment was determined between 26 good responders and 13 non-responders calculating expression change calls and fold-changes. In addition a specific HLA allele was identified as relevant pre-selection marker for therapy prediction. Hierarchical clustering of discriminating mRNA and miRNA candidates was performed using the Gene Expression 'Similarity Suite' including and excluding medium responders (n=13). A clear discrimination with a 100% sensitivity in the negative HLA-DRB subgroup and in the HLA-DRB positive subgroup and specificity rates of 100% and 93.5% between responders and non- responders to future MTX treatment were achieved.
In total, equally n=16 MTX pre-selection markers were identified in the HLA-DRB positive and the HLA-DRB negative subsets with parameters of change ≥80% and a Fold-Change of ≥|1.5|. These mRNA biomarkers were validated using a technical independent qPCR platform. In addition, further 6 predictive miRNA candidates were identified.

Conclusions:
Identified and defined mRNA and miRNA biomarker subsets open new avenues for diagnostic test development and might also allow a translation using other technical platforms. Early prediction of response to MTX therapy using mRNA, as well as miRNA candidates, identified in genome-wide microarray analyses, is an opportunity for effective individual medication and therefore allows preventing side effects and socio-economic reducing costs.