Differential expression analysis was performed with Expression Suite software and selected miRNAs candidates were validated in the validation study by real-time PCR with LNA™ microRNA qPCR assays and analyzed with 2^{-DCt} method. Kruskal-Wallis test, Dunns post-test and linear regression were used for statistical analyses.

Results: In the discovery study we were able to measure 379 (50%) of the miRNAs represented in the array. We observed that 10 miRNAs (miRNA-Let-7a, miRNA-96, miRNA-381, miRNA-451a, miRNA-518d, miRNA-425-5p, miRNA-572, miRNA-190b, miRNA-708, and miRNA-1180) were expressed at the same level in RA and AMI patients but were significantly downregulated compared with controls. These 10 miRNAs were selected as potentially miRNAs associated with the increase risk of CVD in RA patients. Four of those miRNAs were expressed at very low level and were discarded for the validation study. In the validation study with 214 plasma samples from RA patients, we observed that two of the six candidate miRNAs (miRNA-425-5p and miRNA-451a) were significantly associated with cIMT. Thus, adjusted multivariable linear regression analysis showed that miRNA-425-5p and miRNA-451a independently explained 1.4% of the cIMT variability. Furthermore, adjusted regression estimates of the effect of miRNA-425-5p and miRNA-451a on cIMT were $\beta = 0.029\text{mm}$; $p = 0.037$ and $\beta = -0.035\text{ mm}$; $p = 0.039$, respectively. No other miRNA candidate exhibited association with cIMT values. Furthermore we observed that miRNA-425-5p was significantly correlated with ESR ($r = -0.136$; $p = 0.024$) and miRNA-451a with DAS28 ($r = 0.19$; $p = 0.003$), ESR ($r = 0.23$; $p = 0.0001$), CRP ($r = 0.15$; $p = 0.016$) and fibrinogen ($r = 0.28$; $p = 0.0001$). miRNAs concentrations were not affected by any of the AR treatments. No association was observed between the presence of carotid plaque and the expression level of the microRNAs tested.

Conclusion: In the present study, we have identified miRNA-425-5p and miRNA-451a as potentially miRNAs involved in the CVD risk observed in RA patients.

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FRI0008

ADDRESSING THE DIAGNOSTIC GAP IN RHEUMATOID ARTHRITIS BY COMPLEMENTING THE SEROLOGY WITH GENETIC INFORMATION

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Background: Rheumatoid arthritis (RA) is caused by an interaction of inherited and environmental factors. The genetic component was revealed in different twin studies and the shared epitope was identified as main contributor. During several genome-wide association studies (GWAS) different genes have been highlighted as relevant for developing RA but so far, none of these have been used for a diagnostic approach to address the diagnostic gap for RA.

Objectives: The aim of this study was to identify genes within pathways relevant for developing RA and to combine these genetic risk factors with serologic data to improve the diagnosis of RA, especially in regards to CCP negative RA patients.

Methods: The cohort consists of 804 RA patients, 159 Disease controls and 495 Healthy individuals. Serology data were obtained using the EliA™ CCP IgG, ELIA RF IgM and ELIA RF IgA (Phadia AB, Uppsala, Sweden) tests. The genetic measurements were performed using the Next-Generation-Sequencing technology AmpliSeq™ on the Ion GeneStudio™ instruments (Thermo Fisher Scientific, Carlsbad, USA). The combined dataset was analysed by machine learning focusing on multivariate supervised models and discrete models.

Results: The Disease Research Area database (Thermo Fisher Scientific, Carlsbad, USA) uses a proprietary algorithm to create connections between genes and a specific disease highlighting relevant genes and pathways using the available datasets from NCBI, DisGeNet, ClinVar and further databases. Based on this information we were able to identify genes and variants within relevant pathways for RA and designed a sequencing panel specific for diagnosis of RA. The results from the

targeted sequencing approach and the serologic data of the patients were analysed by an algorithm resulting in an improved diagnosis. Each variant within the algorithm has a specific weighting score resulting in a combination of protective and destructive variants, which are used together with the serology to calculate a RA risk score.

In this study the combination of genetic and serology leads to an improved sensitivity of over 10% compared to CCP alone, with a comparable specificity. This increase in sensitivity results in the identification of >20% CCP-negative RA patients. Additionally, a genetic pattern was identified distinguishing between CCP-negative and CCP-positive RA patients.

Conclusion: Usage of genetic variants in combination with serologic data improves the diagnosis of RA within the measured cohort of Caucasian RA patients. Furthermore, a genetic pattern specific for CCP negative RA patients was accentuated and could be used to improve the diagnosis of CCP negative RA patients in the future. The combination of serology and genetic information for the diagnosis of RA patients could lead to an earlier diagnosis, especially for CCP negative RA patients.

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FRI0009

MOLECULAR PROFILING OF CIRCULATING B-LYMPHOCYTES REVEALS THE SUPERIOR PERFORMANCE OF METHYLOME OVER TRANSCRIPTOME DATA FOR DISCRIMINATING RHEUMATOID ARTHRITIS PATIENTS IN AN EARLY ARTHRITIS CLINIC: IMPLICATIONS FOR TRANSLATING "BIG DATA" INTO CLINICALLY USEFUL TOOLS

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Background: Defining optimal strategies for translating "big datasets" of transcriptome and methylome data into clinically valuable tools in RA will benefit from comparisons where potential confounders – including therapeutic background, disease phase and cell substrate heterogeneity – are controlled as far as possible.

Objectives: We have obtained paired B- and CD4+ T-lymphocyte whole genome expression and methylation data from drug-naïve early arthritis clinic patients at a single centre. Focussing on B-lymphocyte data we here ask which of these datasets has most value in discriminating early RA – and the additive value of combining them.

Methods: CD19+ B-lymphocytes were isolated by positive selection from fresh peripheral blood of 90 drug-naïve patients attending the Newcastle Early Arthritis Clinic (NEAC), comprising 36 RA patients and 54 disease controls matched, so far as possible, for age, sex and acute phase response. Paired RNA and DNA extracted. Gene expression was profiled using the Human HT12 v4 BeadChip, and DNA methylation at >850,000 CpG sites quantified with the MethylationEPIC array (both Illumina). Gene expression and/or DNA methylation classifiers for RA prediction were developed based on a combined approach of classification algorithm

selection and hyperparameter optimisation. The Sequential Model-Based Optimization procedure was used to automatically select the best model configuration amongst 10 feature selection filters, 2 class imbalance correction techniques, 3 data cleaning methods and 4 classification algorithms along with their respective hyperparameters. Model optimisation was performed using a 10-fold Cross Validation (CV) while a 10 times repeated 10-fold CV was used for the predictive performance evaluation. Receiver operating characteristic (ROC) and precision recall (PR) curves were used to directly compare the classification potential of molecular approaches.

Results: 12,035 gene expression features and 714,486 methylation features were available for classifier building after quality control in our early arthritis B-lymphocyte dataset. Performance estimation showed the expression profile of B-lymphocytes alone to have no significant discriminatory utility for RA in this setting (average area under [AU] ROC 0.48). Methylation profiles were significantly superior for discriminating RA (average AU ROC 0.71). Interestingly, when a combined transcriptome/methylome model was generated, further enhancement of discriminatory function was observed, although this was modest (AU ROC 0.74).

Conclusion: Methylation profiling of B-lymphocytes affords better discrimination for RA than does transcriptional profiling. This may differ in other cell types. Combining data from molecular platforms may be an optimal approach. Functional analyses of the resultant B-lymphocyte signatures may yield insight into molecular pathobiology.

Figure A. Table summarizing results of performance estimation of B-cell gene expression and methylation profiles using 20 x 10-fold Cross Validation (1000 paired training-testing samples). The performance of a combined model is also given. The ROC curve depicting discriminatory ability of the combined model is shown.

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FRI0010 TCR-CD3Z GENE EXPRESSION PROFILE IN A COHORT OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Advances in understanding the pathogenesis of RA support the contribution of T cells in the disease process through the recognition of the arthritogenic antigen (Ag) and maintenance of chronic inflammatory response [1].

Previous gene association studies have demonstrated that CD247 gene expressions are associated with a variety of autoimmune disease. As regard Rheumatoid Arthritis (RA), this relationship has not been fully clarified yet.

Objectives: We aimed to evaluate the T-cell receptor-CD3 zeta chain (TCR-CD3 ζ) gene expression profile in a cohort of patients with RA.

Methods: A case-control study on 150 consecutive RA patients diagnosed according to 2010 ACR/EULAR criteria and 150 matched healthy controls without family history of RA or other autoimmune diseases. RA patients with other autoimmune diseases, viral hepatitis B or C, malignancy or hematological disorders were excluded from the study.

All participants were subjected to history taking, clinical examination, assessment of disease activity (in RA patients) using DAS-28 and HAQ, routine laboratory investigations, inflammatory markers level, serological tests, as well as molecular analysis for TCR-CD3 ζ mRNA expression by quantitative real-time polymerase chain reaction.

Results: TCR-CD3 ζ gene expression was significantly lower in RA cases than in healthy controls ($p < 0.05$) (Table 1). Expression of TCR-CD3 ζ revealed a significant negative correlation with RA disease duration, RF, and ESR ($p < 0.05$) in RA cases. The level of TCR-CD3 ζ also showed a significant lower expression in +ve RF patients than in -ve RF patients ($p < 0.05$). The AUC of TCR-CD3 ζ level showed a moderate level of accuracy (AUC = 0.840, $p = 0.025$) (Figure 1), and the calculated cutoff value (≤ 0.077) can precisely discriminate subjects with RA from those without RA with 93.5% sensitivity and 75% specificity (Table 2).

Table 1. Comparison between RA cases and control group as regard CD247 (mRNA levels for TCR-CD3 ζ gene expression)

TCR-CD3 ζ	RA cases (n=150)	Healthy Controls (n=150)	Test of sig.	P
Min. – Max.	0.037– 12.467	0.034– 11.314	Z=-3.047*	0.002*
Mean $\pm$ SD.	0.663 $\pm$ 1.90	0.816 $\pm$ 1.64		
Median (Q1-Q3)	0.178 (0.109-0.314)	0.315 (0.198-0.922)		

Z: Z-score of Mann-Whitney test

*: Statistically significant at $p \leq 0.05$

Table 2. Analysis of ROC curve for TCR-CD3 ζ level as a predictor of RA (high RF)

Variable	Cutoff point (ng/ml)	AUC	P	Sensitivity (%)	Specificity (%)	PPV	NPV	Accuracy (%)
TCR-CD3 ζ	≤ 0.077	0.840*	0.025**	93.5	75.0	78.9	92.0	84.3

AUC: Area under the curve

*AUC ≥ 0.5

** Statistically significant at $p \leq 0.05$

PPV: Positive Predictive Value

NPV: Negative Predictive Value

Figure 1. ROC curve for CD247 (TCR-CD3 ζ) level as a predictor of Rheumatoid arthritis

Conclusion: Our results demonstrated a lower expression of TCR-CD3 ζ in RA patients than in healthy controls. We suggested that CD247 gene down-regulation might contribute in the susceptibility to RA and help understanding the mechanisms responsible for deficient T-cell responses in RA patients.

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