A154 A MULTIPLEX MICROARRAY FOR THE DETECTION OF RHEUMATOID ARTHRITIS-ASSOCIATED AUTOANTIBODIES

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Background and objectives Autoantibodies, for example, directed against citrullinated proteins/peptides (ACPA) are highly specific and predictive of development of rheumatoid arthritis (RA). Different subgroups of RA patients have different autoantibody specificities relating to, for example, genetic subsets and disease prognosis. Further autoantibody subgrouping of RA patients can give further information about disease subsets, which may have different prognosis and require different treatments. We have developed a microarray for the simultaneous detection of multiple RA-associated autoantibodies.

Materials and methods The microarray is based on Phadia's ImmunoCAP ISAC system, where reactivity to more than 100 antigens can be analysed simultaneously using small volumes (3 µl) of sera. Antigens are immobilised in an arrayed fashion onto a chemically modified glass slide allowing a 3-dimensional layer with high binding capacity. Thirty µl of diluted serum from RA patients and healthy controls are applied onto reaction sites on the glass slides, followed by fluorescent-labelled antihuman IgG antibody, and analysed by capturing fluorescence intensity images in a laser scanner. Results are analysed with a customised image analysis software. The assay has been optimised concerning serum dilution and choice of anti-IgG conjugate. Each individual antigen is optimised concerning antigen concentration, binding (biotinylation or not) and choice of spotting buffer. A large number of antigens, citrullinated and non-citrullinated peptides as well as full proteins, are being investigated and sequentially added to the chip after individual validation against antigen-specific ELISAs.

Results Until now 16 antigens representing citrullinated and non-citrullinated vimentin, fibrinogen, α enolase, collagen type II and filaggrin have been validated. Strong correlations between ISAC and ELISA results were found for the individual validated antigens (R between 0.73 and 0.93). Results from a pilot study using ISAC imply that subtraction of fluorescence for the corresponding arginine-containing peptide from ACPA results will both increase diagnostic sensitivity as well as strengthen the genetic association to the HLA-DRB1* shared epitope. Such correction for non-citrullinated antigen background is not done with conventional ACPA ELISAs.

Conclusions We have developed a multiplex array for detection of RA-associated antibodies, and validated against ELISA results for the individual antibody specificities. Currently

eight citrullinated antigens and their non-citrullinated counterparts have been individually validated, and new antigens are continuously added to the array. This platform might be of great benefit in studies of RA pathogenesis, diagnosis and potentially as a guide to individualised treatment. The first clinical study using the presently validated antigens is planned for December 2010.