Optimizing the hemagglutination-mediator for rapid detection of anti-citrullinated protein antibodies in rheumatoid arthritis patients

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Anti-citrullinated protein antibodies (ACPA) are the most specific serological marker of rheumatoid arthritis (RA). Several ACPA-detection assays are available for clinical use, which are almost all based on ELISA(-like) assays with citrullinated peptides (CCP2; CCP3) or proteins (MCV). To facilitate ACPA-detection in low-volume laboratories and resource-poor environments, we aimed to develop a rapid and easy to perform test.

An agglutination mediator was generated by protein engineering. This mediator consists of an anti-glycophorin A, one of the major surface proteins of erythrocytes, single-chain antibody fragment (scFv) and a citrullinated synthetic peptide, which is linked to the scFv by sortase A-mediated conjugation. Addition of this mediator to (diluted) whole blood samples results in hemagglutination when ACPA are present, which can be detected by the naked eye. The applicability was assessed by the analysis of fresh blood samples from 204 RA patients, 77 psoriatic arthritis (PsA) patients and 100 healthy individuals (Kruis et al., 2025).

ACPA-dependent erythrocyte agglutination was observed in up to 61% of the RA samples, which correlated well with the results obtained with a standardized anti-CCP2 ELISA (63-67%). Depending on the minimal agglutination score – the level of agglutination was scored 1 (very low) to 4 (very high) – agglutination was observed with only 3-21% of the PsA samples and with 1% of the healthy controls.

Because scFv's have a slight tendency to dimerize, which is for obvious reasons not desired for an agglutination mediator, may be relatively unstable, and the production of the anti-glycophorin A scFv is not very efficient, we generated an alternative agglutination mediator using an anti-glycophorin A nanobody. This nanobody can be very efficiently produced in HEK293 cell cultures. The synthetic citrullinated peptide was conjugated to the nanobody by sortase A-mediated conjugation. The resulting nanobody-based mediator binds to erythrocytes and is recognized by ACPA in RA patient sera. Its capacity to mediate ACPA-dependent agglutination is currently being explored.

We conclude that ACPA detection by erythrocyte agglutination represents a rapid and efficient ACPA detection method using human whole blood samples. The nanobody-based agglutination mediator may be an attractive alternative for the scFv-based mediator, because of production and stability issues.

Reference:

Kruis, I., Kumari, J., Van der Heijden, A., Weijn, A., Vree Egberts, W., Peeters, I.R., Van Herwaarden, N., Salden, M. and Pruijn, G.J.M. (2025) Anti-citrullinated protein antibody detection by hemagglutination. Rheum. Adv. Pract. 9, rkaf010.