**Development of a novel bead-based Luminex assay for simultaneous detection of autoantibodies against acute phase proteins**

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**Background** **and Aims**

Serum amyloid A1 (SAA1), alpha 1 acid glycoprotein (AGP) and alpha 1 antitrypsin (AAT) are three acute phase proteins (APPs) in humans. Although thought to have protective physiological roles, their persistently elevated levels in patients with chronic inflammatory diseases can cause tissue damage. Autoantibodies against SAA1 and AAT have already been identified in healthy and in autoimmune sera, respectively. Currently however, no method exists for simultaneous detection of antibodies (Abs) against multiple APPs. So, our aim was to develop a bead-based triplex Luminex assay for detection of anti-SAA1, anti-AGP and anti-AAT Abs in human sera.

**Methods**

SAA1, AGP and AAT were coupled to fluorescently labeled MagPlex microspheres using an Ab coupling kit according to manufacturer’s instructions (Luminex Corp). Specifically, 4 μg SAA1, 12 ug AGP and 12 ug AAT were used per 106 beads. Coupling confirmation was performed for each bead region using serially diluted mouse monoclonal anti-SAA1, anti-AGP and anti-AAT Abs, with PBS-1%BSA serving also as background control. 50 μl of each serial dilution was added to a 96-well microtiter plate and incubated with SAA1-, AGP- and AAT-coupled microspheres (2000 beads each) in 50 μl per well for 2h. Following 3 washes with PBS-1%BSA, 1 μl of PE-conjugated anti-mouse IgG Ab in 100 μl PBS-1%BSA was added to each well and incubated for 30 min. The plates were then washed twice, resuspended in PBS-1%BSA and read on MagPix system with a minimum of 50 bead reads per well. Cross reactivity was also evaluated.

Sera samples were collected from healthy blood donors (HBD; n=52), patients with giant cell arteritis (GCA; n=20), early rheumatoid arthritis (ERA; n=20) and systemic lupus erythematosus (SLE; n=15), diluted 1:20 and analysed using the same protocol, as defined for monoclonal Abs, with the exception of 2.5 μl secondary PE-labeled anti-human IgG Ab in 100 μl PBS-1%BSA added to each well before washing and reading. For the inter-assay variability between plates, 30 samples were analyzed in 2 separate runs. For the intra-assay variability, 10 samples were tested in triplicates. The coeﬃcients of variation (CV) were calculated.

**Results**

Serially diluted Abs against SAA1, AGP and AAT generated curves saturating at median fluorescence intensity (MFI) above 10.000. No cross reactivity between anti-SAA1, anti-AGP and anti-AAT Ab was observed (the mean CV between singleplex and triplex was 4% for anti-SAA1, 3% for anti-AGP and 8% for anti-AAT) and no signal was detected in the absence of specific primary Ab.

The mean CV for intra-assay variation within a plate was 5, 8 and 8% for anti-SAA1, anti-AGP and anti-AAT, respectively. The mean CV for the inter-assay variation was 11% for anti-SAA1, 16% for anti-AGP and 19% for anti-AAT. We could observe that GCA patients had significantly higher anti-SAA1 (median (IQR) MFI was 2470 (1247-3554); p<0.0001) and anti-AAT Ab (340 (160-571); p<0.01) levels as compared to HBD (719 (546-1478) for anti-SAA1 and 165 (87-264) for anti-AAT), while no difference in anti-AGP Ab levels was observed between HBD and the tested groups of patients.

**Conclusion**

Serum IgG Abs to SAA1, AGP and AAT can be simultaneously measured using a customized triplex Luminex assay. These natural autoAbs could be endogenous immune-regulators of the acute phase response.

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