Targeted quantitative mass spectrometric immunoassay for analysis of serum amyloid A (SAA) in human plasma

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As a result of alternative splicing, single-nucleotide polymorphisms (SNPs) and posttranslational modifications (PTMs), proteins can exist as multiple proteoforms *in vivo*. These proteoforms may play important physiological roles, with possible implications in disease development. Targeted (immuno-based) mass spectrometric (MS) methods of detection hold great promise for analysis and quantification of these proteoforms. Presented here is the development and characterization of one such targeted mass spectrometric immunoassay (MSIA) [1, 2] for quantitative determination of serum amyloid A (SAA) proteoforms in human plasma samples.

SAA is an acute phase protein whose concentrations are known to increase in numerous inflammatory diseases as well as several chronic conditions, such as diabetes and chronic kidney disease [3-5]. As a result of differences in allelic manifestation and posttranslational processing (sequence truncations) SAA exists as several proteoforms *in vivo* [6]. In order to detect all these proteoforms in a single assay, we developed a quantitative MSIA utilizing beta lactoglobulin (BL) as an internal reference standard for quantification. The assay utilized affinity pipettes derivatized with anti-SAA and anti-BL antibodies, and followed a general immunoaffinity capture assay protocol, with the added benefit of a MS detection of the extracted and eluted proteoforms. SAA proteoforms concentration were determined using standard curves, generated by plotting the intensities ratio of the normalized SAA proteoforms and BL signals, against SAA standards concentration.

The intra- and inter-day precision and recovery characteristics of the assay were established, yielding CVs<10%. The new assay was also benchmarked against existing SAA ELISA, producing 2.2% Altman-Bland bias. Finally, the assay was utilized to determine the individual concentrations of the SAA proteoforms across a cohort of ~ 300 samples, revealing 7 different

SAA genetic polymorphic types and a total of 18 different proteoforms. The proteoforms were identified as products of *SAA1* allelic gene – SAA 1.1 and SAA 1.3 and their *N*-terminally truncated proteoforms: des-R 1.1, des-RS 1.1, des-RSFF 1.1, des-RSFFS 1.1, des-R 1.3, des-RS 1.3, des-RSFF 1.3 and des-RSFFS 1.3, and products of *SAA2* allelic gene – SAA 2.1 and SAA 2.2 and their *N*-terminally truncated proteoforms: des-R 2.1, des-RS 2.1, des-RSFF 2.1, des-RSFFS 2.1, des-R 2.2 and des-RS 2.2 (Figure 1).

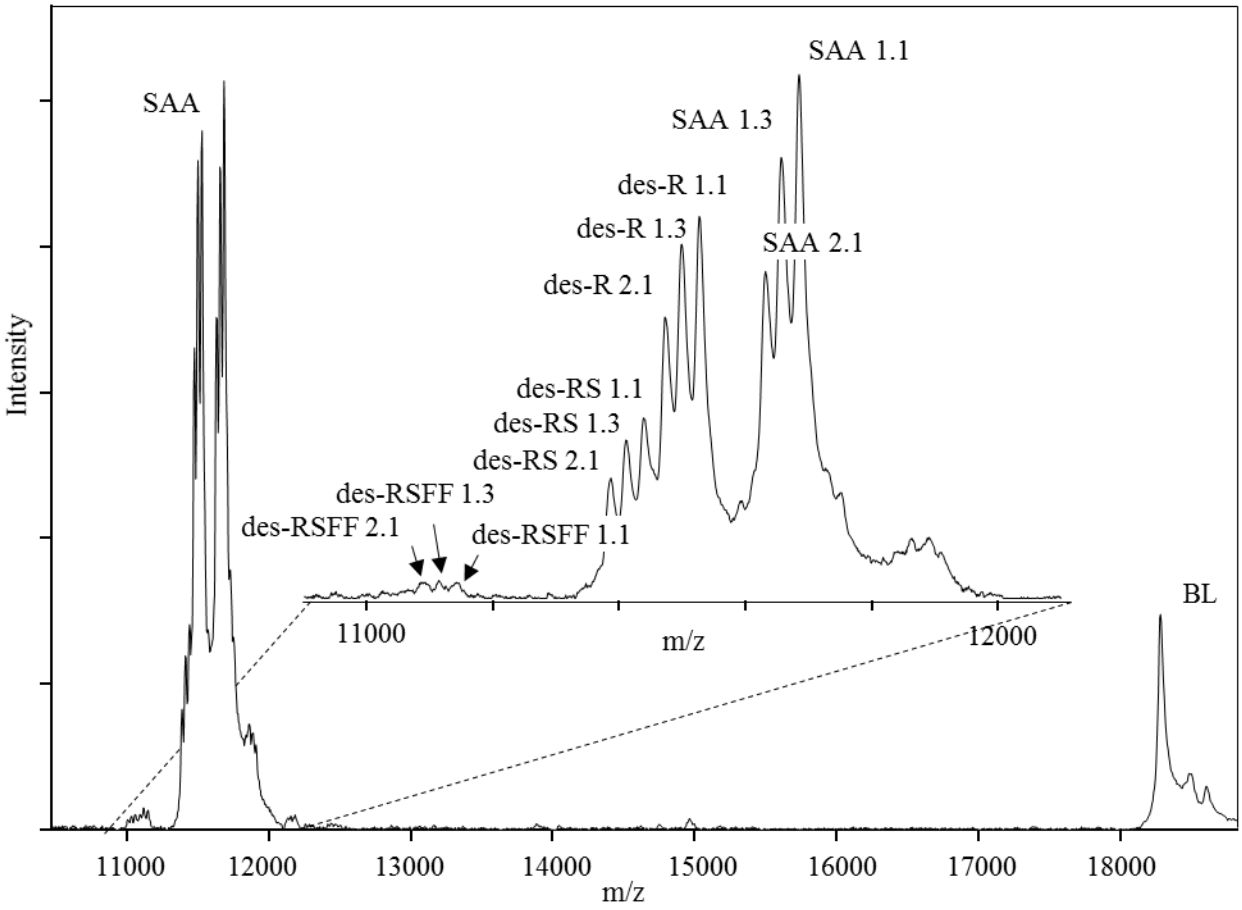

Figure 1: Example mass spectra from human plasma sample presenting multiple SAA proteoforms, identified using developed MSIA. Labeled signals represent SAA polymorphic and *N*-truncated proteoforms as explained in text.

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