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Quantitative analysis of soluble costimulatory molecules as potential diagnostic biomarkers for rheumatoid arthritis using LC-MS/MS in MRM mode

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Abstract: Background and aims Rheumatoid arthritis (RA) is a chronic autoimmune disease. RA-induced immunological responses are coordinated by T-cell stimulation. The costimulatory signal CD28-B7 is essential for T-cell activation by interacting CD28 with CD80 and CD86 costimulatory proteins. CTLA4 is another costimulatory protein that binds to CD80 and CD86 to inhibit T-cell activity. The soluble costimulatory proteins: sCD80, sCD86, sCD28, and sCTLA-4 were detected and quantified in human plasma and correlated with RA development. As potential diagnostic biomarkers for RA, developing a sensitive, specific, and reproducible method for quantifying these costimulatory molecules in human plasma and establishing quantitative ranges for each protein in healthy and RA patients' plasma is essential for advancing the clinical diagnostic and health outcomes. Materials and methods A novel quantitative liquid chromatography-tandem spectrometry (LC-MS/MS) technique using multiple reaction monitoring (MRM) modes was developed and validated to measure soluble costimulatory molecules sCTLA4, sCD28, sCD80, and sCD86 in human plasma samples. Furthermore, the method was applied to determine sCTLA4, sCD28, sCD80, and sCD86 levels in plasma samples from RA patients (n = 23) and healthy controls (n = 21). Results The method was successfully developed and validated according to international inter- and intra-assay precision and accuracy guidelines. The linearity of the method was achieved between 0.5 nM and 100 nM for each protein with a correlation coefficient of > 0.998. The plasma level of sCTLA4, sCD80, and sCD86 in RA patients was significantly elevated compared to controls. RA patients had 63.32 ? 17.63 nM sCTLA4 and controls 36.05 ? 18.83 nM; p < 0.0001. The performance of the four proteins was determined using ROC curves, where sCTLA4 showed the highest diagnostic and clinical performance compared to the others. Conclusions This study reports the first use of LC-MS/MS in MRM