Poster

[P25-7] P25-7: Immunosuppressive drugs (2): Monoclonal antibody and genotyping

Chair: Toru Hashida, Japan

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[P25-7-1] Quantification of active infliximab in human serum with LC-MS/MS

Mohsin El Amrani (University Medical Center Utrecht)

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Background

Infliximab (IFX), a tumor necrosis factor alpha (TNF-a) blocking agent, has been approved for the treatment of a wide range of autoimmune diseases. However, approximately 10% of patients develop anti-infliximab antibodies rendering the treatment ineffective. Early detection of underexposure to unbound IFX would result in a timely switch of therapy which could aid in the treatment of this disease. Traditionally, TDM of monoclonal antibodies such as IFX is performed by enzyme linked immunosorbent assays (ELISA). However, ELISA may lack selectivity due to a higher risk of cross reactivity as was demonstrated in a recent publication where different ELISA assays for IFX determination were compared (Vande Casteele etal, 2012 and J. Marini etal, 2015). Furthermore, LC-MS/MS has several advantages over ELISA such as wider linear dynamic range, shorter validation times and higher throughput and might be favorable for IFX quantification. Therefore, we have developed a quantitative LC-MS/MS method with immunoaffinity pre-analytical sample purification to determine the biological active IFX concentration in serum.

Methods

Biotinylated-TNF-a was coupled to streptavidin coated 96 well plate to selectively extract the active IFX fraction from 2ml serum. Stable isotope labeled Infliximab biosimilar was used as internal standard to correct for loss during purification, digestion and analysis. Sample and standard extracts were treated with 5mM Dithiothreitol, followed by an overnight digestion with 0.25mg trypsin. The liberated peptides were separated and the signature peptide was analyzed on a LC-MS/MS.

Results

The assay was successfully validated according to European Medicines Agency (EMA) guidelines and was found to be linear in a range of 0.5 –20 g/mL (r^2=0.994). Lower limit of quantification for the assay (<20% CV) was 0.5 g/mL, requiring only 2 L of sample. Cross-validation against enzyme-linked immunosorbent assay (ELISA) resulted in a high correlation between methods (r^2=0.95 with a ρ_c=0.83) and the accuracy was in line with previously published results.

Conclusions

In conclusion, a sensitive, robust and cost-effective method was developed for the bio-analysis of IFX with LC-MS/MS by means of a target-based pre-analytical sample purification. Moreover, low volume and costs of consumables per sample promote its feasibility in (pre)clinical studies and in therapeutic drug monitoring.