NEW METHODOLOGY TO RAPIDLY IDENTIFY RESPONDERS DURING INFliximab TREATMENT IN INFLAMMATORY DISEASES

Csaba Váradi1 and András Gutman1,2
1 Horváth Laboratory of Bioseparation Sciences, University of Debrecen, Hungary; 2 Sciex Separations, Brea, CA 92822

ABSTRACT
Prediction of responsiveness in biological therapies is an important and challenging issue in different diseases. Analyzing glycosylation pattern changes of key serum glycoproteins is one of the possible avenues to follow disease remission. The aim of this study was to investigate the changes of serum IgG glycoforms in Crohn’s disease and Rheumatoid arthritis patients in response to anti-tumor necrosis factor alpha (TNFα) treatment. IgG was isolated from patient serum samples using Protein A affinity pull-down, followed by the release of N-glycans with peptide-N-glycanase F. The released glycans were fluorescently tagged with aminopyrene-trisulfonate and analyzed by capillary gel electrophoresis with laser induced fluorescent detection. Significant alterations were detected between responders and non-responders in both disease groups. In Crohn’s disease patients, disease specific alteration was found in response to anti-TNFα therapy, which was also confirmed by transcriptomics data analysis of the corresponding glycosyltransferases and glycosidases.

METHODS

RESULTS

As it shown in Figure 2 (A, B, C), three low (>2%) abundant structures of A2(3)G1, A2B(3)G1 and F2AB(3)G1 were found to be significantly different between responders (R) and non-responders (NR). RA patients before anti-TNFα treatment. Higher galactosidase activity was found in non-responders suggesting the reason of the detected lower galactosylation level compared to responders (Figure 2D).

CONCLUSIONS
Prediction of patient response for any therapy is critical in inflammatory diseases such as CD and RA, as currently used scoring systems require months for responder identification. The importance of reliable biomarkers in these diseases is essential as the efficacy of biological therapies can vary between patients. In this paper a highly sensitive and high resolution CE-LIF based method was applied to examine serum IgG glycosylation changes in autoimmune diseases (CD and RA) before and two weeks after anti-TNFα therapy in order to identify responders and non-responders. Utilizing the high resolving power of CE-LIF, 26 glycoforms were separated and relatively quantified. In RA, three low abundant galactosylated structures were found to be significantly different before the treatment where in all of the cases responders showed higher galactosylation level. No significant alteration was detected in RA in response to the treatment. In CD significant differences were detected in galactosylation level between responders and non-responders before the treatment (higher in the responder group). FA2G2S1 level was significantly increased in response to anti-TNFα therapy, thus being a possible candidate marker for responder identification. The level of this structure was not significantly altered in any of the RA groups suggesting disease specificity for CD. Our findings were also supported by transcriptomics analysis of the corresponding glycosyltransferase and glycosidase activity, as higher sialyltransferase and lower sialidase activity were found.

REFERENCES

ACKNOWLEDGMENT
The authors acknowledge the help of Zsofia Holló, Szilárd Póliska, László Nagy, Zoltán Szekanecz, Andrea Vánca, Károly Palatka.

TRADEMARKS/LICENSING
For Research Use Only. Not for use in diagnostic procedures. © 2014 AB SCIEX. SCIEX is part of AB SCIEX. The trademarks mentioned herein are the property of AB SCIEX Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.