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

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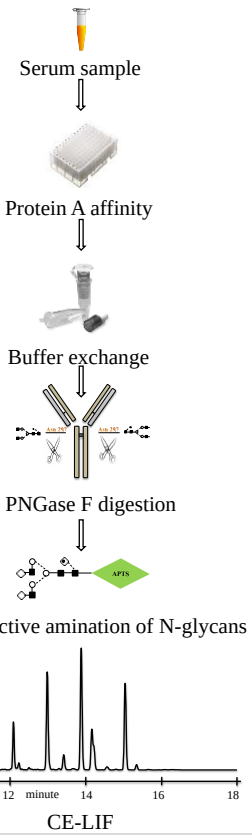
ABSTRACT

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 glycosyl-transferases and glycosidases.

METHODS



Scheme 1. Serum IgG glycosylation analysis workflow

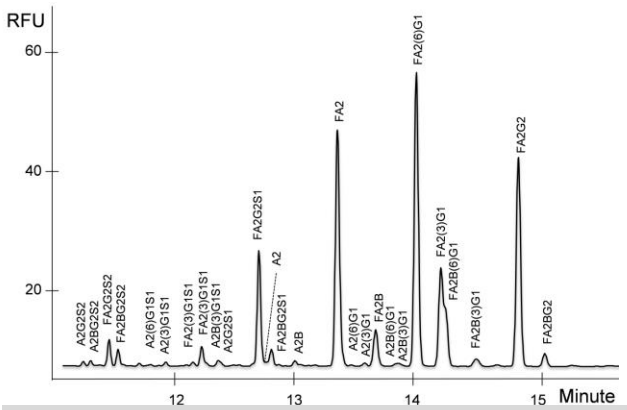


Figure 1. CE-LIF trace of IgG N-glycan structures from human serum. Conditions: 50 cm effective length (60 cm total) N-CHO coated, 50 µm i.d. capillary columns filled with N-CHO Carbohydrate Separation Gel Buffer, E=500 V/cm, reversed polarity, Samples were injected by pressure: 1 psi (6.89 kPa) for 5 sec.

RESULTS

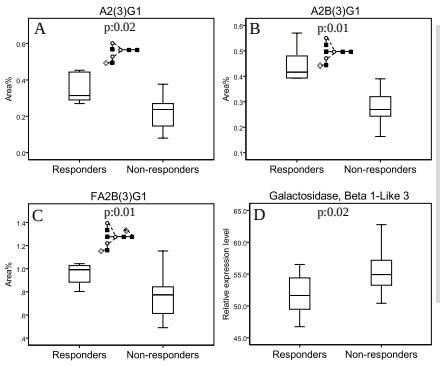


Figure 2. Glycosylation and gene expression based differences between responders and non-responders in RA before anti-TNFα treatment.

As it shown in Figure 2 (A, B, C), three low (>2%) abundant structures of A2(3)G1, A2B(3)G1 and FA2B(3)G1 were found to be significantly different between responders (R) and non-responders (NR) of 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).

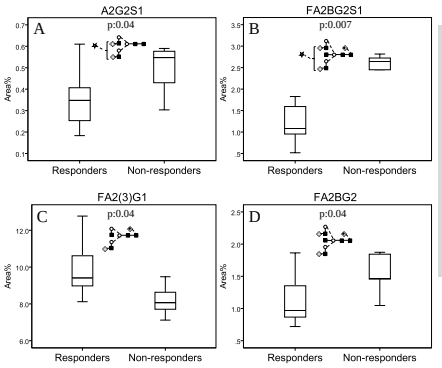


Figure 3. Differences of IgG glycoform levels in CD between responders and non-responders before anti-TNFα treatment.

Similarly to RA, even before the start of the treatment significant differences were revealed between the two subgroups (R vs NR). Using Mann-Whitney pairwise comparison, A2G2S1 (Figure 3A), FA2BG2S1 (Figure 3B), FA2(3)G1 (Figure 3C) and FA2BG2 (Figure 3D) were found to be significantly different although it has to be noted that among these, only the FA2(3)G1 is not a low abundant structure.

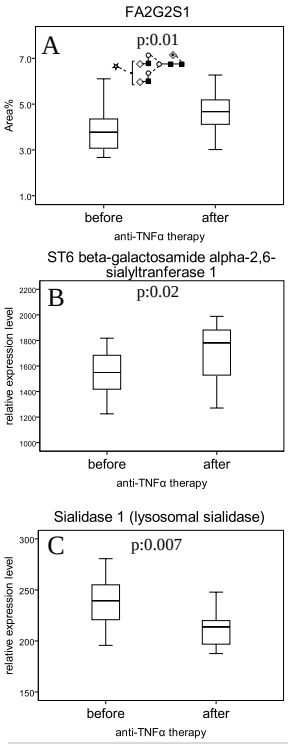


Figure 4. Significant glycosylation and trans-criptomics changes in response to anti-TNFα therapy.

The main goal of this study was to find glycosylation markers that were significantly altered in response to anti-TNFα treatment and thereby identify responders. We have successfully found an IgG glycoform, which level was significantly altered 2 weeks after the therapy. In the responder group of CD, FA2G2S1 was found to be significantly (p=0.01) higher 2 weeks after anti-TNFα treatment (Figure 4A). To confirm our findings, the expression levels were determined for the relevant sialyl-transferases and sialidases. Significant differences were found only in the responder group of CD where higher sialyl-transferase activity (Figure 4B) and lower sialidase activity (Figure 4C) was detected 2 weeks after the infliximab treatment.

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 glycosyl-transferase and glycosidase enzymes, as higher sialyl-transferase and lower sialidase activity were found.

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