Serum IgG N-glycans act as novel serum biomarkers of ankylosing spondylitis

Ankylosing spondylitis (AS) is a chronic inflammatory disease with poorly defined aetiologies and no curative treatments. The average delay in the diagnosis of AS is 6–8 years.[1] Human leukocyte antigen B27 (HLA-B27) is a key laboratory marker for AS presenting in at least 90% of patients with AS.[2] However, 63%–90% of patients with reactive arthritis[3] and 19.2% of patients with psoriatic arthritis (PsA)[4] are also positive for HLA-B27, indicating low specificity of HLA-B27. The risk of development of AS in an HLA-B27-positive individual is only 2%–10%,[5] which suggests the limited value of HLA-B27 in supporting an AS diagnosis. Moreover, reported serum biomarkers for AS have generally exhibited low sensitivity or specificity[6] (<60%). Novel serum biomarkers with high prediction capacity remain needed.

The changed IgG glycosylation in autoimmune and inflammatory conditions, as well as the broad roles for specific IgG glycoforms in maintaining immune homeostasis, have been well documented.[7 8] However, specific glycan biomarkers on IgG for AS have not been fully identified. In our previous study, a specialised microfluidic titanium dioxide-porous graphitised carbon chip was developed; this approach enabled the quantification of low-abundance and trace acidic glycans that are often biologically important species. In glycomic analyses of serum IgG in patients with rheumatoid arthritis (RA), two sulfated N-glycans were identified as promising biomarkers for seronegative RA.[9] In the current study, we used this glycomic approach to analyse serum IgG in patients with AS and identified potential N-glycan biomarkers of AS for the first time.

Eighty patients who exhibited definite AS that fulfilled the modified New York criteria (1984) from three hospitals in China and 80 age-matched and gender-matched healthy volunteers were enrolled in this study. The determined levels of individual N-glycans[9] were used as variations for the classification. In total 160 samples were divided into a training set (n=56) and a validation set (n=104) (online supplementary table 1).

By using the feature selection methods in WEKA,[9] 11 neutral and 6 acidic N-glycans were selected as potential biomarkers for the classification of AS (online supplementary table 2). Two of the 17 biomarkers, 5_5_1_0 and 6_5_0_3-a (figure 1A,D), demonstrated relatively high prediction capacity for AS, with area under the curve (AUC), sensitivity and specificity greater than 70% for both the training and validation sets (figure 1B,E). Of note, significantly higher AUCs (0.823 and 0.911), sensitivities (75% and 86.5%) and specificities (82.1% and 80.8%) in training and validation sets, respectively, were observed for a combination of these two N-glycan biomarkers (online supplementary table 2). Univariate analysis showed significant differences in the levels of these two markers between the control and AS groups (figure 1C,F), while no significant alterations were observed in patients with PsA (online supplementary figure 1, online supplementary tables 3 and 4). Moreover, we noted a correlation between the levels of glycan 5_5_1_0 and erythrocyte sedimentation rate (ESR) ($|r|=0.42$, $p=0.0001$), and observed more significant reduction of this glycan in the subgroup with elevated ESR (online supplementary figure 2). No such correlation was observed for glycan 6_5_0_3-a ($|r|=0.11$, $p=0.3328$). Influence from impurity (IgA and IgM) was proved to be slight (<5%; online supplementary table 5).

In conclusion, we identified N-glycan-based biomarkers for patients with AS for the first time. Two N-glycans which are overwhelmingly from IgG exhibited relatively high sensitivity and specificity for the classification of AS. Given the crucial roles of N-glycans of IgG for immune homeostasis and inflammation, the identified biomarkers could serve as additional measures of disease phenotype, predict patients' responsiveness to treatment and provide new insight into the pathogenesis for AS. We anticipate that large-scale studies on the roles of N-glycans in AS could be profoundly conducted further.

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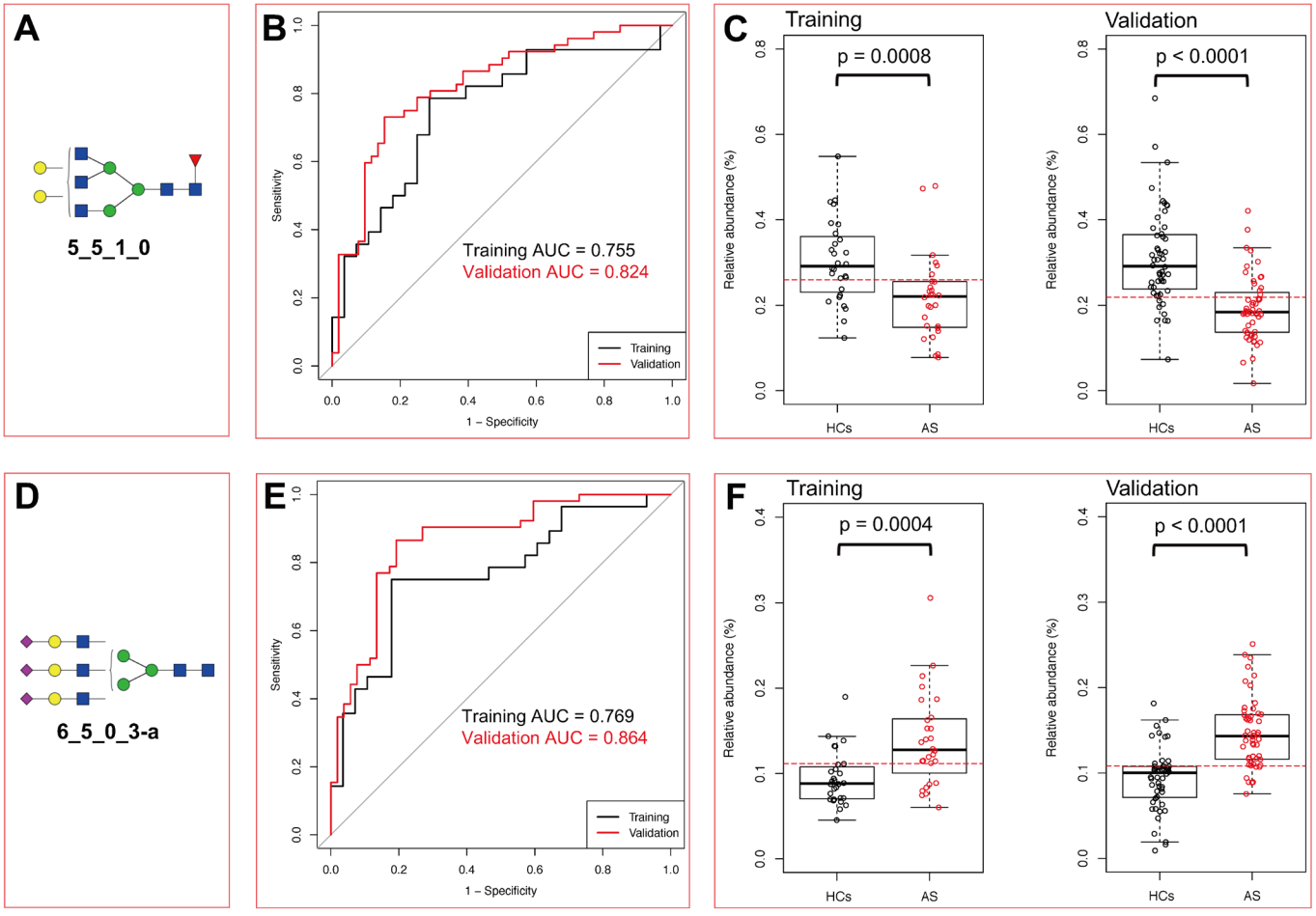

Figure 1 Performance and relative abundances of the two potential N-glycan biomarkers for ankylosing spondylitis (AS) in the training set (AS, n=28; healthy controls (HCs), n=28) and validation set (AS, n=52; HCs, n=52). A and D show the symbols depicting N-glycan biomarkers identified in the current study. B and E show the receiver operating characteristic curves of biomarkers for the classification of AS and HCs. C and F show the boxplots for the levels of the biomarkers in AS and HCs. The red dotted lines in the figures represent the cut-off values determined based on the maximum values generated using the formula, sensitivity+specificity – 1, in our analyses. A and D were drawn using GlycoWorkbench V.2.1 stable (build: 157) (developed by Alessio Ceroni, KAI Maass, and David Damerell, European carbohydrates database, Europe), and B, C, E and F were drawn using RStudio V.1.0.153 (RStudio, Boston, USA). AUC, area under the curve.

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