The role of Fc-glycosylation of anti-HLA antibodies in platelet refractoriness

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Abstract

Prophylactic and supportive platelet transfusions significantly reduce mortality and hemorrhagic complications in patients with thrombocytopenia. However, in approximately 5-15% of patients with chronic platelet support, rapid clearance of the transfused platelets (platelet refractoriness) is observed, a process which is mainly attributable to anti-HLA antibodies. These antibodies are responsible for the rapid clearance of donor platelets, theoretically via several immunological pathways, including IgG-Fc Receptor (FcγR)-mediated phagocytosis and complement activation. For unknown reasons, not all patients with anti-HLA antibodies develop refractoriness to unmatched platelet transfusions. Therefore, the Fc glycosylation of these antibodies could play an important role in the disease outcome, as it is known to affect the effector functions of antibodies.

In this study, we analyzed the Fc glycosylation of anti-HLA A2, A24 and B7 antibodies from patients receiving platelet transfusions, who were included in the PREPAReS Trial, using nLC-MS. We found IgG-Fc glycosylation of anti-HLA antibodies to be highly variable between patients, especially with respect to galactosylation and sialylation, which were significantly increased for the majority of the patients. Furthermore the level of galactosylation and sialylation of anti-HLA antibodies correlated with the transfusion outcome (CCIs). Next, we have produced recombinant glycoengineered anti-HLA monoclonal antibodies with varying galactosylation and sialylation levels and studied their effect on the classical complement pathway. We observed that anti-HLA monoclonal antibodies with different specificities, binding simultaneously to the same HLA-molecules, interact synergistically with the first component of the classical pathway, C1q. Furthermore, elevated Fc galactosylation increased the complement activating properties of anti-HLA mAbs significantly and a small additional effect was observed for elevated Fc sialylation. We propose that these factors can shift the balance from a relatively benign response to clinically relevant response, leading to complement dependent lysis and the rapid clearance of donor platelets in platelet refractoriness.

1 Increased Fc-Galactosylation and Sialylation of anti-HLA-I antibodies observed in patients receiving platelets transfusions

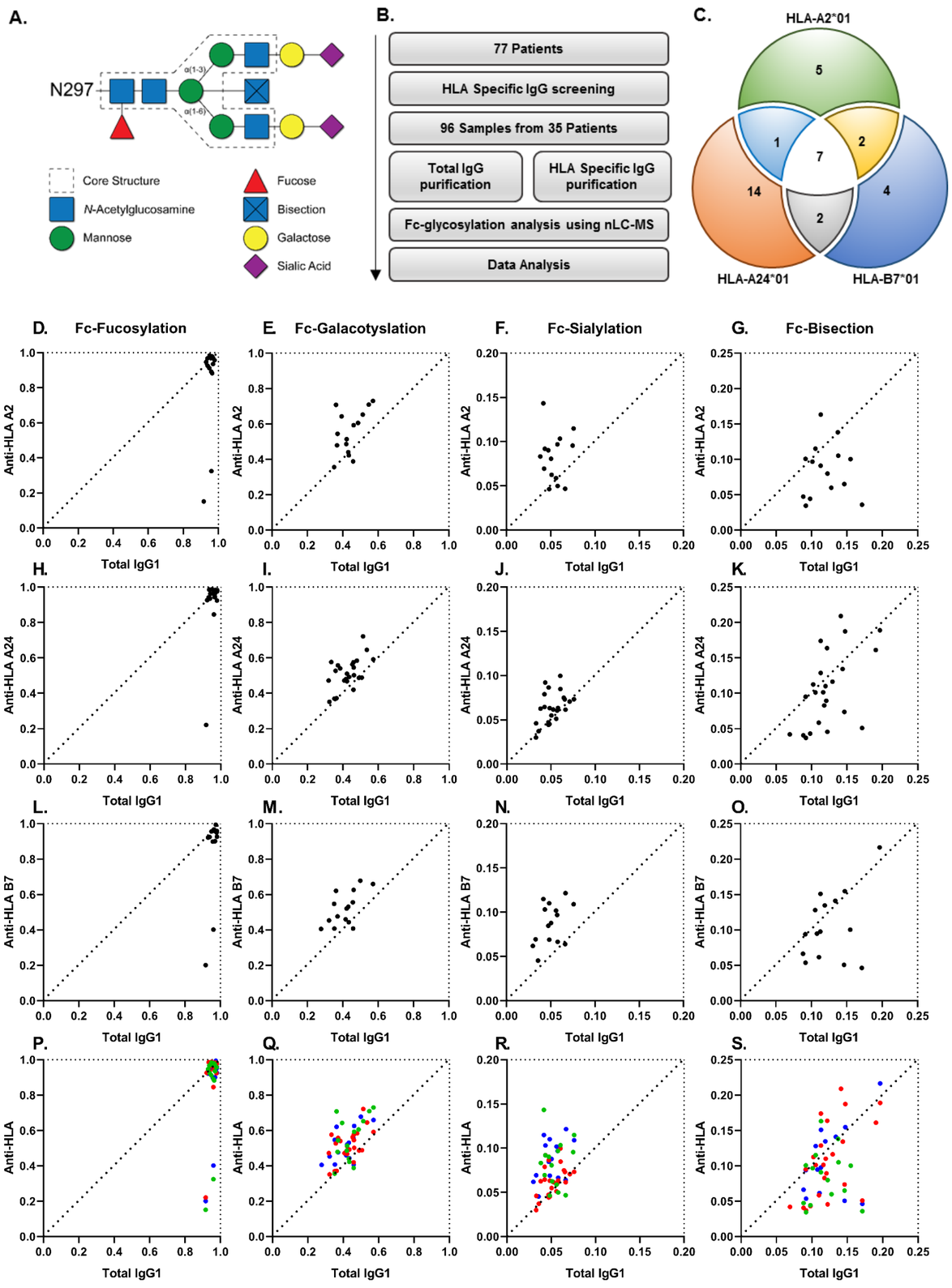


Figure 1 | Fc glycosylation analysis of total and anti-HLA specific IgG1 A) Depiction of the N297-linked glycan structure with symbols depicting individual monosaccharides. **B)** Flow chart of the entire workflow. **C)** Venn Diagram showing the HLA-specific antibodies shared between 35 investigated patients. **D-S) Fc glycosylation profiles of anti-HLA specific (y-axis) and total IgG1 (x-axis) for 35 patients analyzed by nLC-MS** The glycosylation profile was divided into the major glycan traits fucosylation, galactosylation, sialylation and bisection. Each dot represents a patient. The mean percentage was calculated when multiple serum samples were available per patient. The statistical difference was calculated using the paired t test. Fc galactosylation and sialylation was significantly higher for anti-HLA antibodies compared to the total IgG1. Bisection was decreased for the majority of the patients and two patients showed lower levels of fucosylation of anti-HLA antibodies.

2 Fc Galactosylation and Sialylation correlates with transfusion outcome

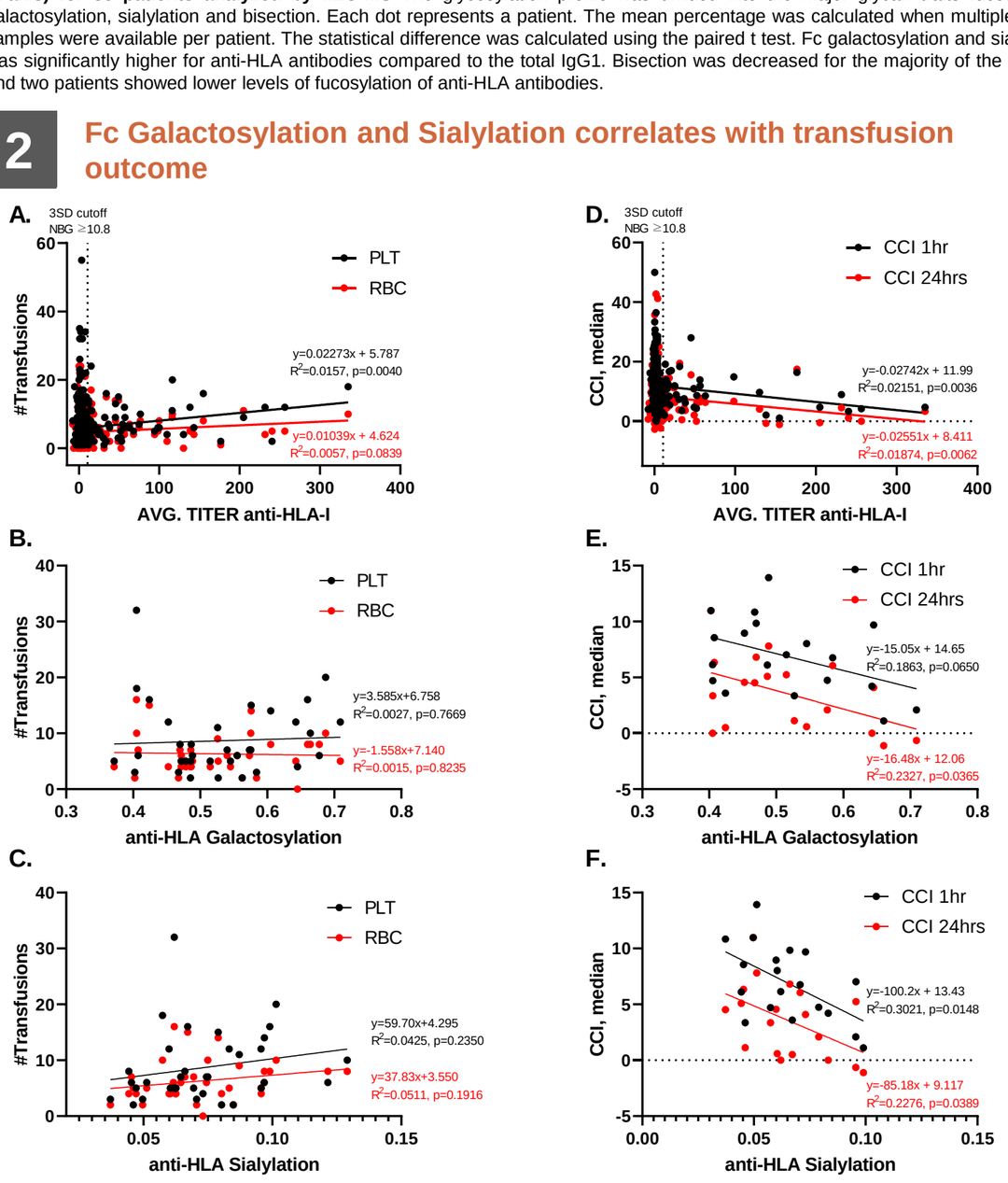
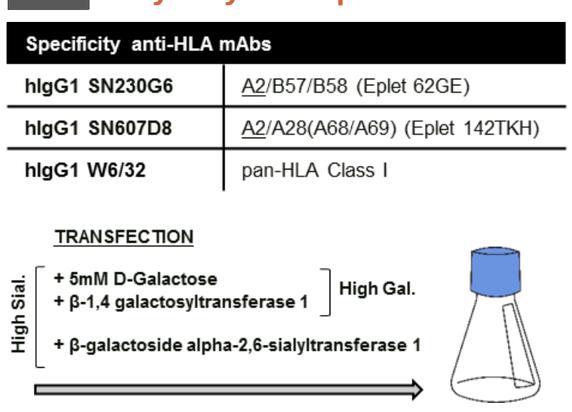


Figure 2 | Correlation between the number of transfusions, Corrected Count Increments (CCI 1 and 24hrs) and anti-HLA titer and Glycosylation. The amount of platelet and red blood cell transfusions received during the study were plotted against **A)** the total anti-HLA-I levels **B)** the anti-HLA-I Fc Galactosylation and **C)** the anti-HLA-I Fc Sialylation. The median corrected count increments (CCI) at 1hr and 24hrs are plotted against **D)** the total anti-HLA-I levels **E)** the anti-HLA-I Fc Galactosylation and **F)** the anti-HLA-I Fc Sialylation. Each dot represents a patient and the relationship between parameters were modeled using linear regression models.

3 Production of anti-HLA monoclonal antibodies with altered Fc-Glycosylation profiles

Specificity anti-HLA mAbs	
hIgG1 SN230G6	A2/B57/B58 (Eplet 62GE)
hIgG1 SN607D8	A2/A28(A68/A69) (Eplet 142TKH)
hIgG1 W6/32	pan-HLA Class I



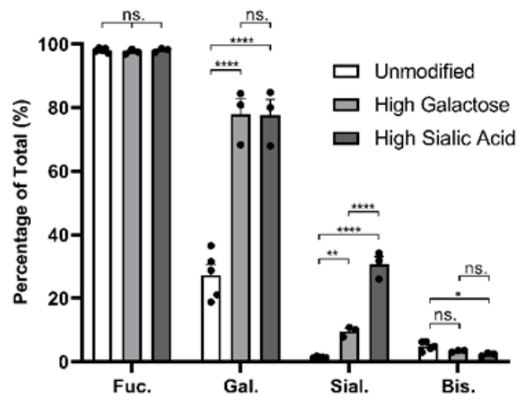


Figure 3 | A) Antibody specificity of the anti-HLA monoclonal antibodies B) Glycoengineering techniques to produce mAbs with increased Fc-galactosylation and –sialylation, via the addition of relevant substrates and constructs coding for enzymes prior/during transfection. C) Fc-glycosylation profiles of produced anti-HLA mAbs using different glycoengineering techniques to increase Fc galactosylation and sialylation, analysed by mass spectrometry.

4 Synergistic properties of anti-HLA mAbs on complement activation, which is further enhanced by Fc galactosylation and sialylation

Figure 4 | HLA-matched Platelets were incubated with glycoengineered anti-HLA mAbs in the presence of complement rich serum. IgG-Binding, C1q-Binding and C3b-Deposition was measured using Flow Cytometry (MFI). A-C) Properties of singular anti-HLA mAbs. **D-F)** Differences between combinations of unmodified anti-HLA mAbs. **G-O)** Differences in IgG-Binding and Complement Activation Properties of combinations of glycoengineered anti-HLA mAbs. The data represents three independent experiments (N=3). The combinations of anti-HLA mAbs induce enhanced complement activation, which is further increased by elevated Fc-Galactosylation and Fc-Sialylation.

5 Fc galactosylation promotes hexamerization of anti-HLA antibodies, leading to enhanced classical complement activation

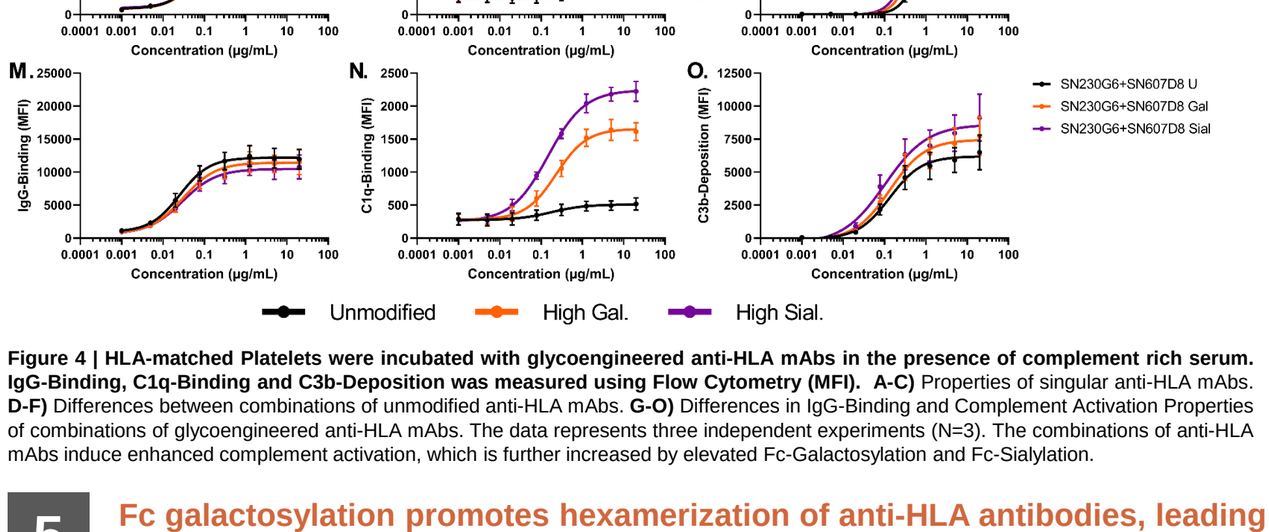


Figure 5 | A-D) Incompatible platelet transfusions result in the formation of anti-HLA antibodies. A polyclonal antibody response will lead to the binding of multiple anti-HLA antibodies, recognizing different epitopes, to a singular HLA-Class I Molecule on the surface op platelets. Fc-glycosylation impacts the potential of antibodies to form hexamers with neighbouring Fc-tails, which forms the optimal platform C1q to bind. This leads to activation of the C1q-R_2-S_2 complex, starting the complement cascade by depositing C4b and C3b in the process, which ultimately leads to the formation of the membrane attack complex (MAC) and lysis of platelets.