Altered glycosylation profile of anti-HPA-1a-specific antibodies: insights from a prospective fetal and neonatal alloimmune thrombocytopenia cohort

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https://doi.org/10.3324/haematol.2025.288701

Received: July 14, 2025.
Accepted: November 28, 2025.
Early view: December 4, 2025.

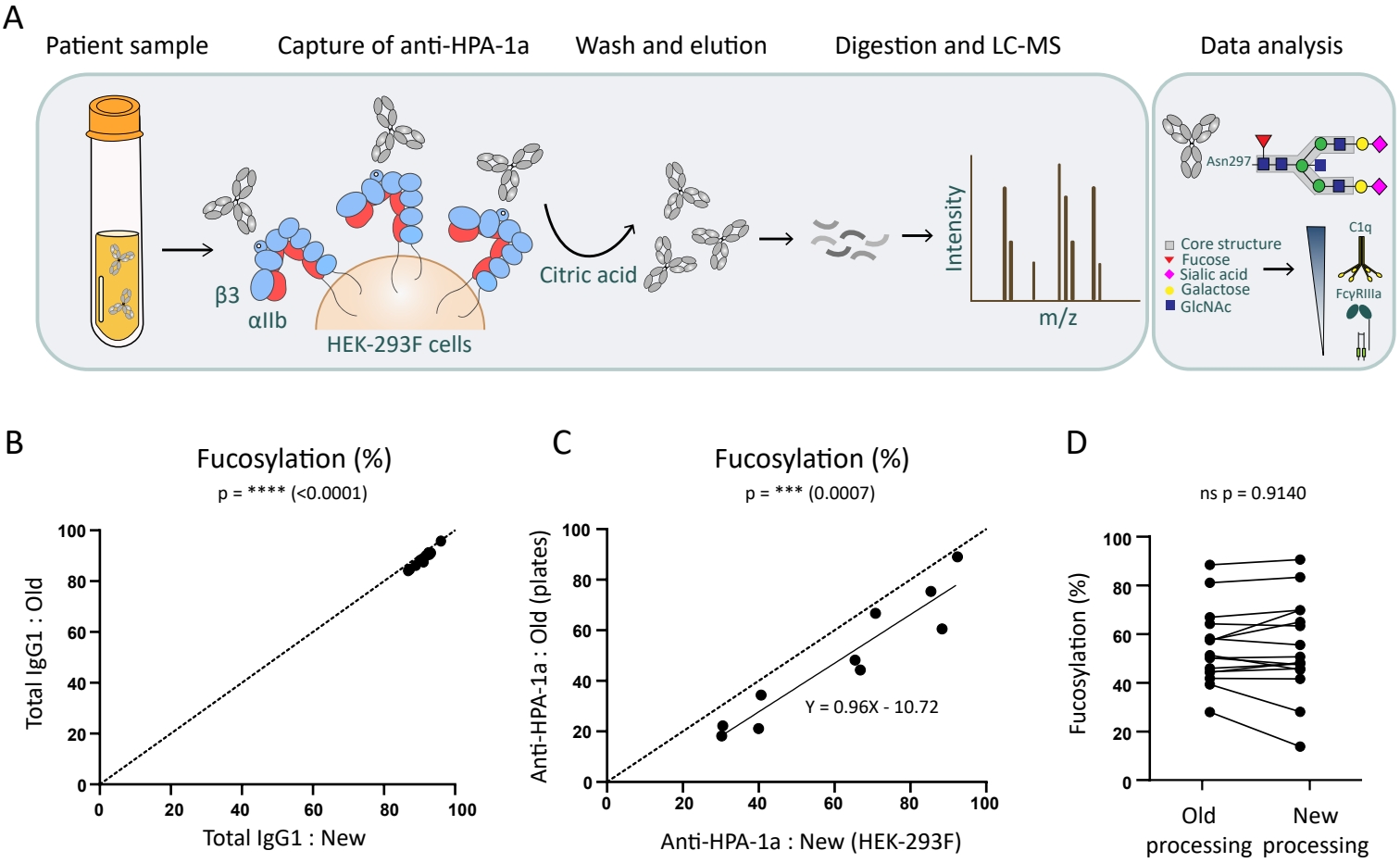
Supplementary figure legends

Supplementary figure 1: Isolation of anti-HPA-1a antibodies using HEK-293F cells expressing integrin αIIbβ3. (A) Schematic overview of antibody isolation. A total of 5x105 HEK293F cells expressing either αIIbβ3 or αvβ3 were seeded and incubated with 50µl (HIP samples) or 20µl (retrospective samples) serum for 30min at 4°C to absorb all specific antibodies. Cells were washed extensively afterwards to remove aspecific proteins and unbound antibodies. Elution of bound IgG was performed with citric acid buffer (pH2.8) and subsequent neutralization was performed with Tris (pH9.0). After digestion, IgG-Fc glycans were analyzed by mass spectrometry. LC-MS signals were integrated in LaCyTools (version 2.1.0, build 20230525) and further analyzed in GlycoDash (version 1.5.3). Settings of LaCyTools and GlycoDash processing report is provided as Supplementary Information. The Fc-glycan structures of antibodies influence the binding strength to C1q, the first component of the complement system, and FcγR's, which facilitates antibody-dependent cellular cytotoxicity and phagocytosis. **(B)** Antibodies were isolated from 10 retrospective FNAIT samples from the Finnish cohort. The fucosylation degree of total and **(C)** specific IgG were compared to previously obtained data from an antigen-coated plate-based isolation method. The dotted line represents the equal fucosylation degree of HPA-1a-specific IgG between the old isolation method and current isolation method. **(D)** The current automated data processing workflow was compared to the previous largely manual data processing to observe the effect of the processing on IgG-Fc fucosylation outcome. We tested if differences in data processing were responsible for these minor alterations in glycosylation values compared with our previous work. After re-analyzing the previous original ion trap data with the new software-based workflow, no significant differences were observed. Simple linear regression was performed in (C) and slopes of the indicated equation in (C) and Y=X were compared which were not significantly different. Statistical analysis was performed using two-tailed paired t-test analysis comparing specific and total IgG glycosylation. Statistically significant differences are indicated by asterisks: ***<0.001, ****<0.0001, ns; not significant.

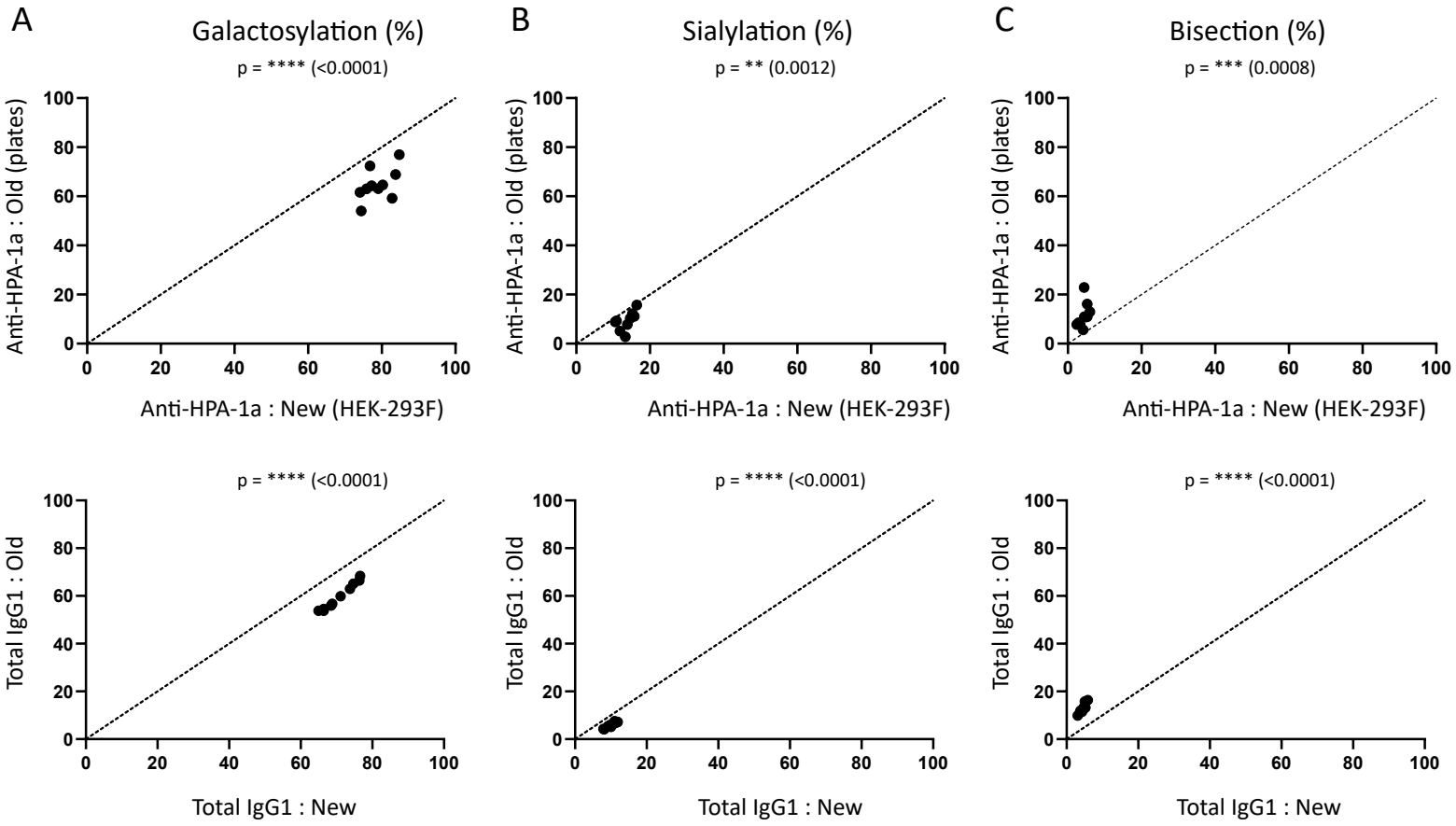
Supplementary figure 2: Glycosylation traits in the newer method are slightly higher than in the older method except for bisection. The glycosylation traits of 10 Finnish samples are compared between the new isolation method using HEK-293F cells and the old method using antigen-coated plates. **(A)** Galactosylation, **(B)** sialylation and **(C)** bisection are compared of specific IgG (upper panels) and total IgG (lower panels). The dotted line represents the equal glycosylation degree of HPA-1a-specific IgG between the old isolation method and current isolation method. Statistical analysis was performed using two-tailed paired t-test analysis comparing specific and total IgG glycosylation. Statistically significant differences are indicated by asterisks:**<0.01, ***<0.001, ****<0.0001, ns; not significant.

Supplementary figure 3: Glycosylation traits of the HIP samples. The **(A)** fucosylation, **(B)** galactosylation, **(C)** sialylation and **(D)** bisection degree of the HIP samples. The black dots indicate cases with no clinical symptoms, orange dots represent mild cases and the red dot represents the severe FNAIT case. The dotted line represents the equal glycosylation degree of HPA-1a-specific IgG and total IgG. Statistical analysis was performed using two-tailed paired t-test analysis comparing specific and total IgG glycosylation. Statistically significant differences are indicated by asterisks:***<0.001, ****<0.0001.

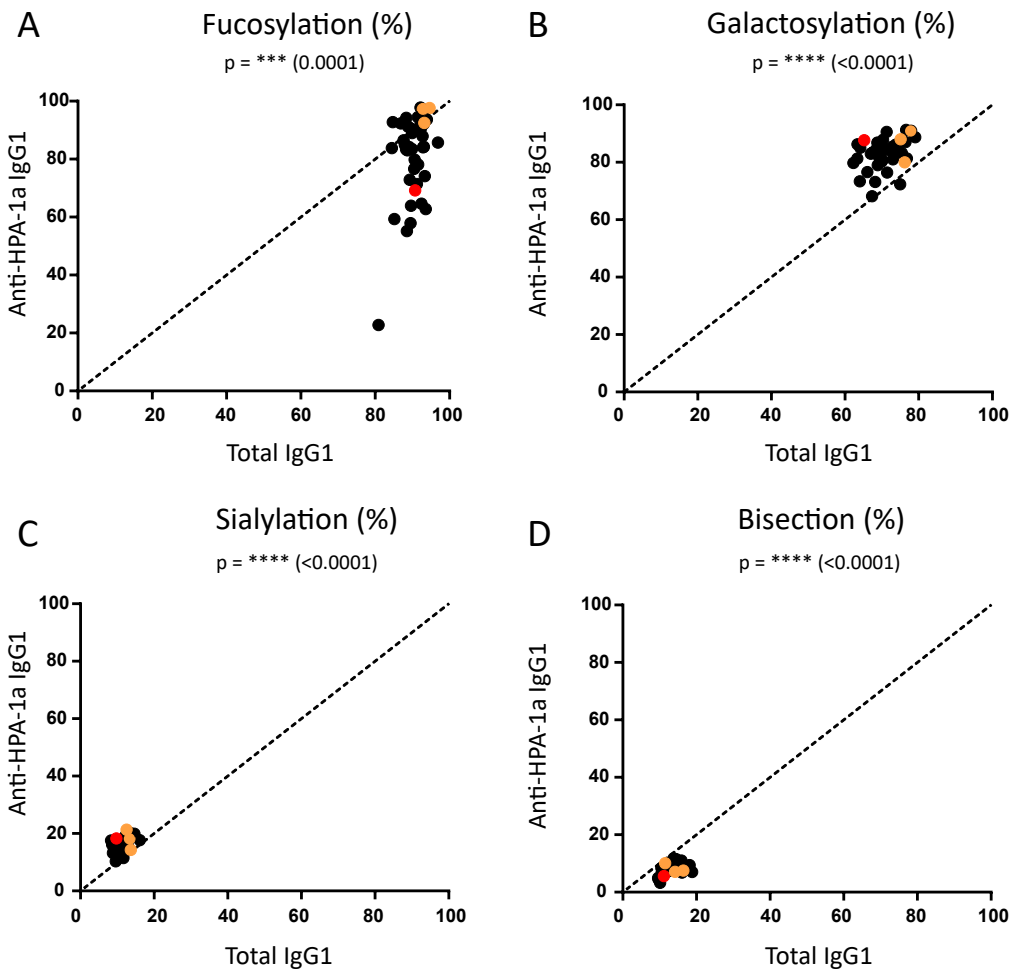
Supp Fig 1


A
Patient sample
Capture of anti-HPA-1a
Wash and elution
Digestion and LC-MS
Data analysis
β3
αIIb
HEK-293F cells
Citric acid
Intensity
m/z
Asn297
Core structure
Fucose
Sialic acid
Galactose
GlcNAc
C1q
FcγRIIIa
B
Fucosylation (%)
p = **** (<0.0001)
Total IgG1 : Old
Total IgG1 : New
C
Fucosylation (%)
p = *** (0.0007)
Anti-HPA-1a : Old (plates)
Anti-HPA-1a : New (HEK-293F)
Y = 0.96X - 10.72
D
ns p = 0.9140
Fucosylation (%)
Old processing
New processing

Supp Fig 2


A
Galactosylation (%)
p = **** (<0.0001)
Anti-HPA-1a : Old (plates)
Anti-HPA-1a : New (HEK-293F)
B
Sialylation (%)
p = ** (0.0012)
Anti-HPA-1a : Old (plates)
Anti-HPA-1a : New (HEK-293F)
C
Bisection (%)
p = *** (0.0008)
Anti-HPA-1a : Old (plates)
Anti-HPA-1a : New (HEK-293F)
p = **** (<0.0001)
Total IgG1 : Old
Total IgG1 : New
p = **** (<0.0001)
Total IgG1 : Old
Total IgG1 : New
p = **** (<0.0001)
Total IgG1 : Old
Total IgG1 : New

Supp Fig. 3


A
Fucosylation (%)
p = *** (0.0001)
Anti-HPA-1a IgG1
Total IgG1
B
Galactosylation (%)
p = **** (<0.0001)
Anti-HPA-1a IgG1
Total IgG1
C
Sialylation (%)
p = **** (<0.0001)
Anti-HPA-1a IgG1
Total IgG1
D
Bisection (%)
p = **** (<0.0001)
Anti-HPA-1a IgG1
Total IgG1
0
20
40
60
80
100