Changes in IgG and total plasma protein glycomes in acute systemic inflammation

Mislav Novokmet, Edita Lukić, Frano Vučković, Željko Đurić, Toma Keser, Katarina Rajšl, Daniel Remondini, Gastone Castellani, Hrvoje Gašparović, Olga Gornik and Gordan Lauc

Supplementary Information (Supplementary figure 1; Supplementary figure 2; Supplementary table 1; Supplementary table 2; Supplementary table 3)

GP1 GP2 GP3 GP4 GP5 GP6

GP7 GP8 GP9 GP10 GP11 GP12

GP13 GP14 GP15 GP16 GP17 GP18

GP19 GP20 GP21 GP22 GP23 GP24

GP1n GP2n GP4n GP5n GP6n GP7n

GP8n GP9n GP10n GP11n GP12n GP13n

GP14n GP15n % G0n % G1n % G2n % Fn total

% FG0n total/G0n % FG1n total/G1n % FG2n total /G2n % Fn % FG0n/G0n % FG1n/G1n

% FG2n/G2n % FBn % FBG0n/G0n % FBG1n/G1n % FBG2n/G2n FBn/Fn

FBn/Fn total Fn/(Bn + FBn) Bn/(Fn + FBn) ‰ FBG2n/FG2n FBG2n /(FG2n + FBG2n) FG2n/(BG2n + FBG2n)

BG2n/(FG2n + FBG2n) ‰ FGS/(FG+FGS) FBGS/(FBG+FBGS) FGS/(F+FG+FGS) FBGS/(FB+FBG+FBGS) FG1S1/(FG1+FG1S1)

FG2S1/(FG2+FG2S1+FG2S2) FG2S2/(FG2+FG2S1+FG2S2) FBG2S1/(FBG2+FBG2S1+FBG2S2) FBG2S2/(FBG2+FBG2S1+FBG2S2) FtotalS1/FtotalS2 FS1/FS2

FBS1/FBS2 FBStotal/FStotal FBS1/FS1 FBS1/(FS1+FBS1) FBS2/FS2 FBS2/(FS2+FBS2)

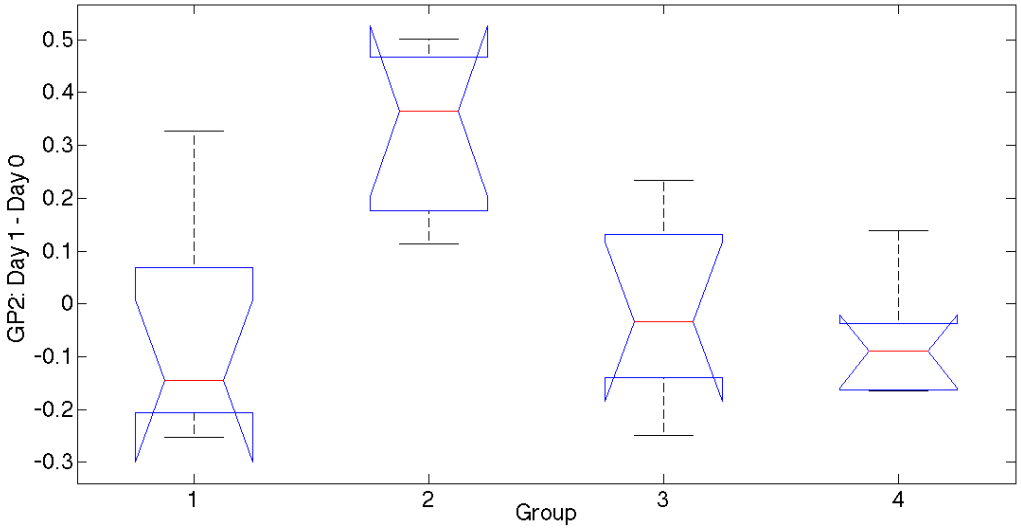
value

Day

Patient
100
75
50
25

Supplementary figure 1. Changes in *N*-linked IgG glycans through the initial phase of acute inflammatory response induced by cardiac surgery. Day 0 represents level of glycan group before surgical procedure, day 1 on the first day after the surgical procedure and day 3 on the third day after the surgery. The same patient is not represented by the same colour in all graphs, but a same spectrum is used for better visualisation.
Derived glycosylation traits were approximated from the ratios of glycan peaks (G1-G24) each of which combined the glycans with the same structural characteristics (see Figure 4). The percentage of sialylation of fucosylated galactosylated structures without bisecting GlcNAc in total IgG glycans-FGS / (FG + FGS) = SUM (GP16 + GP18 + GP23) / SUM (GP16 + GP18 + GP23 + GP8 + GP9 + GP14)*100; the percentage of sialylation of fucosylated galactosylated structures with bisecting GlcNAc in total IgG glycans-FBGS / (FBG + FBGS) = SUM (GP19 + GP24) / SUM (GP19 + GP24 + GP10 + GP11 + GP15)*100; the percentage of sialylation of all fucosylated structures without bisecting GlcNAc in total IgG glycans-FGS / (F + FG + FGS) = SUM (GP16 + GP18 + P23) / SUM (GP16 + GP18 + GP23 + GP4 + GP8 + GP9 + GP14)*100; the percentage of sialylation of all fucosylated structures with bisecting GlcNAc in total IgG glycans-FBGS / (FB + FBG + FBGS) + SUM (GP19 + GP24) / SUM (GP19 + GP24 + GP6 + GP10 + GP11 + GP15)*100; the percentage of monosialylation of fucosylated monogalactosylated structures in total IgG glycans-FG1S1 / (FG1 + FG1S1) = GP16 / SUM (GP16 + GP8 + GP9)* 100; the percentage of monosialylation of fucosylated digalactosylated structures in total IgG glycans-FG2S1 / (FG2 + FG2S1 + FG2S2) = GP18 / SUM (GP18 + GP14 + GP23)* 100; the percentage of disialylation of fucosylated digalactosylated structures in total IgG glycans-FG2S2 / (FG2 + FG2S1 + FG2S2) = GP23 / SUM (GP23 + GP14 + GP18)* 100; the percentage of monosialylation of fucosylated digalactosylated structures with bisecting GlcNAc in total IgG glycans-FBG2S1 / (FBG2 + FBG2S1 + FBG2S2) = GP19 / SUM (GP19 + GP15 + GP24)* 100; the percentage of disialylation of fucosylated digalactosylated structures with bisecting GlcNAc in total IgG glycans-FBG2S2 / (FBG2 + FBG2S1 + FBG2S2) = GP24 / SUM (GP24 + GP15 + GP19)* 100; ratio of all fucosylated (± bisecting GlcNAc) monosialylated and disialylated structures in total IgG glycans-$F^{total}S1/F^{total}S2$ = SUM (GP16 + GP18 + GP19) / SUM(GP23 + GP24); ratio of fucosylated (without bisecting GlcNAc) monosialylated and disialylated structures in total IgG glycans-FS1/FS2 = SUM (GP16 + GP18) / GP23; ratio of fucosylated (with bisecting GlcNAc) monosialylated and disialylated structures in total IgG glycans - FBS1 / FBS2 = GP19 / GP24; ratio of all fucosylated sialylated structures with and without bisecting GlcNAc-FBS^{total} / FS^{total} = SUM (GP19 + GP24) / SUM (GP16 + GP18 + GP23); ratio of fucosylated monosialylated structures with and without bisecting GlcNAc-FBS1 / FS1 = GP19 / SUM (GP16 + GP18); the incidence of bisecting GlcNAc in all fucosylated monosialylated structures in total IgG glycans-FBS1 / (FS1 + FBS1) = GP19 / SUM (GP16 + GP18 + GP19); ratio of fucosylated disialylated structures with and without bisecting GlcNAc - FBS2 / FS2 = GP24 / GP23; the incidence of bisecting GlcNAc in all fucosylated disialylated structures in total IgG glycans - FBS2 / (FS2 + FBS2) = GP24 / SUM (GP23 + GP24). The following derived traits were approximated only from the ratios of glycan peaks containing neutral glycan as a major structure. First, the percentage of each neutral glycan peak ($GP1^n$-$GP15^n$) was calculated from the total neutral glycan fraction (SUM(GP1:GP15)) and then traits were defined as: the percentage of agalactosylated structures in total neutral glycan fraction - $G0^n$ = SUM ($GP1^n$ + $GP2^n$ + $GP4^n$ + $GP6^n$); the percentage of monogalactosylated structures in total neutral glycan fraction - $G1^n$ = SUM ($GP7^n$ + $GP8^n$ $GP9^n$ + $GP10^n$ + $GP11^n$); the percentage of digalactosylated structures in total neutral glycan fraction - $G2^n$ = SUM

($GP12^n + GP13^n + GP14^n + GP15^n$); the percentage of all fucosylated (± bisecting GlcNAc) structures in total neutral glycan fraction - $F^{n\ total}$ = SUM ($GP1^n + GP4^n + GP6^n + GP8^n + GP9^n + GP10^n + GP11^n + GP14^n + GP15^n$); the percentage of fucosylation of agalactosylated structures-$FG0^{n\ total}$ / G0 = SUM ($GP1^n + GP4^n + GP6^n$) / G0n*100; the percentage of fucosylation of monogalactosylated structures-$FG1^{n\ total}$ / $G1^n$ = SUM ($GP8^n + GP9^n + GP10^n + GP11^n$) / G1n*100; the percentage of fucosylation of digalactosylated structures-$FG2^{ntotal}$ / $G2^n$ = SUM($GP14^n + GP15^n$) / $G2^n$*100; the percentage of fucosylated (without bisecting GlcNAc) structures in total neutral glycan fraction-F^n = SUM ($GP1^n + GP4^n + GP8^n + GP9^n + GP14^n$); the percentage of fucosylation (without bisecting GlcNAc) of agalactosylated structures - $FG0^n$ / $G0^n$ = SUM ($GP1^n + GP4^n$)/$G0^n$* 100; the percentage of fucosylation (without bisecting GlcNAc) of monogalactosylated structures -$FG1^n$/$G1^n$ = SUM($GP8^n + GP9^n$)/$G1^n$* 100; the percentage of fucosylation (with-out bisecting GlcNAc) of digalactosylated structures-$FG2^n$/$G2^n$ = $GP14^n$ / $G2^n$* 100; the percentage of fucosylated (with bisecting GlcNAc) structures in total neutral glycan fraction-FB^n = SUM ($GP6^n + GP10^n + GP11^n + GP15^n$); the percentage of fucosylation (with bisecting GlcNAc) of agalactosylated structures - $FBG0^n$ / $G0^n$ = $GP6^n$ / $G0^n$*100; the percentage of fucosylation (with bisecting GlcNAc) of monogalactosylated structures - $FBG1^n$ / $G1^n$ = SUM($GP10^n + GP11^n$)/$G1^n$* 100; the percentage of fucosylation (with bisecting GlcNAc) of digalactosylated structures - $FBG2^n$/$GP2^n$ = $GP15^n$ / $G2^n$* 100; ratio of fucosylated structures with and without bisecting GlcNAc - FB^n / F^n = FB^n / F^n; the incidence of bisecting GlcNAc in all fucosylated structures in total neutral glycan fraction-FB^n / $F^{n\ total}$ = FB^n/F^n total* 100; ratio of fucosylated non-bisecting GlcNAc structures and all structures with bisecting GlcNAc-F^n / ($B^n + FB^n$) = F^n / ($GP13^n + FB^n$); ratio of structures with bisecting GlcNAc and all fucosylated structures (± bisecting GlcNAc)- B^n / ($F^n + FB^n$) (‰) = $GP13^n$ / (F^n + % FB^n) * 1000; ratio of fucosylated digalactosylated structures with and without bisecting GlcNAc-$FBG2^n$ / $FG2^n$ = $GP15^n$ / $GP14^n$; the incidence of bisecting GlcNAc in all fucosylated digalactosylated structures in total neutral glycan fraction - $FBG2^n$ / ($FG2^n + FBG2^n$) = $GP15^n$ / ($GP14^n + GP15^n$) + 100; ratio of fucosylated digalactosylated nonbisecting GlcNAc structures and all digalactosylated structures with bisecting GlcNAc - $FG2^n$ / ($BG2^n + FBG2^n$) = $GP14^n$ / ($GP13^n + GP15^n$); ratio of digalactosylated structures with bisecting GlcNAc and all fucosylated digalactosylated structures (± bisecting GlcNAc) - $BG2^n$ / ($FG2^n + FBG2^n$) (‰) = $GP13^n$ / ($GP14^n + GP15^n$) * 1000.



Supplementary figure 2. Shows the boxplot for the 4 patient groups with respect to the GP2 peak in the data obtained by the difference in values at Day 1 and Day 0.
x axis: group label (1-4); y axis: values of the differences between Day 1 and Day 0 for GP2 for each sample.

Supplemental table 1. Average values for each HILIC-UPLC separated glycan group released from total plasma proteins, for each studied day. Statistically significance was set at P<0.05.

Glycan	Day 0	Day 1	Day 3	p (Day 0 - Day 1)	p (Day 0 - Day 3)
GP7	9.66	8.31	7.34	<0.01	<0.01
GP8	11.06	10.02	8.02	<0.01	<0.01
GP9	51.55	58.65	60.71	<0.01	<0.01
GP10	7.76	7.23	6.22	<0.01	<0.01
GP11	2.19	1.53	1.42	<0.01	<0.01
GP12	1.44	0.85	0.91	<0.01	<0.01
GP13	4.82	4.09	3.71	<0.01	<0.01
GP14	8.73	7.63	10.14	<0.01	<0.01
GP15	0.40	0.31	0.31	<0.01	<0.01
GP16	1.62	2.23	3.06	<0.01	<0.01

Supplementary table 2. Average values for each HILIC-UPLC separated glycan group released from IgG, for each studied day are given. The P values indicate that for majority of glycan groups statistically significant difference(P<0.05) occur between day 0 and day 1, which is not the case for the difference between day 3 and day 0. These results show that majority glycans released from IgG, change during first 24 hours of inflammatory response and then come back to their preinflammatory levels.

Glycan group	Day 0	Day 1	Day 3	p (Day 0 - Day 1)	p (Day 0 - Day 3)
GP1	0.08	0.09	0.09	<0.001	<0.001
GP2	0.49	0.48	0.50	0.50	0.35
GP3	0.34	0.34	0.39	0.57	0.01
GP4	22.03	21.53	22.03	0.02	1.00
GP5	0.35	0.32	0.35	<0.001	0.91
GP6	5.93	5.71	5.63	<0.001	<0.001
GP7	0.51	0.52	0.51	0.25	0.87
GP8	18.32	18.41	18.24	0.26	0.39
GP9	9.45	9.72	9.54	<0.001	0.04
GP10	5.71	5.64	5.51	0.14	<0.001
GP11	0.82	0.84	0.81	<0.001	0.36
GP12	0.55	0.58	0.56	0.02	0.46
GP13	0.39	0.40	0.40	0.16	0.25
GP14	11.16	11.80	11.01	<0.001	0.25
GP15	1.49	1.54	1.47	<0.001	0.25
GP16	3.03	3.13	3.28	0.03	<0.001
GP17	1.07	0.90	0.96	<0.001	<0.001
GP18	8.18	8.50	8.45	<0.001	0.02
GP19	2.00	2.08	2.04	0.01	0.24
GP20	0.42	0.34	0.44	<0.001	0.24
GP21	0.92	0.75	0.89	<0.001	0.38
GP22	0.17	0.16	0.17	0.78	0.87
GP23	1.45	1.46	1.55	0.88	0.03
GP24	1.70	1.78	1.76	0.15	0.27

Supplemental table 3. Coefficients of variations in studied population for each glycan group on day 0 and day 1. Δ CV % indicates for how many percentages CV has changed between day 0 and day 1. It is clearly seen that, for majority of glycans, CV decreased on day 1.

Glycan Group	Day 0 CV %	Day 1 CV %	Δ CV %
GP1	47.42	58.51	23.4
GP2	50.05	45.70	-8.7
GP3	30.93	29.87	-3.4
GP4	25.05	23.17	-7.5
GP5	19.60	19.47	-0.7
GP6	26.29	25.56	-2.8
GP7	43.47	37.75	-13.2
GP8	10.26	9.29	-9.4
GP9	14.35	12.49	-12.9
GP10	21.12	18.07	-14.4
GP11	17.51	15.47	-11.7
GP12	58.27	44.94	-22.9
GP13	28.72	25.19	-12.3
GP14	29.29	26.12	-10.8
GP15	24.90	21.67	-13.0
GP16	14.37	15.62	8.7
GP17	24.23	20.62	-14.9
GP18	24.10	23.07	-4.3
GP19	21.51	18.85	-12.4
GP20	31.35	30.52	-2.6
GP21	30.35	27.95	-7.9
GP22	51.40	57.98	12.8
GP23	29.56	33.35	12.8
GP24	50.21	35.80	-28.7

Supplementary Table 4. Pearson's correlation coefficients between glycan peaks and Euroscore at all times (Day 0, 1, 3) and time differences (Day 1 - Day 0, Day 3 - Day 0). Only the significant correlations (P<0.05) are shown. The rightmost column shows P values after post-hoc Bonferroni correction, highlighting the most significant correlations. As it can be shown, some peaks appear significant at all times (GP4, GP14) even after Bonferroni correction (GP9).

Time	GP	r (Pearson)	r^2 (Pearson)	P value	P value (Bonferroni)
Day 0	GP1	0.27511	0.075685512	0.00830917	0.9971004
	GP3	0.242369	0.058742732	0.0206286	2.475432
	GP4	0.386882	0.149677682	0.000151725	0.018207
	GP5	0.282441	0.079772918	0.00667719	0.8012628
	GP6	0.330448	0.109195881	0.00137873	0.1654476
	GP9	-0.460705	0.212249097	4.32E-06	0.000518641
	GP13	-0.333366	0.11113289	0.00124206	0.1490472
	GP14	-0.393533	0.154868222	0.000113804	0.01365648
	GP15	-0.270782	0.073322892	0.0094294	1.131528
	GP16	-0.215448	0.046417841	0.0402684	4.832208
	GP18	-0.320824	0.102928039	0.0019316	0.231792
Day 1	GP4	0.330882	0.109482898	0.00135757	0.1629084
	GP6	0.291523	0.08498566	0.00505227	0.6062724
	GP9	-0.386724	0.149555452	0.000152753	0.01833036
	GP13	-0.331961	0.110198106	0.00130624	0.1567488
	GP14	-0.298485	0.089093295	0.00405534	0.4866408
	GP15	-0.226889	0.051478618	0.0305604	3.667248
	GP18	-0.260826	0.068030202	0.0125208	1.502496
	GP20	-0.263958	0.069673826	0.0114648	1.375776
Day 3	GP4	0.338907	0.114857955	0.00101587	0.1219044
	GP6	0.303541	0.092137139	0.00344551	0.4134612
	GP9	-0.468507	0.219498809	2.82E-06	0.000338474
	GP14	-0.323664	0.104758385	0.00175062	0.2100744
	GP15	-0.209288	0.043801467	0.0464818	5.577816
	GP16	-0.243865	0.059470138	0.0198353	2.380236
	GP18	-0.255544	0.065302736	0.0144943	1.739316
Day 1 - Day 0	GP4	-0.207474	0.043045461	0.0484566	5.814792
	GP9	0.313843	0.098497429	0.00245028	0.2940336
	GP14	0.266998	0.071287932	0.0105151	1.261812
	GP21	-0.23279	0.054191184	0.0263797	3.165564
Day 3 - Day 0	GP5	-0.210312	0.044231137	0.0453973	5.447676
	GP14	0.23025	0.053015063	0.0281153	3.373836
	GP21	-0.225364	0.050788932	0.0317277	3.807324