High-throughput mass spectrometric N-glycomics

Reiding, K.R.

Chapter 7

Serum protein *N*-glycosylation changes with rheumatoid arthritis disease activity during and after pregnancy

Research article

Karli R. Reiding[1]*, Gerda C. M. Vreeker[1], Albert Bondt[1,2], Marco R. Bladergroen[1], Johanna M. W. Hazes[3], Yuri E. M. van der Burgt[1,4], Manfred Wuhrer[1], Radboud J. E. M. Dolhain[3]

[1]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands;

[2]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands;

[3]Department of Rheumatology, Erasmus University Medical Center, Rotterdam, The Netherlands;

[4]Department of Clinical Chemistry, Leiden University Medical Center, Leiden, The Netherlands;

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7.1 Abstract

Objective: Symptoms of rheumatoid arthritis (RA) improve during pregnancy, a phenomenon that was found to be associated with *N*-glycosylation changes of immunoglobulin G (IgG). Recent advances in high-throughput glycosylation analysis allow the assessment of the *N*-glycome of human sera as well. The aim of this study was to identify new protein *N*-glycosylation properties that associate with changes in RA disease activity during and after pregnancy.

Methods: A longitudinal cohort of serum samples was collected during 285 pregnancies (32 control individuals and 253 RA patients). Per individual one sample was collected before conception, three during pregnancy, and three after delivery. Released serum protein *N*-glycans were measured by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF-MS) after employing chemical modification of the sialic acids to allow discrimination of sialic acid linkage isomers.

Results: Serum protein *N*-glycosylation was strongly modified during pregnancy, with similar changes visible in control individuals and RA pregnancies. A decrease in bisection and an increase in galactosylation in diantennary glycans were found, as well as an increase in tri- and tetraantennary species and α2,3-linked sialylation thereof. The change in RA disease activity (DAS28(3)-CRP) proved negatively associated with the galactosylation of diantennary *N*-glycans, and positively with the sialylation of triantennary fucosylated species.

Conclusion: We have identified novel changes in the sialylation of triantennary *N*-glycans (A3FGS) to be associated with RA disease activity during pregnancy. While the protein source of A3FGS is thus far unknown, future studies into the glycosylation phenotype are recommended to improve our understanding of RA disease severity.

7.2 Introduction

Rheumatoid arthritis (RA) is a prevalent autoimmune disease affecting up to 1% of the adult population in developed regions[1,2]. Its main characteristic is a symmetrical polyarthritis involving predominantly the hand- and foot joints, although every organ system may be involved. The disease is more frequently diagnosed in women than in men, and is more common with increasing age[1,3]. The potential causes of RA are diverse in nature[4,5], but a role for T-cells, antibody producing B-cells, but also monocytes/ macrophages has been suggested[1,6]. Interestingly, in many female RA patients an improvement in RA disease severity is reported during pregnancy, as well as a relapse thereof after delivery[7-10]. The reasons for these changes in disease activity are still poorly understood, but are of importance to understand the etiology of RA, and may possibly provide leads for new modes of (personalized) treatment.

Glycosylation, the process of co- and posttranslational protein modification with complex carbohydrates, plays an important role in the interaction, function, and solubility of proteins[11-13]. It is expected that more than half of all proteins is glycosylated with one or more *N*-glycans[14], and commonly observed glycoforms range from high-mannose- to complex-type with two to four antennae (branching *N*-acetylglucosamines) (**Figure 1**)[12,15]. These structures may be extended by additional monosaccharides such as a bisecting *N*-acetylglucosamine, as well as galactoses, *N*-acetylneuraminic acids (sialic acids) and fucoses in a variety of different positions and linkages. This leads to a large *N*-glycan diversity, and may also lead to the formation of specific epitopes such as sialyl-Lewis X, which can be recognized by E-selectin[12,16].

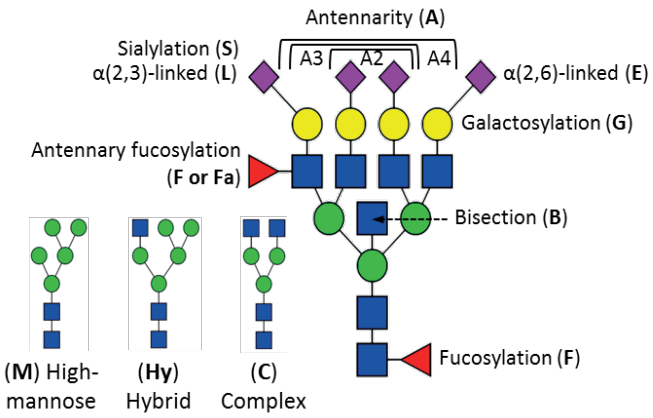

Figure 1. Schematic overview of glycosylation traits derived from human serum proteins. *N-glycan structures are generalized into high-mannose type (M), hybrid type (Hy) and complex type (C) by the number of mannoses (green circle) and antennary N-acetylglucosamines (blue square). High-mannose-type N-glycans can have up to nine mannoses, whereas each antennary N-acetylglucosamine can be terminally substituted with a galactose (yellow circle), and sialic acid (magenta diamond). Sialic acids (S) can either be α2,3-linked (L) or α2,6-linked (E). N-glycans can further be modified with a fucose (F), optionally at an antennary N-acetylglucosamine (Fa), and structures may as well be bisected (B). In case of derived traits the subject of the calculation is represented by the last letter, e.g. sialylation (S), and the group on which it is calculated by the preceding letters, e.g., triantennary fucosylated species (A3F). This, for instance, translates A3FGS into the sialylation per galactose within triantennary fucosylated species.*

Various proteins have already been found to display altered glycosylation with RA and its disease activity. For instance, the *N*-glycosylation of the fragment-crystallizable (Fc) portion of immunoglobulin G (IgG) shows to differ in galactosylation, bisection and fucosylation[17-23], and the acute-phase protein alpha-1-acid glycoprotein (also known as orosomucoid) shows differences in antennarity (*i.e.*, the ratio between di-, tri- and tetraantennary glycans) and fucosylation with RA, as well as changes throughout pregnancy[21,22]. Although such an analysis of single proteins is highly informative, additional insights may be gained by a systemic glycomics approach that covers a broad range of protein-linked glycan modifications.

The total serum *N*-glycome (TSNG) comprises the *N*-glycans from all serum proteins, which are to a large extent liver- (acute-phase proteins) and plasma cell-derived (antibodies)[15,24]. Interestingly, the TSNG has been shown, in a small sample set, to differ between healthy individuals and those with RA, and undergoes clear alteration throughout pregnancy and the following postpartum period[13,14]. However, it is hitherto unknown which TSNG characteristics are associated with the disease activity of RA, and changes thereof throughout pregnancy. Recent developments in mass spectrometry (MS)-based high-throughput glycosylation analysis have provided the opportunity to acquire information on TSNG *N*-glycan complexity, antennarity, galactosylation, fucosylation, as well as on the presence and linkage of sialic acids (α2,6- versus α2,3-linkage)[23, 24]. The latter appears to be of high immunological relevance, since the α2,3-linked sialic acids are required for sialyl-Lewis X formation implicated in the interaction with selectins[25,26].

The objective of the work presented here is to assess the changes in serum *N*-glycosylation throughout pregnancy and the postpartum period in RA patients, and to identify the glycosylation properties associated with the accompanying changes in disease activity (DAS28(3)-CRP). To this end, we studied the *N*-glycosylation of sera from 253 RA and 32 control pregnancies at seven time points before, during and after pregnancy by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS, and report the disease- and pregnancy-associated changes of 78 *N*-glycan species and 91 glycosylation traits derived thereof.

7.3 Methods

Study population and sample collection

The current research is embedded in the PARA study, a nationwide prospective cohort on pregnancy and RA[21,2721,2721, 27]. The cohort consisted of serum samples of 253 pregnancies from 219 RA patients, collected between 2002 and 2009[27]. In addition, 32 pregnancies of healthy Caucasian volunteers without adverse obstetric histories were included and followed from the 1st trimester of pregnancy. Of each patient at least one sample was obtained during pregnancy and one postpartum, with a minimum of three samples per

patient. Only completed pregnancies were included and all patients fulfilled the 1987 ACR criteria for RA. Disease activity was assessed using the disease activity score (DAS) in 28 joints, incorporating the swollen joint count, the tender joint count and the C-reactive protein level (DAS28(3)-CRP). The study was in compliance with the Helsinki Declaration and was approved by the Ethics Review Board at the Erasmus University Medical Center, Rotterdam, the Netherlands. For quality control purposes, 111 plasma standards (Visucon-F frozen normal control plasma, pooled from 20 human donors, citrated, and buffered with 0.02 M HEPES, obtained from Affinity Biologicals, Ancaster, ON) and 40 PBS blanks were distributed across the 21 96-wells sample plates.

Table 1. Study population characteristics	**Control pregnancies (n = 32)**	**Patient pregnancies (n = 253)**
Age at delivery in years, mean (SD)	32.1 (4.4)	32.8 (3.7)
Duration of pregnancy in weeks, mean (SD)	40.1 (1.4)	39.2 (1.9)
Disease duration at first visit in years, mean (SD)	-	7.7 (6.3)
ACPA positive patients, n (%)	-	153/253 (60.5)
RF positive patients, n (%)	-	161/240 (67.1)
Erosive disease, n (%)	-	149/229 (65.1)
Response during pregnancy, n (%)	-	72/137 (52.6)
Flare during postpartum period, n (%)	-	72/232 (31.0)
Disease activity score (DAS28(3)-CRP) at first trimester of pregnancy, mean (SD)	-	3.6 (1.1)

Sample preparation, measurement and analysis

For a full description of the analytical procedure, see **Supplementary Methods**. In short, *N*-glycans were enzymatically released from 6 µL of serum, derivatized by ethyl esterification, and measured by MALDI-TOF-MS using a largely automated approach[26]. Glycan compositions were assigned on the basis of literature, and data were processed and curated using MassyTools[28], resulting in a set of 78 *N*-glycans that were quantified.

Mixed linear regression was used to establish the associations between glycosylation and respectively pregnancy, RA and DAS28(3)-CRP. Linear regression was used to confirm the intra-individual DAS28-CRP associations between the time frames (ΔDAS28-CRP). For the whole study a significance threshold was maintained of $\alpha = 3.03 \cdot 10^{-2}$, allowing detection of effects under a false-discovery rate of 5%. The threshold was calculated using the Benjamini-Hochberg procedure[29].

7.4 Results

To explore the association between serum protein *N*-glycosylation and improvement of RA disease activity during pregnancy, we investigated by MALDI-TOF-MS the TSNG of 285 pregnancies embedded in the PARA study (**Table 1**)[27]. This MS methodology allowed us to obtain information on 78 glycan compositions (**Figure 2**; **Supplementary Table S1**), including discrimination between sialic acid linkage isomers, and to calculate biologically relevant ratios between subsets of glycans in the form of 91 derived traits (**Supplementary Table S2**)[25]. Data quality was confirmed by the repeated measurement of a standard sample (**Supplementary Figure S1**, **Supplementary Table S3**).

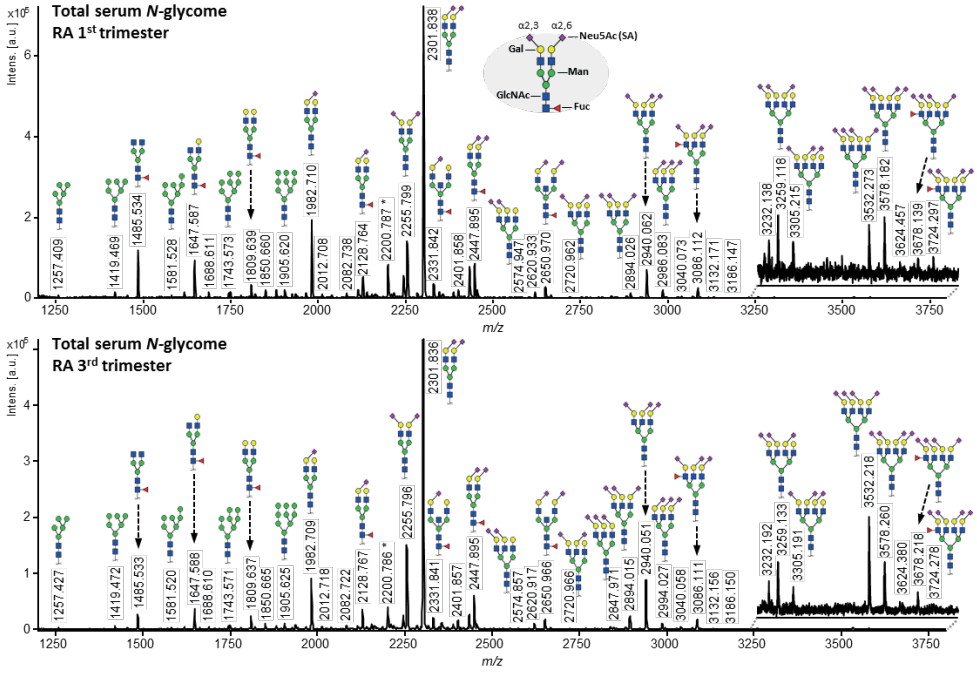

Figure 2. Comparison of MALDI-TOF-MS spectra obtained from released N-glycans from serum proteins after linkage-specific sialic acid esterification and HILIC enrichment. *The shown spectra are derived from an individual with rheumatoid arthritis who has been diagnosed with ACPA and RF, and has been classified as a responder by the EULAR response criteria. Notable differences between the 1st trimester (**top**) and 3rd trimester (**bottom**) spectra include galactosylation (e.g., between m/z 1485.5, 1647.6 and 1809.6), antennarity (e.g. m/z 2940.1 vs. 2301.8), α2,3-linked sialylation (e.g. m/z 2255.8 vs. 2301.8), and α2,6-linked sialylation (e.g. m/z 1982.7 vs. 2301.8).*

Pregnancy-associated glycosylation changes

The association of pregnancy and the postpartum period with serum protein *N*-glycosylation was established by mixed linear regression. Within individuals, comparison was made between the 1st and 3rd trimester (representative of pregnancy; respectively

coded 0 and 1; adjusted for individual), 3rd trimester and 6 weeks postpartum, and 3rd trimester and 26+ weeks postpartum (**Supplementary Table S4**).

With progression of pregnancy, a marked alteration in overall number of antennae per *N*-glycan was observed (**Figure 3**, top row). During pregnancy, within the complex-type glycans (C), the relative abundance of tri- and tetraantennary *N*-glycans (CA3, CA4) showed to increase at the expense of diantennary species (CA2) (β_{CA2} = -1.05 standard error (SE) ± 0.06; β_{CA3} = 1.08 ± 0.06; β_{CA4} = 0.70 ± 0.06), followed by a slow recovery after delivery (β_{CA2} = 0.94 ± 0.07; β_{CA3} = -0.98 ± 0.07; β_{CA4} = -0.56 ± 0.08; **Supplementary Table S4**).

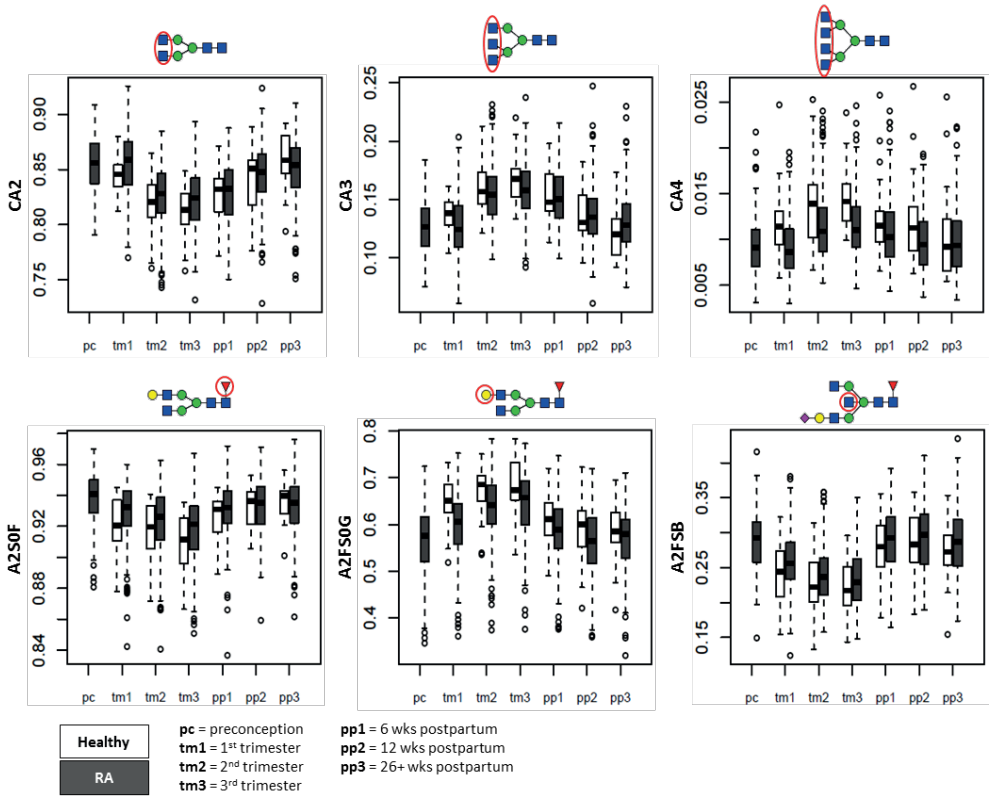

Figure 3. Overall glycosylation changes throughout pregnancy and the postpartum period shown in boxplots. *Displayed are the glycan traits related to antennarity (**top row**), and the glycosylation traits likely to be of immunoglobulin origin (**bottom row**)[15,24]. Depicted are healthy controls (in white) and patients with RA (in grey) at preconception (pc), trimesters 1 through 3 (tm1, tm2, tm3), 6 weeks postpartum (pp1), 12 weeks postpartum (pp2) and 26+ weeks postpartum (pp3). CA2 = diantennary species within complex-type; CA3 = triantennary species within complex-type; CA4 = tetraantennary species within complex-type; A2S0F = fucosylation of nonsialylated diantennary species; A2FS0G = galactosylation of nonsialylated fucosylated diantennary species; A2FSB = bisection of sialylated fucosylated diantennary species.*

For the serum *N*-glycosylation characteristics likely originating from the Fc portion of IgG (diantennary *N*-glycans without sialylation; A2S0)[15,24], we confirmed with progressing pregnancy a decrease in fucosylation (A2S0F, β_{A2S0F} = -0.59 ± 0.05) and increase in galactosylation (A2FS0G, β_{A2FS0G} = 0.59 ± 0.04), both rapidly reversing after delivery (β_{A2S0F} = 0.67 ± 0.04; β_{A2FS0G} = -0.81 ± 0.04) (**Figure 3**, **bottom row**).

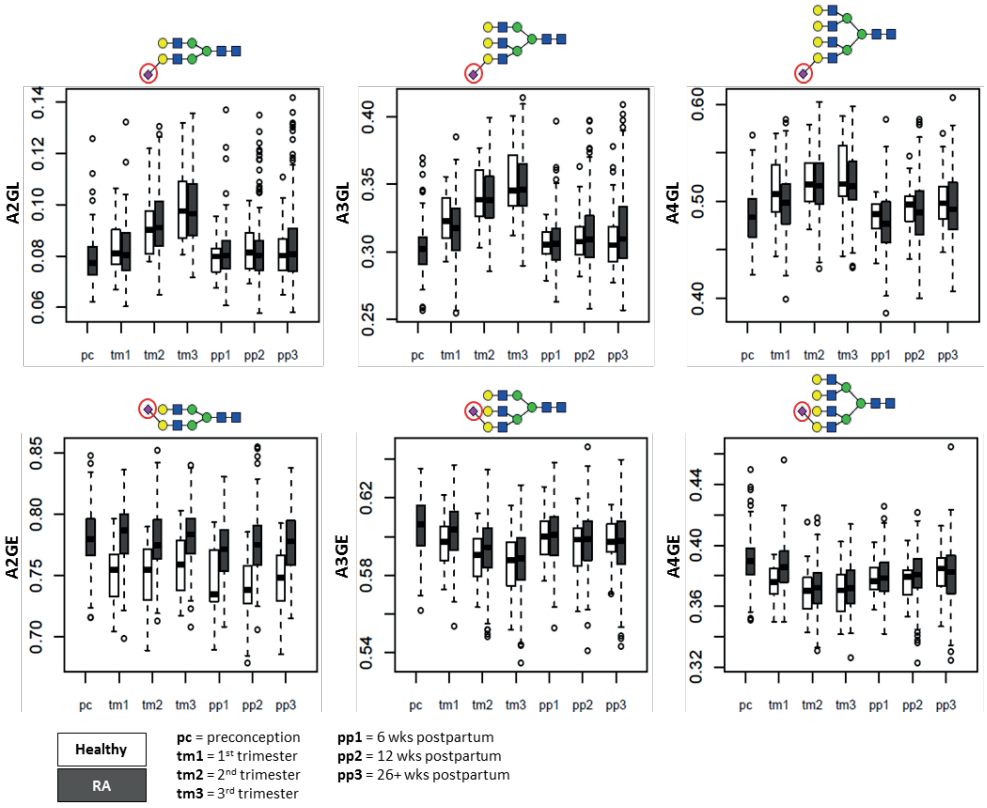

Figure 4. Sialylation changes throughout pregnancy and the postpartum period shown in boxplots. *Whereas α2,3-linked sialylation (L) displays the most prominent change throughout pregnancy (**top row**), α2,6-linked sialylation shows the most distinction between patients and controls (**bottom row**). Separation is made between pregnancies of patients with RA (in grey) and healthy controls (in white). A2GL = α2,3-linked sialylation per galactose of diantennary species; A3GL = α2,3-linked sialylation per galactose of triantennary species; A4GL = α2,3-linked sialylation per galactose of tetraantennary species; A2GE = α2,6-linked sialylation per galactose of diantennary species; A3GE = α2,6-linked sialylation per galactose of triantennary species; A4GE = α2,6-linked sialylation per galactose of tetraantennary species.*

For the serum *N*-glycosylation characteristics likely originating for a large part from IgG-Fab, IgM and IgA (fucosylated diantennary *N*-glycans with sialylation; A2FS)[15,24], we observed a decrease in bisection with trimester progression (A2FSB, β_{A2FSB} = -0.56 ± 0.04) and a rapid return to pre-pregnancy levels at 6 weeks postpartum (β_{A2FSB} = 1.11 ± 0.03).

Notably, whereas α2,6-linked sialylation remained relatively stable throughout pregnancy (**Figure 4**, **bottom row**), a drastic increase of α2,3-linked sialylation (e.g. per galactose on di- or triantennary glycans, respectively A2GL and A3GL) was observed up to the 3rd trimester (β_{A2GL} = 1.11 ± 0.05; β_{A3GL} = 1.16 ± 0.05), followed by a rapid return to baseline levels at the first time point after delivery (β_{A2GL} = -1.19 ± 0.05; β_{A3GL} = -1.42 ± 0.04) (**Figure 4**, **top row**).

Differences between healthy and RA

By mixed linear regression, glycosylation values were compared between individuals with RA and controls (healthy = 0, RA = 1; adjusted for pregnancy) (**Supplementary Table S5**). For immunoglobulin-type glycosylation we observed in RA a lower galactosylation (A2FS0G, β_{A2FS0G} = -0.44 ± 0.07), higher bisection (A2FS0B, β_{A2FS0B} = 0.55 ± 0.08), and a higher sialylation per galactose (A2FGS, β_{A2FGS} = 0.75 ± 0.08). In addition, differences were found in several non-fucosylated diantennary traits (higher A2F0G, A2F0GS and A2GE, lower A2F0B, A2F), all mainly driven by elevated levels of H5N4E2 (β_{H5N4E2} = 0.97 ± 0.07) in RA patient serum. For tri- and tetraantennary *N*-glycosylation we observe with RA a higher fucosylation (e.g. A3F, β_{A3F} = 0.34 ± 0.08; A4F, β_{A4F} = 0.56 ± 0.08) particularly within species with α2,3-linked sialylation (A3LF, β_{A3LF} = 0.35 ± 0.08; A4LF, β_{A4LF} = 0.58 ± 0.08), suggesting an increase in sialyl-Lewis X/A.

Association of glycosylation with DAS28(3)-CRP

In addition, we compared by mixed linear regression the association of glycosylation with RA disease activity, as assessed by DAS28(3)-CRP, both between and within individuals with RA (respectively adjusted for time point and individual) (**Supplementary Figure S2**, **Supplementary Table S6**). The increase in DAS28(3)-CRP associated with both the decrease in IgG-Fc-type galactosylation (*e.g.*, A2S0G, β_{A2S0G} = -0.34 ± 0.02) and the decrease in fucosylation of non-α2,3-sialylated triantennary species (A3L0F, β_{A3L0F} = -0.13 ± 0.03). Increasing with DAS28(3)-CRP were the bisection of IgG-Fc-type glycans (A2F0S0B, $\beta_{A2F0S0B}$ = 0.25 ± 0.03) as well as the sialylation and fucosylation of tri- and tetraantennary species (*e.g.*, A3FGS, β_{A3FGS} = 0.26 ± 0.02; A4F, β_{A4F} = 0.13 ± 0.03; A4GS, β_{A4GS} = 0.17 ± 0.02). Glycosylation features associating with DAS28(3)-CRP only within (and not between) individuals throughout pregnancy were the fucosylation of (α2,6-)sialylated diantennaries (A2SF, β_{A2SF} = -0.12 ± 0.02) and α2,6-sialylation of triantennary nonfucosylated species (A3F0GE, β_{A3F0GE} = 0.14 ± 0.02).

Association of glycosylation with the improvement of DAS28(3)-CRP during pregnancy

Lastly, between trimesters 1 and 3 (response timeframe) as well as between trimester 3 and 12 weeks postpartum (flare timeframe), we employed linear regression to confirm the within-individual association of DAS28(3)-CRP (ΔDAS28(3)-CRP) with glycosylation (Δglycosylation) (**Supplementary Table S7**). In both timeframes, ΔDAS28(3)-CRP showed a

negative association with the Δgalactosylation of IgG-Fc type species (ΔA2S0G, responder, $\beta_{\Delta A2S0G}$ = -0.29 ± 0.07; flare, $\beta_{\Delta A2S0G}$ = -0.35 ± 0.07; ΔA2FS0G, responder, $\beta_{\Delta A2FS0G}$ = -0.29 ± 0.07; flare, ΔA2FS0G; $\beta_{\Delta A2FS0G}$ = -0.33 ± 0.07), whereas the sialylation of triantennary fucosylated species showed in both cases a positive association (ΔA3FGS, responder, $\beta_{\Delta A3FGS}$ = 0.26 ± 0.07; flare, $\beta_{\Delta A3FGS}$ = 0.35 ± 0.07) (**Figure 5**). The flare timeframe additionally showed a positive association of ΔDAS28(3)-CRP with the (α2,6)-sialylation of (fucosylated) diantennary glycans (ΔA2GE, $\beta_{\Delta A2GE}$ = 0.27 ± 0.07; ΔA2FGS, $\beta_{\Delta A2FGS}$ = 0.24 ± 0.07).

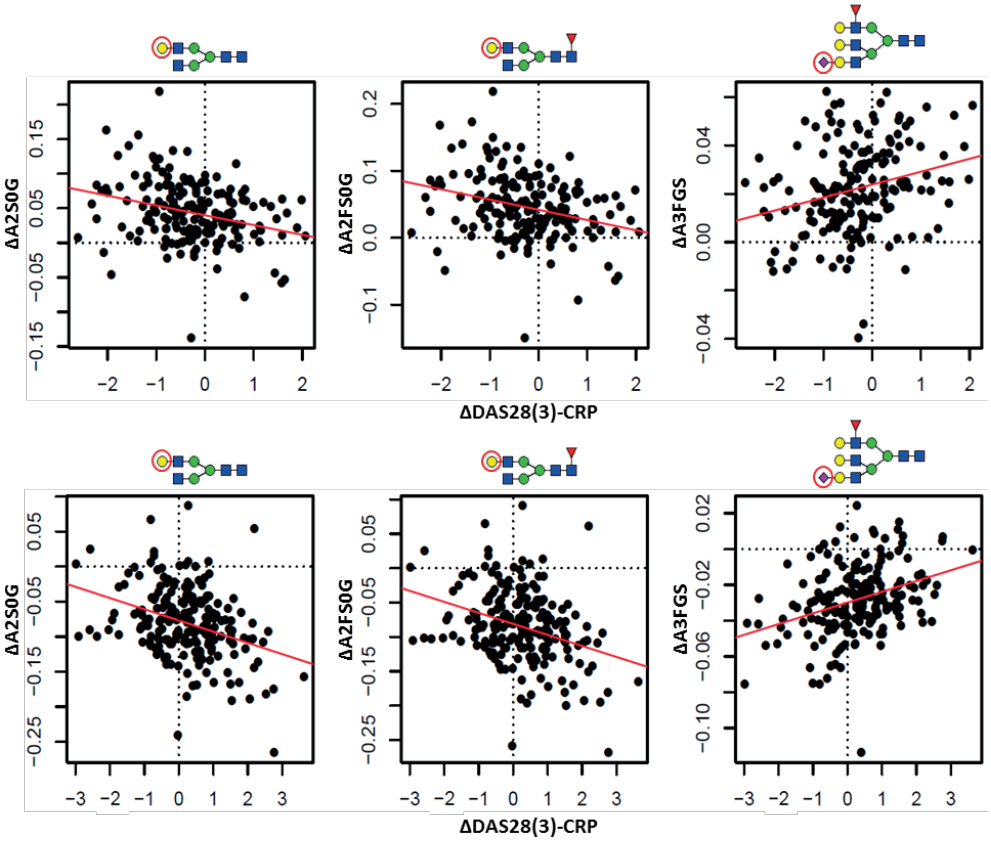

Figure 5. The relation between changing RA disease activity (ΔDAS28(3)-CRP) and changing glycosylation. *Distinction is made between the timeframes to assess responder status (the change between 1st and 3rd trimester;* ***top row****) and flare status (the change between 3rd trimester and 12 weeks postpartum;* ***bottom row****). ΔA2S0G = the change in galactosylation per antenna of diantennary nonsialylated species; ΔA2FS0G = the change in galactosylation of diantennary fucosylated nonsialylated species; ΔA3FGS = the change in sialylation per galactose of triantennary fucosylated species.*

7.5 Discussion

The decrease of RA disease activity during pregnancy and the flare following delivery are reproducible clinical observations that are mechanistically poorly understood[8-10]. To expand our understanding of protein glycosylation that associates with RA disease activity, we studied the total serum *N*-glycosylation changes occurring throughout pregnancy and the postpartum period of 253 pregnancies of patients with RA, along with 32 control pregnancies.

Interpretation of glycomics data

To interpret the information contained in this study, several aspects of our MALDI-TOF-MS analysis need to be kept in mind. First, mass spectrometry assesses glycan chemical compositions and not structures, although sialic acid-linkage information is provided by the employed derivatization technique[25]. Other structural characteristics are presumed based on a wide array of literature on biosynthetic pathways, and on experiments with enzymatic digestion, nuclear magnetic resonance spectroscopy, and MS fragmentation[12,15,30-32]. Second, the changes observed in the released *N*-glycan samples could have originated from changes in the glycosylation of proteins, but also from changes in the abundance of those glycoproteins. Derived traits have been constructed to reflect differences in biosynthesis, and our current-day understanding of the relative contribution of specific glycoproteins and tissues to the serum *N*-glycome[15,24]. Third, the mass spectrometric analysis did not provide quantitative ratios of *N*-glycosylation, but the direction and magnitude of observed changes is expected to be biologically representative, as suggested by method comparisons[33].

Glycosylation changes with pregnancy

Previously, we found glycosylation changes throughout healthy pregnancies, and have in RA patients studied the specific glycosylation of IgG-Fc throughout pregnancy[20,21,34-37]. In the current study, the first application of a similar MALDI-MS approach with automated sample preparation, we achieved the analysis of the total serum *N*-glycosylation throughout the pregnancies of both healthy controls and RA patients, in total leading to the analysis of 1770 clinical samples[26]. Since the current cohort also contains the control individuals (without RA) from prior studies, these previous results were confirmed, but we could also for the first time show that comparable TSNG changes can be observed in RA patients.

As such, we detected with the progression of pregnancy an increase of galactosylation (A2FS0G) as prior reported for the Fc part of IgG, as well as a decrease in bisection (A2FSB)[18,20,21]. Additionally, we observed with pregnancy an overall increase in glycan branching (from CA2 to CA3 and CA4) and an increase of α2,3-linked sialylation (L, in part at the expense of α2,6-sialylation, E). In all cases the postpartum period led to the return to the values before or at the beginning of pregnancy. The increased branching observed in the TSNG could have originated from the abundance and antennarity of acute-phase proteins such as alpha-1-acid glycoprotein, for which an increased serum level and *N*-glycan

branching has been reported with pregnancy[38,39]. On the other hand, we did not observe the decrease in fucosylation reported for the same protein, potentially obscured by the increased fucosylation of other proteins, and have yet to identify the source of the substantial increase of α2,3-linked sialylation up to the 3rd trimester. In literature it has been reported that IgG and alpha-1-acid glycoprotein glycosylation may be affected by estrogens[40,41]. known to change significantly throughout pregnancy[42], and it is conceivable that other proteins within the TSNG are under similar high-level control.

Glycosylation differs between RA patients and healthy controls

The total serum *N*-glycosylation changes with pregnancy showed remarkably comparable between RA patients and healthy controls, but baseline differences could be detected. For example, RA patients displayed a lower degree of IgG-Fc-type galactosylation and higher bisection when compared to controls (A2FS0G, A2FS0B). A decreased IgG-Fc galactosylation and increased bisection are well-known to associate with a variety of inflammatory conditions, including RA, inflammatory bowel disease, and aging[18,43,44], and the same glycosylation phenotypes appear detectable in our TSNG study as well[20,21]. The mechanisms by which IgG-Fc glycosylation may affect inflammatory processes remain for a large part to be elucidated, but increased galactosylation and decreased fucosylation have been implicated in increased FcγRIIa and FcγRIIIa binding and antibody-dependent cellular cytotoxicity[45-47].

Furthermore, RA patients showed in our study a higher α2,6-sialylation and lower fucosylation of diantennary glycans (A2GE, A2F) compared to healthy controls, mainly driven by the *N*-glycan composition H5N4E2, as well as a substantially higher (multi-)fucosylation of tri- and tetraantennary glycans (A3F(a), A4F(a)). The increased A3/A4 fucosylation strongly suggests the upregulation of sialyl-Lewis X, which has been reported with inflammatory arthritis for several acute-phase proteins, *e.g.*, alpha-1-acid glycoprotein, haptoglobin, alpha-1-antichymotrypsin, and transferrin[48-52]. The sialyl-Lewis X on glycans is known to bind to E-selectin, an inducible receptor expressed by endothelial cells[16]. This interaction is implicated in the homing of immune cells to a site of inflammation[53], as well as cancer metastasis[54], and may play a role in RA as well. For instance, the alpha-1-acid glycoprotein observed within RA synovial fluid is thought to be of hepatic origin[55], meaning its circulatory variant might make use of a glycan-mediated mechanism for transportation towards the inflamed synovial tissue.

Glycosylation associates with RA disease activity

Next to a negative association with the galactosylation of IgG-Fc-type *N*-glycans, we additionally report a positive association between (Δ)DAS28(3)-CRP and the sialylation of triantennary fucosylated glycans (A3FGS), notably of the α2,6-linked variety. This is in contrast to the TSNG changes throughout pregnancy, which shows marked increase of α2,3-linked sialylation but not an association with disease activity. A recent study, which

compared total plasma *N*-glycosylation with the levels of various metabolic and inflammatory markers, has indicated a link between A3FGS and C-reactive protein (CRP), suggesting that the CRP component of DAS28(3)-CRP may in part be responsible for the association found within the current study[56]. While the protein source of A3FGS remains unknown, future studies will have to reveal its biomarker potential and facilitate our understanding of RA disease severity.

7.6 Summary

To summarize, by performing MS-based total serum protein *N*-glycosylation analysis we detected 1) changes in protein glycosylation throughout pregnancy, 2) glycosylation differences between healthy individuals and RA patients, and 3) glycosylation traits coinciding with the pregnancy-associated changes in RA disease activity. While we confirmed in serum the IgG glycosylation phenotypes that were prior reported to associate with RA disease activity, our glycomics approach has additionally allowed the detection of changes that are presumably independent from IgG, namely the sialylation of fucosylated triantennary *N*-glycans (A3FGS).

7.7 Acknowledgments

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7.8 Additional information

Supplementary information

Supplementary information accompanies this paper.

Competing interests

The authors declare no competing financial interest.

7.9 References

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