Published in final edited form as:

Lupus. 2016 March ; 25(3): 296–300. doi:10.1177/0961203315617842.

GlycA, a Novel Marker of Inflammation, is Elevated in Systemic Lupus Erythematosus

Cecilia P. Chung, M.D., M.P.H.¹, Michelle J. Ormseth, M.D., M.S.C.I.¹, Margery A. Connelly, Ph.D.², Annette Oeser, M.L.A.S.¹, Joseph F. Solus, Ph.D.¹, James D. Otvos, Ph.D.², Paolo Raggi, M.D.³, and C. Michael Stein, M.D.¹

¹Department of Medicine, Vanderbilt University, Nashville, TN, USA

²LabCorp, Raleigh, NC, USA

³Department of Medicine, University of Alberta, Edmonton, AB, Canada

Abstract

Background—GlycA is a novel marker of systemic inflammation detected by nuclear magnetic resonance spectroscopy (NMR). In the general population, GlycA is correlated with inflammatory markers such as C-reactive protein (CRP) and associated with coronary heart disease and diabetes. The utility of GlycA in patients with systemic lupus erythematosus (SLE) has not been defined. Therefore, we tested the hypothesis that GlycA concentrations are elevated in patients with SLE and associated with other markers of inflammation and coronary atherosclerosis.

Methods—We compared concentrations of GlycA, detected by NMR, in 116 patients with SLE and 84 control subjects frequency-matched for age, sex, and race. SLE disease activity index (SLEDAI) and the SLE Collaborating Clinics damage index (SLICC) were calculated. Acute phase reactants, a panel of cytokines, and a lipid panel were measured. Electron beam computer tomography (EBCT) was used to quantify coronary artery calcification, a measure of coronary artery atherosclerosis.

Results—Patients with SLE had higher concentrations of GlycA [398 (350–445)] than control subjects [339 (299–391)] $\mu\text{mol/L}$, $p < 0.001$. In patients with SLE, concentrations of GlycA were significantly associated with sedimentation rate ($\rho = 0.43$), C-reactive protein ($\rho = 0.59$), e-selectin ($\rho = 0.28$), intracellular adhesion molecule-1 ($\rho = 0.30$), triglycerides ($\rho = 0.45$), all $p < 0.0023$ to account for multiple comparisons, but not with creatinine, SLEDAI, SLICC, or coronary calcium scores.

Conclusions—Concentrations of GlycA are higher in patients with SLE than control subjects and associated with markers of inflammation but not with SLE disease activity or chronicity scores or coronary artery calcification.

Keywords

systemic lupus erythematosus; GlycA; inflammation

Corresponding author: Cecilia P. Chung, M.D., M.P.H., 1161 21st Avenue South, T-3113 MCN, Nashville, TN 37232, Phone: 615-322-4746, Fax: 615-322-6248, c.chung@vanderbilt.edu.

Disclosures: MAC and JDO are employees of LabCorp

GlycA is a novel marker of systemic inflammation detected by nuclear magnetic resonance (NMR) spectroscopy. It arises from the N-acetyl methyl signals from glycosylated acute phase proteins.¹ Several glycoproteins, including α 1-acid glycoprotein, α -1 antichymotrypsin, haptoglobin, α -1-antitrypsin, and transferrin contribute significantly to increase the GlycA signal during inflammation.¹ GlycA concentrations correlate positively with high-sensitivity C-reactive protein (hs-CRP) and interleukin (IL)-6 concentrations.¹ Thus, GlycA may have utility as a marker of disease activity in patients with chronic inflammatory diseases. In addition, GlycA has been associated with incident cardiovascular disease in the Women's Health Study, respectively.²

In 1987, the intensity of an NMR peak of glycosylated proteins that included the peak now known as GlycA was noted to be higher in patients with rheumatoid arthritis (RA) than in control subjects, but the clinical significance of these findings was unclear.³ Recently, we reported that GlycA concentrations were higher in patients with RA than in control subjects and correlated strongly with RA disease activity and inflammatory markers,⁴ but its role in patients with systemic lupus erythematosus (SLE) has not been defined.

Despite increasing awareness and multiple efforts, there are no good laboratory markers of SLE disease activity for use in clinical practice;⁵ similarly, our ability to estimate cardiovascular risk in patients with SLE is limited. Therefore, we set out to test the hypothesis that GlycA concentrations were elevated in patients with SLE and associated with disease activity, other markers of inflammation, and coronary atherosclerosis.

Methods

Patients

Patients with SLE (n=116) and control subjects (n=84), who have contributed to ongoing studies of risk factors associated with coronary atherosclerosis, were evaluated. Details regarding enrollment and study procedures have been described previously.^{6, 7} In summary, patients met the 1997 American College of Rheumatology classification criteria for SLE⁸ and were recruited from the practices of local rheumatologists, through a Lupus Foundation newsletter, and by advertisements. Control subjects were recruited from the patients' acquaintances, by advertisement, and from a database of volunteers from the Vanderbilt General Clinical Research Center. The study was approved by the institutional review board at Vanderbilt University, and all study participants signed an informed consent.

Clinical evaluation

All patients with SLE and control subjects underwent a structured interview, physical examination, laboratory tests, and review of medical records. Blood pressure was recorded as the mean of two measurements obtained 5 minutes apart after subjects had rested. In patients with SLE, disease activity and disease damage were ascertained with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)⁹ and the Systemic Lupus International Collaborating Clinics (SLICC) damage index,¹⁰ respectively. Electron beam computer tomography (EBCT) was used to quantify coronary atherosclerosis.¹¹

Laboratory tests and inflammatory markers

Blood was collected after an overnight fast for the measurement of total cholesterol, high-density lipoprotein cholesterol (HDL), and triglycerides. Low-density lipoprotein cholesterol (LDL) was calculated. In patients with lupus, Westergren erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), 50% hemolytic complement (CH₅₀) and creatinine concentrations were measured in the clinical laboratory. Glomerular filtration rate was calculated using the Modification of Diet in Renal Disease (MDRD) equation.¹² IL-6, tumor necrosis factor- α (TNF- α), e-selectin, vascular adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1), concentrations were measured using an enzyme linked immunoabsorbent assay (ELISA) (Millipore, Billerica, MA).

GlycA quantification

NMR spectra were acquired from EDTA plasma samples as previously described for the *NMR LipoProfile*[®] (lipoprotein particle) test at LabCorp (formerly LipoScience, Inc., Raleigh, NC).¹³ The NMR Profiler platform is comprised of a 9.4 T (400 MHz ¹H frequency) spectrometer (Bruker Biospin) with an integrated fluidics sample delivery system. The GlycA signal (2.00 ppm) was quantified using proprietary deconvolution software that uses a non-negative linear least squares algorithm to fit the experimental signal to individual spectral components, including protein and lipoproteins as well as signals representing the GlycA NMR resonance.¹ Reported intra and inter-assay coefficients of variation are 1.9 and 2.6% and results are presented in $\mu\text{mol/L}$.¹

Statistical analysis

Demographic characteristics are presented as mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables, and frequency (percentage) for categorical variables. Statistical differences between cases and controls were tested with the Wilcoxon rank-sum or the Fisher's exact test, as appropriate. Spearman correlations were used to examine the association between concentrations of GlycA, ESR and CRP with patient characteristics. All analyses used a 2-sided significance level of 5% and Bonferroni adjustments were done to account for multiple comparisons. The analyses were performed with Stata 12.0 (Stata Corp., College Station, TX, USA) (Stata Corp., College Station, TX, USA).

Results

Subjects characteristics

Patients with SLE and control subjects had similar age, systolic and diastolic blood pressure, total and HDL cholesterol and creatinine. The majority of patients with SLE and control subjects were female and Caucasian. As previously reported, inflammatory markers, coronary artery calcium scores⁶ and triglyceride concentrations were higher in patients with SLE than control subjects.¹⁴ (Table 1) In patients with SLE, the median (IQR) ESR was 17 (8–36) mm/hr, CRP 4.0 (3.0–7.0) mg/L, and IL-6 5.8 (2.4–26.3) pg/ml. Patients had a median disease duration of 7 (3–12) years, a median SLEDAI score of 4 (0–6) and SLICC

damage index of 1 (0–2). Their median cumulative corticosteroid use was 12.3 (2.8–28.3) grams.

GlycA in patients and controls and association with SLE characteristics

Concentrations of GlycA were higher in patients with SLE [median 398, IQR (350–445) $\mu\text{mol/L}$] than in control subjects [339 (299–391) $\mu\text{mol/L}$], $p<0.001$ (Figure). After correction for multiple comparisons, GlycA concentrations correlated positively with ESR ($\rho=0.43$, $p<0.001$), CRP ($\rho=0.59$, $p<0.001$), CH50 ($\rho=0.30$, $p=0.001$), ICAM-1 ($\rho=0.30$, $p=0.001$), e-selective ($\rho=0.28$, $p=0.002$), and triglyceride concentrations ($\rho=0.45$, $p<0.001$) in patients with SLE. There were no statistically significant associations between GlycA and age, coronary calcium scores, creatinine, glomerular filtration rate, SLEDAI, SLICC, and VCAM-1 (Table 2).

Discussion

The two novel findings of this study are that GlycA concentrations were higher in patients with SLE than in control subjects and that GlycA concentrations correlated with acute phase reactants, multiple inflammatory markers, and lipid concentrations.

There is an increased recognition of the need for better markers of SLE disease activity in clinical practice.⁵ Several characteristics of GlycA made it a potentially interesting marker in SLE. First, it may be a more stable marker of inflammation, less susceptible to alterations by both day to day variability and by non-inflammatory conditions such as age, anemia, and BMI than CRP or ESR.¹ In keeping with this, we found that while the correlation between ESR and hemoglobin was strong, GlycA was not associated with hemoglobin. Similarly, the correlation between CRP and BMI was strong, but GlycA only correlated weakly with BMI.

Second, GlycA concentrations correlated with disease activity in patients with RA.⁴ However, despite the fact that GlycA concentrations were higher in SLE than controls and were associated with several acute phase reactants and adhesion molecules, they were not associated with lupus disease activity. One possible explanation is that C3, an acute phase protein, is a minor contributor to the GlycA signal. However, due to activation of the complement cascade, low complement levels are a feature of lupus disease activity. As expected, we found both a significant negative correlation between CH50 and SLEDAI; however, there was a significant positive correlation between GlycA and both CRP and CH50.

We found that both CRP and ESR were weakly correlated with SLEDAI. Consistent with our findings, elevated ESR has been associated with higher lupus activity in other studies. A report from a large cohort of patients with SLE showed that patients with $\text{ESR}>75$ mm/hr had a two-fold increase in the systemic lupus activity measure (SLAM) score than those with an $\text{ESR}<25$ mm/hr.¹⁵ A similar relationship was found between ESR and the SELENA-SLEDAI in another large cohort.¹⁶

In addition to examining GlycA as a marker of lupus activity, we explored its role as marker of coronary atherosclerosis. Three reasons supported the hypothesis that GlycA could be

associated with coronary atherosclerosis in SLE. First, in the general population² and in patients with RA⁴ GlycA concentrations were associated with coronary atherosclerosis. Second, a panel of four non-traditional biomarkers (pro-inflammatory HDL function, leptin, sTWEAK, and homocysteine) was recently described as a tool to predict carotid atherosclerosis in patients with SLE.¹⁷ Third, the ability of GlycA to capture many glycosylated products at once could be advantageous to mimic a multibiomarker approach to assessing cardiovascular risk. However, despite the association of GlycA with systolic and diastolic blood pressure, triglyceride, and HDL concentrations, it was not associated with CAC scores. This does not exclude that GlycA may be associated with non-calcified atherosclerosis that would not be detected by a non-contrast enhanced CT scan.

This study needs to be interpreted in the light of some limitations. The sample size is relatively small, the design is cross-sectional, and there were multiple comparisons. However, after using the Bonferroni correction and resetting the threshold for statistical significance to a $p < 0.0023$, the association between GlycA with CRP, ESR, e-selectin, ICAM-1, and triglycerides remained significant. Also, we studied a group of SLE patients with relative low disease activity and almost normal renal function. Therefore, the utility of GlycA in patients with more severe disease, with renal involvement, and its role during clinical course or on cardiovascular event prediction remain to be determined.

In conclusion, GlycA is increased in patients with SLE and associated with inflammatory markers and lipids, but is not associated with SLE disease activity or coronary artery calcium as a marker of subclinical atherosclerosis.

Acknowledgements

This study was supported by grants (P60AR056116, K23AR064768, and GM5M01-RR00095) from the National Institutes of Health, Grant UL1 RR024975-01, now at the National Center for Advancing Translational Sciences, Grant 2 UL1 TR000445-06, the Vanderbilt Physician Scientist Development Award, and the Arthritis Foundation Clinical to Research Transition Award.

Reference List

1. Otvos JD, Shalaurova I, Wolak-Dinsmore J, et al. GlycA: A Composite Nuclear Magnetic Resonance Biomarker of Systemic Inflammation. Clin Chem. In press.
2. Akinkuolie AO, Buring JE, Ridker PM, Mora S. A Novel Protein Glycan Biomarker and Future Cardiovascular Disease Events. J Am Heart Assoc. 2014; 3:e001221. [PubMed: 25249300]
3. Bell JD, Brown JC, Nicholson JK, Sadler PJ. Assignment of resonances for 'acute-phase' glycoproteins in high resolution proton NMR spectra of human blood plasma. FEBS Lett. 1987; 215(2):311–315. [PubMed: 2438159]
4. Ormseth M, Chung CP, Oeser A, et al. Utility of a novel inflammatory marker, GlycA, for assessment of rheumatoid arthritis disease activity and coronary atherosclerosis. Arthritis Res Ther. In press.
5. Ahearn JM, Liu CC, Kao AH, Manzi S. Biomarkers for systemic lupus erythematosus. Transl Res. 2012; 159(4):326–342. [PubMed: 22424435]
6. Asanuma Y, Oeser A, Shintani AK, et al. Premature coronary-artery atherosclerosis in systemic lupus erythematosus. N Eng J Med. 2003; 349(25):2407–2415.
7. Asanuma Y, Chung CP, Oeser A, et al. Increased concentration of proatherogenic inflammatory cytokines in systemic lupus erythematosus: relationship to cardiovascular risk factors. J Rheumatol. 2006; 33(3):539–545. [PubMed: 16463434]

8. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997; 40(9):1725. [PubMed: 9324032]
9. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum.* 1992; 35(6):630–640. [PubMed: 1599520]
10. Gladman D, Ginzler E, Goldsmith C, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum.* 1996; 39(3):363–369. [PubMed: 8607884]
11. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Vaimonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol.* 1990; 15(4): 827–832. [PubMed: 2407762]
12. Botev R, Mallie JP, Couchoud C, et al. Estimating glomerular filtration rate: Cockcroft- Gault and Modification of Diet in Renal Disease formulas compared to renal inulin clearance. *Clin J Am Soc Nephrol.* 2009; 4(5):899–906. [PubMed: 19406960]
13. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med.* 2006; 26(4):847–870. [PubMed: 17110242]
14. Chung CP, Oeser A, Raggi P, et al. Lipoprotein subclasses and particle size determined by nuclear magnetic resonance spectroscopy in systemic lupus erythematosus. *Clin Rheumatol.* 2008; 27(10): 1227–1233. [PubMed: 18421545]
15. Vila LM, Alarcon GS, McGwin G Jr, Bastian HM, Fessler BJ, Reveille JD. Systemic lupus erythematosus in a multiethnic cohort (LUMINA): XXIX. Elevation of erythrocyte sedimentation rate is associated with disease activity and damage accrual. *J Rheumatol.* 2005; 32(11):2150–2155. [PubMed: 16265693]
16. Stojan G, Fang H, Magder L, Petri M. Erythrocyte sedimentation rate is a predictor of renal and overall SLE disease activity. *Lupus.* 2013; 22(8):827–834. [PubMed: 23761098]
17. McMahon M, Skaggs BJ, Grossman JM, et al. A panel of biomarkers is associated with increased risk of the presence and progression of atherosclerosis in women with systemic lupus erythematosus. *Arthritis Rheumatol.* 2014; 66(1):130–139. [PubMed: 24449580]

Figure.
GlycA concentrations in patients with SLE and control subjects

Table 1

Clinical characteristics of patients and control subjects

	Patients with SLE	Control subjects	p-value
Age (yrs)	40±12	41±12	0.71
Female sex, %	91%	86%	0.25
Caucasian race, %	71%	68%	0.64
Systolic blood pressure (mm/hr)	121±18	117±14	0.36
Diastolic blood pressure (mm/hr)	74±14	71±10	0.16
Coronary calcium score (Agatston units)	71±287	4±27	<0.001
Total cholesterol (mg/dl)	172±46	179±41	0.10
HDL cholesterol (mg/dl)	47±15	49±16	0.54
LDL cholesterol (mg/dl)	101±38	111±35	0.02
Triglycerides (mg/dl)	121±61	99±56	0.004
Creatinine (mg/dl)	1.0±1.5	0.8±0.1	0.94
Glomerular filtration rate (mL/min per 1.73 m ²)	89±29	91±20	0.55
C-reactive protein (mg/l)	7.2±8.4	N/A	N/A
Interleukin-6 (pg/ml)	20.2±32.9	6.7±16.1	<0.001
Tumor necrosis factor (pg/ml)	6.3±6.2	N/A	N/A
E-selectin (ng/ml)	24.9±11.8	18.5±7.2	<0.001
Intracellular adhesion molecule-1 (ICAM-1), (ng/ml)	192.9±73.5	153.3±57.8	<0.001
Vascular adhesion molecule-1 (VCAM-1), (ng/ml)	1092.1±329.1	988.8±224.8	0.02

Table 2

GlycA, erythrocyte sedimentation rate, and C-reactive protein: correlations with disease characteristics, markers of inflammation and coronary atherosclerosis in patients with SLE

	GlycA, rho	ESR, rho	CRP, rho
Age (years)	-0.08	-0.24*	0.11
Body mass index (kg/m ²)	0.25	0.23*	0.41*
Hemoglobin (g/dl)	-0.06	-0.46*	-0.07
Creatinine (mg/dl)	-0.04	0.01	-0.02
Glomerular rate filtration (mL/min per 1.73 m ²)	0.09	0.08	<0.01
Erythrocyte sedimentation rate (mm/hr)	0.43*	--	0.34*
C-reactive protein (mg/l)	0.59*	0.34*	--
Interleukin-6 (pg/ml)	0.27	0.37*	0.27
Tumor necrosis factor (pg/ml)	0.18	0.33*	0.21
E-selectin (pg/ml)	0.28*	0.26*	0.23
Intracellular adhesion molecule-1 (ICAM-1)	0.30*	0.08	0.40*
Vascular adhesion molecule-1 (VCAM-1)	0.02	-0.04	0.09
Total cholesterol (mg/dl)	0.17	-0.12	0.15
LDL cholesterol (mg/dl)	0.17	0.03	0.16
HDL cholesterol (mg/dl)	-0.26	-0.42*	-0.21
Triglycerides (mg/dl)	0.45*	0.17	0.37*
Systolic blood pressure (mm Hg)	0.23	0.12	0.16
Diastolic blood pressure (mm Hg)	0.21	0.01	0.08
Coronary calcium score (Agatston units)	0.14	0.02	0.20*
SLEDAI	0.15	0.20	0.20*
SLICC damage index	0.02	0.01	-0.08
CH50	0.30*	-0.05	0.22

* p<0.0023 (statistically significant p-value after correction for multiple comparisons)

LDL: Low density lipoprotein

HDL: High density lipoprotein

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index

CH50: hemolytic complement

SLICC: Systemic Lupus International Collaborating Clinics