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BRIEF REPORT

# Plasma levels of GlycA, a pro-inflammatory glycoprotein biomarker, associate with an increased risk of microvascular complications in patients with type 2 diabetes (Zodiac-62)

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## Abstract

**Purpose** GlycA, a pro-inflammatory glycoprotein biomarker, associates with newly developed type 2 diabetes (T2D). We determined the association of plasma GlycA with the development of microvascular complications in patients with established T2D.

**Methods** Plasma GlycA was measured by nuclear magnetic resonance spectrometry in T2D patients without microvascular complications at baseline ($n = 145$) participating in a longitudinal cohort study of primary care-treated T2D patients (Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study). Associations of GlycA with incident microvascular complications including nephropathy, retinopathy, and neuropathy, were determined by Cox proportional hazards regression analyses.

**Results** After a median follow-up of 3.2 (interquartile range [IQR]: 2.9–3.4) years, 49 patients (33.8%) developed one or more microvascular complications. Median GlycA levels were 453.5 (IQR: 402.0–512.8) µmol/l. GlycA was associated with incident microvascular complications (hazard ratio [HR] per 1-SD increment: 1.28 [95% confidence interval [CI]:1.00–1.63], $P = 0.048$]), even after adjustment for potential confounders and high-sensitive C-reactive protein (hs-CRP), HR 1.79 [95%CI:1.25–2.57], $P = 0.001$). In contrast, hs-CRP levels were not significantly associated with the risk of developing microvascular complications ($P = 0.792$).

**Conclusion** Higher plasma GlycA is associated with an increased risk of developing microvascular complications in T2D patients. Altered *N*-glycan branching associated with acute-phase reactive proteins may represent a preferred biomarker of systemic low-grade inflammation in predicting diabetic complications.

**Keywords** Type 2 diabetes · GlycA · Inflammation · Microvascular complications · hs-CRP

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## Introduction

Low-grade systemic inflammation is a commonly observed phenomenon in non-communicable inflammatory diseases such as cardiovascular diseases, autoimmune diseases, cancer and type 2 diabetes (T2D) [1–5]. Furthermore, low-grade systemic inflammation is enhanced by lifestyle factors like smoking behavior, alcohol consumption, dietary habits and physical exercise [6]. A widely used biomarker of low-grade systemic inflammation is high-sensitive C-reactive protein (hs-CRP), an acute-phase protein released from hepatocytes [7]. More recently, plasma levels of a composite biomarker of acute-phase protein glycosylation, called GlycA, have been documented to be associated with unfavorable cardiometabolic outcomes [5, 8]. GlycA is

associated with all-cause mortality, reduced life expectancy and incident cardiovascular disease in the general population [6, 9–11]. Although GlycA and hs-CRP are found to be moderately interrelated, GlycA seems to be a less variable biomarker of low-grade systemic inflammation, presumably because of its composite reflection of the acute-phase response [6, 8]. GlycA can be measured on a high-throughput basis using nuclear magnetic resonance (NMR) spectroscopy, where signals are detected from *N*-acetyl methyl groups embedded in the antennary branches of serum glycoproteins such as the acute-phase proteins haptoglobin, α1-antitrypsin, α1-antichymotrypsin, α1-acid glycoprotein, and transferrin [8]. Accordingly, plasma GlycA levels are correlated with *N*-glycan branching as determined by matrix-assisted laser desorption/ionization-mass spectrometry (Maldi-MS) [12].

Previously, we demonstrated that GlycA is associated with incident T2D independent of traditional clinical risk factors and hs-CRP in the general population [13]. These data indicated that GlycA might be a suitable biomarker for assessing the risk of future T2D development. However, the potential utility of GlycA in already established T2D, particularly in relation to the development of microvascular complications, has remained unexplored.

We hypothesized that circulating levels of GlycA, reflecting low-grade systemic inflammation, may also have potential merit as a prognostic biomarker for the development of microvascular complications in patients with T2D. In this study, we aimed to investigate the association between GlycA and the development of microvascular complications in T2D and comparatively assessed its utility in relation to hs-CRP.

## Materials and methods

Plasma levels of GlycA were determined in samples from patients with T2D participating in the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study, a Dutch longitudinal observational cohort study involving individuals with established T2D treated in primary care [14]. This study was approved by the Institutional Review Board (IRB) of the Isala Hospital in Zwolle, the Netherlands (IRB reference nos. 03.0316 and 07.0335). All patients provided informed consent for study participation. Patients with T2D with available plasma samples at baseline and data on existing and follow-up onset of microvascular complications (i.e., the presence of either diabetic retinopathy, nephropathy, or neuropathy) were selected, from which patients with existing microvascular complications at baseline were subsequently excluded. The development of diabetic retinopathy was determined by fundus images obtained via a retinal camera by experienced ophthalmologists. Diabetic nephropathy was defined as the occurrence of at least two consecutive measurements of albuminuria (albumin-to-creatinine ratio >3.5 mg/mmol for women and >2.5 mg/mmol for men) or as single establishment of albuminuria while the patient was using an angiotensin-converting enzyme-inhibitor or angiotensin-II-receptor antagonist. Diabetic neuropathy was defined as two or more errors made out of three tests of foot sensibility on at least one foot leveraging a 5.07 Semmes-Weinstein monofilament.

Ethylene diamine tetra-acetic acid-anticoagulated plasma samples were stored at −80 °C until analysis. GlycA concentration was assayed on the Vantera® Clinical Analyzer using NMR LipoProfile® Test spectra, where GlycA was quantified as described previously [6]. Briefly, the GlycA NMR signal was derived from *N*-acetyl methyl group of *N*-acetylglucosamine moieties residing in bi-, tri-, and tetra-antennary branches of plasma acute-phase glycoproteins. Coefficients of variation (CVs) of the GlycA assay vary from 1.3–2.3%. Plasma hs-CRP levels were determined by nephelometry (BNII; Dade Behring Diagnostics, Marburg, Germany) with a threshold of 0.18 mg/l and a CV of 3.6%.

To study the association between plasma GlycA and the future occurrence of microvascular complications, we used multivariable Cox proportional hazards regression analyses where results were expressed as hazard ratios (HRs) with corresponding 95% confidence intervals. Plasma GlycA and hs-CRP levels were standardized to allow for the expression of HRs per 1-SD increment (or decrement) in biomarker level. The proportionality of hazards assumption was checked to confirm absence of violation. Kaplan-Meier survival analysis was used to determine survival distributions for patients with below- and above-median GlycA levels, which were compared using log-rank tests. We additionally fitted restricted cubic splines (RCS) with three knots to evaluate potential non-linearity of the observed association in Cox proportional hazards regression analysis. Non-linearity was assessed using likelihood ratio tests, in which nested models were compared with each other using linear or linear and cubic spline terms. Statistical analysis was performed using SPSS Statistics 28.0 software (SPSS Inc., Chicago, IL, USA) and R (v.4.0.1, Vienna, Austria). Two-tailed $P$ values ≤ 0.05 were considered statistically significant.

## Results

In total, 145 patients with T2D without microvascular complications at baseline and available follow-up data were included. Baseline demographic and clinical characteristics of the study population are presented in Table 1. During a

**Table 1** Baseline demographic and clinical characteristics of the study population

| | Total cohort ($n = 145$) | Microvascular complications ($n = 49$) | No microvascular complications ($n = 96$) | $P$ value* |
|---|---|---|---|---|
| Age (years) | 63.7 ± 10.7 | 67.0 ± 10.3 | 61.5 ± 10.4 | 0.003 |
| Sex | | | | 0.121 |
| Male, *n* (%) | 64 (44.1) | 25 (51.0) | 39 (42.4) | |
| Female, *n* (%) | 81 (55.9) | 24 (49.0) | 53 (57.6) | |
| BMI, kg/m$^2$ | 28.7 [25.21–32.0] | 30.1 [25.5–33.9] | 28.6 [24.7–31.5] | 0.110 |
| Disease duration (years) | 2.7 [1.3–6.0] | 3.0 [1.4–6.0] | 2.6 [1.3–5.0] | 0.428 |
| Systolic blood pressure (mmHg) | 145 [140–165] | 150 [140–163] | 145 [135–164] | 0.638 |
| Current smoking, *n* (%) | 22 (15.2) | 6 (12.2) | 16 (17.4) | 0.498 |
| History of macrovascular complications, *n* (%) | 35 (24.1) | 15 (30.6) | 20 (21.7) | 0.261 |
| Diabetes therapy at baseline | | | | <0.001 |
| Diet, *n* (%) | 32 (22.0) | 8 (16.3) | 24 (26.1) | |
| Oral blood glucose-lowering drugs, *n* (%) | 100 (69.0) | 34 (69.4) | 66 (68.8) | |
| Insulin, *n* (%) | 6 (4.1) | 3 (6.1) | 3 (3.1) | |
| Both, *n* (%) | 4 (2.8) | 4 (8.2) | 0 (0.0) | |
| HbA1c (%) | 6.6 [6.0–7.5] | 7.0 [6.3–8.2] | 6.5 [5.9–7.3] | 0.008 |
| HbA1c (mmol/mol) | 48.6 [42.1–59.0] | 53.0 [45.4–66.1] | 47.5 [41.0–56.3] | 0.008 |
| eGFR (ml/min/1.73 m$^2$) | 75.5 [59.3–91.0] | 75.0 [60.0–88.0] | 77.7 [59.2–93.7] | 0.716 |
| Total cholesterol (mmol/l) | 5.4 [4.9–6.1] | 5.5 [5.0–6.1] | 5.3 [4.7–6.1] | 0.369 |
| Triglycerides (mmol/l) | 2.1 [1.5–3.2] | 2.2 [1.5–3.0] | 2.1 [1.5–3.2] | 0.762 |
| GlycA (µmol/l) | 454 [402–513] | 471 [406–535] | 430 [401–493] | 0.037 |
| hs-CRP (mg/l) | 2.5 [1.0–5.4] | 2.5 [1.2–6.2] | 2.2 [0.8–5.5] | 0.443 |

Data are presented as mean ± standard deviation (SD), median [25th–75th percentiles] or as proportions with corresponding percentages (%)

*BMI* body mass index, *eGFR* estimated glomerular filtration rate, *hs-CRP* high sensitive C-reactive protein

*$P$ values for differences between patients who developed microvascular complications or not during follow-up, calculated using independent sample $t$ tests or Mann-Whitney $U$ tests, as appropriate

median follow-up of 3.2 years (interquartile range [IQR]: 2.9–3.4 years), 49 of 145 patients developed one or more microvascular complications (33.8%). Of these 49 patients, 10 developed retinopathy, 19 nephropathy and 29 neuropathy, alone or in combination. Mean age at baseline was 63.7 (±10.7) years and 55.9% of patients were female. Besides diet and lifestyle modification and the use of oral blood glucose-lowering drugs (metformin, sulfonylurea, and other drugs), 6 (4.1%) used insulin monotherapy. Median HbA1c levels were 6.6% (IQR: 6.0–7.5) (48.6 [IQR: 42.1–58.7] mmol/mol). Median GlycA levels were 454 [IQR: 402–513] µmol/l and median hs-CRP levels were 2.5 [IQR: 1.0–5.4] mg/l. Kaplan-Meier survival analysis demonstrated significantly different survival distributions between patients with below- and above-median plasma GlycA levels (log-rank test, $P = 0.032$) (Fig. 1A). In Cox proportional hazards regression analyses, higher GlycA levels were associated with an increased risk of developing one or more microvascular complications (HR per 1-SD increment: 1.28 [95% CI: 1.00–1.63], $P = 0.048$, Table 2). No significant deviation from linear association with the risk of microvascular complications was observed when RCS were fitted ($P = 0.719$, Fig. 1B). After adjustment for potentially confounding factors, including age, sex, HbA1c, total cholesterol, triglycerides, BMI, eGFR, and hs-CRP, this association remained robust and statistically significant. Separate analyses using retinopathy, nephropathy, and neuropathy as individual outcomes showed crude HRs per 1 standard deviation (SD) increment of 1.55 [95% CI: 0.91–2.64], 1.06 [95% CI: 0.70–1.62] and 1.31 [95% CI: 0.95–1.79], respectively, but these associations did not reach statistical significance likely due to lower numbers of events. Plasma levels of GlycA were correlated with hs-CRP (Spearman rank correlation coefficient: 0.39, $P < 0.001$). Notably, in contrast to GlycA, plasma hs-CRP levels were not significantly associated with the risk of developing microvascular complications in any of the studied models (all $P > 0.05$, Table 2).

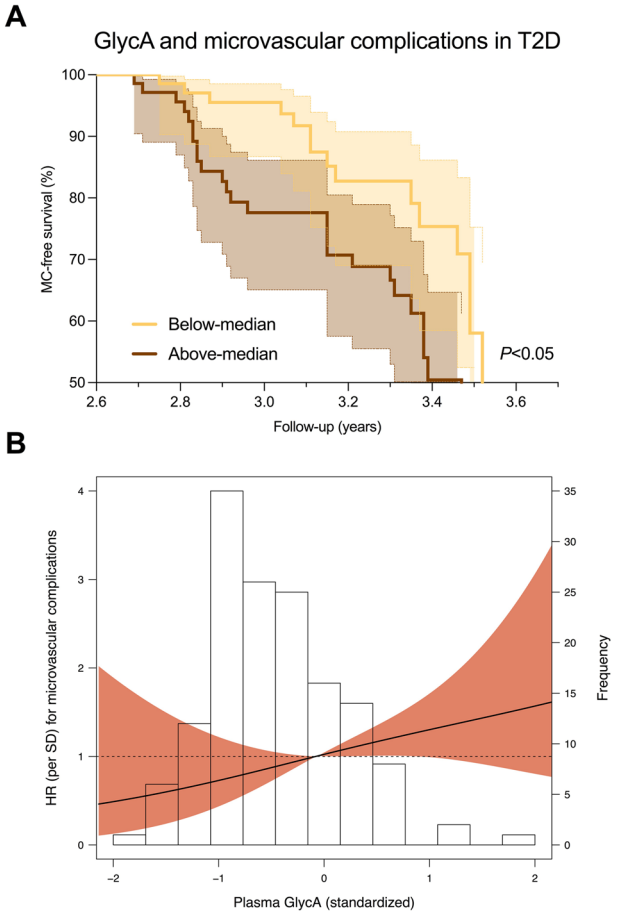


**Fig. 1** Higher plasma GlycA levels associate with increased risk of microvascular complications in type 2 diabetes. **A** Kaplan-Meier survival curve demonstrating significantly differential survival distributions for below- and above-median plasma GlycA levels (log-rank test, $P = 0.032$), with the highest event rate observed in patients with above-median GlycA. **B** Restricted cubic spline (RCS) curve showing the association between plasma GlycA levels and the risk of microvascular complications. The association was estimated from Cox proportional hazards regression and RCS with three knots (set at the 1st, 50th, and 99th percentiles). A likelihood ratio test for non-linearity was non-significant ($\chi^2 = 0.13$, $P = 0.719$). Color-shaded areas represent 95% confidence intervals. Abbreviations: HR, hazard ratio; T2D, type 2 diabetes

## Discussion and conclusions

Taken together, the current findings in a contemporary cohort of T2D patients treated in primary care consent with the hypothesis that higher plasma GlycA levels are associated with an increased risk of developing one or more microvascular complications. Several earlier reports have shown that low-grade systemic inflammation, as evidenced by raised hs-CRP, is associated with prevalent or incident microvascular complications in T2D although such a relationship has not always been demonstrated [7, 15–18]. Notably, we found no association of hs-CRP with incident complications in the present cohort. In fact, the association

**Table 2** Cox proportional hazards regression analyses for associations between circulating GlycA and hs-CRP and the risk of developing microvascular complications in patients with Type 2 diabetes

| | GlycA, per 1 SD increment | | hs-CRP, per 1 SD increment | |
|---|---|---|---|---|
| | HR [95% CI] | *P* value | HR [95% CI] | *P* value |
| Model 1 | 1.28 [1.00–1.63] | 0.048 | 1.04 [0.77–1.42] | 0.792 |
| Model 2 | 1.44 [1.10–1.87] | 0.007 | 1.07 [0.77–1.49] | 0.681 |
| Model 3 | 1.33 [1.01–1.75] | 0.041 | 1.02 [0.70–1.47] | 0.925 |
| Model 4 | 1.49 [1.11–2.02] | 0.009 | 1.04 [0.73–1.47] | 0.841 |
| Model 5 | 1.38 [1.05–1.81] | 0.020 | 0.99 [0.69–1.43] | 0.967 |
| Model 6 | 1.79 [1.25–2.57] | 0.001 | 0.86 [0.61–1.23] | 0.411 |

HRs were expressed a per 1-SD increment (GlycA: SD = 74.5 µmol/l; hs-CRP: SD = 3.7 mg/l). Model 1: crude model. Model 2, model 1 plus age and sex. Model 3, model 2 with additional adjustment for disease duration, HbA1c and previous cardiovascular events. Model 4, model 2 with additional adjustment for triglycerides and total cholesterol. Model 5, model 2 with additional adjustment for BMI and eGFR. Model 6, model 2 with adjustment for hs-CRP (for GlycA) or GlycA (for hs-CRP)

*BMI* body mass index, *hs-CRP* high sensitive C-reactive protein, *HR* hazard ratio, *SD* standard deviation

of plasma GlycA, as a composite biomarker of acute-phase inflammation, remained and was not attenuated after adjustment for hs-CRP, raising the possibility that GlycA, and hence altered *N*-glycan branching associated with acute-phase reactive proteins, could become a preferred biomarker of low-grade systemic inflammation in the context of diabetic complications. Since this patient cohort study is of a longitudinal observational nature, we were not able to establish causality, but instead could only document associations. Still, our present results emphasize the potential utility of plasma GlycA as composite biomarker of low-grade systemic inflammation in relation to disease progression (e.g., the development of microvascular complications) in patients with established T2D. Future studies are warranted to further examine the value of this biomarker in larger and diverse clinical populations in relation to disease progression outcomes of patients with T2D.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

**Ethics approval** This study was performed in accordance with the principles of the Declaration of Helsinki (2013). This study was approved by the Institutional Review Board (IRB) of the Isala Hospital in Zwolle, the Netherlands (IRB reference nos. 03.0316 and 07.0335).

**Consent to participate** All patients provided informed consent for study participation and publication.

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