GlycA, a novel pro-inflammatory glycoprotein biomarker is associated with mortality

Gruppen, E G; Kunutsor, S K; Kieneker, L M; van der Vegt, B; Connelly, M A; de Bock, G H; Gansevoort, R T; Bakker, S J L; Dullaart, R P F

Published in:
Journal of Internal Medicine

DOI:
10.1111/joim.12953

Publication date:
2019

JIM Original Article

doi: 10.1111/joim.12953

GlycA, a novel pro-inflammatory glycoprotein biomarker is associated with mortality: results from the PREVEND study and meta-analysis

E. G. Gruppen[1,2], S. K. Kunutsor[3,4], L. M. Kieneker[1], B. van der Vegt[5], M. A. Connelly[6], G. H. de Bock[7], R. T. Gansevoort[1], S. J. L. Bakker[1] & R. P. F. Dullaart[2]

From the [1]Divisions of, Nephrology; [2]Endocrinology, Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; [3]National Institute for Health Research Bristol Biomedical Research Centre, University Hospitals Bristol NHS Foundation Trust and University of Bristol; [4]Translational Health Sciences, Musculoskeletal Research Unit, Bristol Medical School, Southmead Hospital, University of Bristol, Bristol, UK; [5]Division of Pathology, Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [6]Laboratory Corporation of America® Holdings (LabCorp), Morrisville, NC, USA; and [7]Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Abstract. Gruppen EG, Kunutsor SK, Kieneker LM, van der Vegt B, Connelly MA, de Bock GH, Gansevoort RT, Bakker SJL, Dullaart RPF. GlycA, a novel pro-inflammatory glycoprotein biomarker is associated with mortality: results from the PREVEND study and meta-analysis. *J Intern Med.* 2019; https://doi.org/10.1111/joim.12953

Background. Chronic diseases are associated with an inflammatory response. We determined the association of two inflammatory markers, GlycA and high-sensitivity C-reactive protein (hsCRP), with overall and cause-specific mortality in a cohort of men and women.

Methods. Cox regression analyses were used to examine associations of GlycA and hsCRP with all-cause, cancer and cardiovascular mortality in 5526 subjects (PREVEND cohort; average follow-up 12.6 years).

Results. GlycA was associated with all-cause mortality ($n = 838$), independent of clinical risk factors and hsCRP (hazard ratio 1.43 [95% confidence interval (CI): 1.09–1.87] for top versus bottom quartiles). For hsCRP, the association with all-cause mortality was nonsignificant after adjustment for GlycA. GlycA and hsCRP were associated with cancer mortality in men ($n = 248$), but not in women ($n = 132$). Neither GlycA nor hsCRP was independently associated with cardiovascular mortality ($n = 201$). In a meta-analysis of seven population-based studies, including 8153 deaths, the pooled multivariable-adjusted relative risk of GlycA for all-cause mortality was 1.74 (95% CI: 1.40–2.17) for top versus bottom quartiles. The association of GlycA with all-cause mortality was somewhat stronger than that of hsCRP. GlycA and hsCRP were not independently associated with cardiovascular mortality. The associations of GlycA and hsCRP with cancer mortality were present in men, but not in women.

Conclusions. GlycA is significantly associated with all-cause mortality. GlycA and hsCRP were each not independently associated with cardiovascular mortality. The association of GlycA and hsCRP with cancer mortality appears to be driven by men.

Keywords: C-reactive protein, GlycA, glycoproteins, inflammation, mortality, nuclear magnetic resonance spectroscopyPA.

Introduction

Accumulating evidence shows that there may be a link between systemic low-grade inflammation and major adverse health issues. Numerous studies have shown an association between low-grade inflammation and lifestyle factors such as obesity [1], exercise [2], smoking [3] and diet [4]. In addition, enhanced low-grade inflammation may play a role in the aetiology of chronic diseases such as cardiovascular disease (CVD) [5], type 2 diabetes (T2D) [6] and cancer [7].

GlycA and high-sensitivity C-reactive protein (hsCRP) are both markers of low-grade systemic inflammation. Whilst GlycA is a composite biomarker that senses the glycosylation states of several of the most abundant acute-phase proteins [8], hsCRP is a single marker of low-grade systemic inflammation. GlycA is determined using nuclear magnetic resonance (NMR) spectroscopy; the signal comes from *N*-acetyl methyl groups mostly bound to acute-phase proteins [mainly: α1-acid glycoprotein (oromucosoid), haptoglobin, α1-antitrypsin, α1-antichymotrypsin and transferrin] [8]. GlycA and hsCRP were found to be rather strongly correlated with each other [8–10], but hsCRP is not highly glycosylated, thus it contributes negligibly to the measured GlycA signal.

GlycA has been found to be associated with incident CVD events as well as with new onset T2D in multiple large studies [9,11–15]. Interestingly, its association with CVD and with incident T2D remained present after adjustment for hsCRP, suggesting that the association of GlycA with adverse cardiometabolic outcomes is as at least as strong as that with hsCRP. Of further interest, GlycA has been shown to be associated with cancer incidence in the Women's Health Study (WHS) [16] and with cancer-hospitalization and mortality in the Multi-Ethnic Study of Atherosclerosis (MESA) [11].

We have recently shown that higher levels of GlycA were associated with reduced life expectancy [17]. Furthermore, the association of GlycA with all-cause mortality has been evaluated in a number of studies. GlycA was associated with all-cause mortality in high-risk populations of subjects with established CVD or with several cardiovascular risk factors [18–20]. Comparable results on GlycA and all-cause mortality were found in general population-based studies [11,21]. However, published studies on GlycA and all-cause mortality showed effect sizes ranging from 1.30 to 2.40. The current study will investigate how the variability in effect size might be explained. Further, limited data are available with regard to GlycA and cause-specific mortality.

Hence, the aims of the current study were (i) to examine the associations of GlycA and hsCRP with all-cause, CVD and cancer mortality in the Prevention of Renal and Vascular End-Stage Disease (PREVEND) cohort, a general predominantly Caucasian population of both men and women and (ii) to report on a meta-analysis of published evidence on the association of GlycA with all-cause mortality.

Subjects and methods

Study design and population

The Prevention of Renal and Vascular End-Stage Disease (PREVEND) study was designed to investigate the natural course of increased levels of urinary albumin excretion and its relation to renal and CVD in a large cohort drawn from the general population. In short, in the period from 1997 to 1998, all inhabitants of the city of Groningen (The Netherlands) aged 28–75 years were asked to send in a morning urine sample and to fill out a short questionnaire. Pregnant women and subjects with type 1 diabetes mellitus were excluded. Urinary albumin concentration was assessed in 40 856 (47.8%) responders. Subjects with a urinary albumin concentration of $\geq$10 mg L^{-1} (n = 7768) were invited to participate, of whom 6000 agreed. Furthermore, 3394 randomly selected subjects with a urinary albumin concentration <10 mg L^{-1} were invited and 2592 agreed to participate. These 8592 individuals constitute the actual PREVEND cohort.

For the current study, data were used from the second screening round (2001–2003) in which 6894 subjects participated. GlycA and hsCRP were measured in 5526 subjects of the second screening round (Figure S1). The PREVEND study has been approved by the medical ethics committee of the University Medical Center Groningen, The Netherlands, and was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants gave written informed consent.

Mortality data

The cause of death was obtained by linking the number of the death certificate to the primary cause of death as coded by a physician from the Dutch Central Bureau of Statistics (CBS). Causes of death were coded according to the 10th revision of the International Classification of Diseases. Survival time for the participants was defined as the period from the date of blood collection of the participant at the second screening round to the date of death from any cause or January first 2017, until which date information about specific causes of death follow-up information was available. If a person had moved to an unknown destination, the date on which the person was dropped from the

municipal registry was used as the census date. Cardiovascular mortality was defined as a cardiovascular event leading to or directly causing death. Qualifying cardiovascular events were ICD-10 codes I10-I99, which include myocardial infarction, stroke, abdominal aortic aneurysm, pulmonary embolism, arrhythmias, myocarditis, cardiomyopathy, cardiac arrest, heart failure, cerebrovascular diseases and intraoperative and postprocedural complications and disorders of circulatory system. Cancer mortality was defined as death due to any type of malignancy (ICD-10 codes C00–C97).

Data collection

The procedures at each examination in the PREVEND study have been described in detail previously [22]. In short, before the outpatient clinic visit, all participants completed a questionnaire regarding demographics, cancer, cardiovascular and renal disease history, smoking habits, alcohol consumption and medication use. Cancer incidence was established by computerized record linkage with the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA: Dutch Pathology Registry) [23]. A history of cancer was defined as any type of malignancy, indicated by the patient in the questionnaire or obtained by PALGA.

Information on medication use (including oral contraceptive use and hormone replacement therapy) was combined with information from a pharmacy-dispensing registry, which has complete information on drug usage of >95% of subjects in the PREVEND study. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m). Smoking status was categorized as never, former and current. Alcohol intake was categorized as <10 g or ≥10 g per day. T2D was defined as a fasting serum glucose level >7.0 mmol L^{-1}, a non-fasting plasma glucose level >11.1 mmol L^{-1}, self-report of a physician diagnosis or the use of glucose-lowering drugs, retrieved from a central pharmacy registry. Estimated glomerular filtration rate (eGFR) was calculated using the combined creatinine–cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equation [24].

Laboratory measurements

Plasma samples were sent frozen to LipoScience/LabCorp (Morrisville, NC, USA) for testing on the Vantera® Clinical Analyzer. *NMR LipoProfile®* Test spectra were collected and GlycA values were quantified as previously described [8,14,25]. In short, the GlycA NMR signal comes from the *N*-acetyl methyl group protons of the *N*-acetylglucosamine moieties located on the bi-, tri- and tetra-antennary branches of plasma glycoproteins, mainly α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin and transferrin. The coefficients of variation (CVs) for the GlycA assay ranged from 1.3% to 2.3%. hsCRP was measured by nephelometry with a threshold of 0.18 mg L^{-1} (BNII; Dade Behring, Marburg, Germany). Plasma glucose was measured using standard laboratory protocols [26]. Serum total cholesterol was assayed on an automatic analyser type MEGA (Merck, Darmstadt, Germany) using the CHOD-PAP-method. Measurement of serum creatinine was performed by an enzymatic method on a Roche Modular analyzer (Roche Diagnostics, Mannheim, Germany). Serum cystatin C concentrations were measured by Gentian Cystatin C Immunoassay (Gentian AS, Moss, Norway) on a Modular analyzer (Roche Diagnostics). Urinary albumin concentration was measured by nephelometry with a threshold of 2.3 mg L^{-1}, and intra- and inter-assay CVs of 2.2% and 2.6%, respectively (Dade Behring Diagnostic, Marburg, Germany).

Statistical analysis

SPSS (version 22.0; SPSS Inc. Armonk, NY: IBM Corp) and STATA version 13.1 (StataCorp, College Station, TX: StataCorp LP) were used for data analysis. Results are presented as mean ± SD, median (interquartile range) and percentages. Skewed data were normalized by natural logarithmic (Log_e) transformation before analyses, which was the case for urinary albumin excretion (UAE) and hsCRP. Baseline characteristics were calculated across sex-stratified quartiles of GlycA. *P*-values across quartiles of GlycA were determined by linear regression for continuous variables or chi-square test for categorical variables. To study the association of GlycA and hsCRP with mortality, we fitted Cox proportional hazard models to the data. Tests of trend across quartiles were conducted by assigning the median value for each quartile as its value and treating this as a continuous variable. Results are summarized by hazard ratios (HRs), with 95% confidence intervals. Possible effect modification was explored by including the interaction terms between GlycA or hsCRP and age, sex or smoking in the multivariable-adjusted models. Interaction terms were considered to be

statistically significant at two-sided *P*-values <0.10 [27]. Otherwise, the levels of significance were set at two-sided *P*-values <0.05.

Meta-analysis of published studies

Studies that determined the association between GlycA and all-cause mortality, published in full text before 17 December 2018 (date last searched), were identified through electronic searches not limited to the English language using MEDLINE and EMBASE. Reference lists from included articles were scanned as well. Summary measures were presented as relative risks (RR) with 95% CI intervals and were pooled using a random effects model to minimize the effect of between-study heterogeneity. We assessed heterogeneity using the Cochrane chi-square statistic and the I^2 statistic.

Results

Table 1 shows the baseline characteristics according to sex-stratified quartiles of GlycA. The mean age of the subjects was 53.6 ± 12.1 years at baseline and 52.4% were women. Mean GlycA was 352 ± 62 μmol L^{-1} and median [IQR] hsCRP was 1.36 [0.62–3.08] mg L^{-1}. BMI, blood pressure, glucose, total cholesterol, triglycerides, hsCRP and UAE increased, whereas HDL cholesterol and eGFR decreased when GlycA levels were higher. Subjects with elevated GlycA were more likely to have comorbid conditions such as hypertension, CVD history, cancer history and T2D.

All-cause mortality

During an average follow-up of 12.6 years, 838 deaths were recorded. Associations of GlycA and hsCRP with all-cause mortality are shown in Table 2. GlycA was significantly associated with all-cause mortality in a crude model, as well as after adjustment for age, sex, BMI, alcohol consumption and smoking status. Further adjustment for T2D, blood pressure, use of lipid-lowering drugs, anti-hypertensive medication use and lipids did not substantially change the hazard associated with GlycA. Results remained essentially the same after further adjustment for CVD and cancer history (model 4) and renal function (model 5). Of note, the *P* for trend was still significant after adjustment for hsCRP. Results for hsCRP were comparable to those for GlycA. However, after adjustment for GlycA, the *P* for trend was no longer significant. In addition, there were no statistically significant interactions between GlycA or hsCRP and age, sex or smoking on outcome [interactions: $P > 0.10$ for all].

Cardiovascular mortality

During the follow-up period, 201 subjects died due to CVD events (Table 3). GlycA was significantly associated with CVD mortality in a crude model as well as after adjustment for age, sex, BMI, alcohol intake and smoking status. The association was attenuated after adjustment for T2D, systolic blood pressure, lipid-lowering drugs and anti-hypertensive medication (model 2). In addition, statistical significance was lost after adjustment for lipid levels. This was also true for the analyses with hsCRP as independent variable. There were no significant interactions for each marker with age, sex or smoking with CVD mortality.

Cancer mortality

In total, 380 participants died due to malignancies (248 men and 132 women). Table 4 shows the associations of GlycA and hsCRP with cancer mortality. GlycA was associated with cancer mortality in analyses adjusted for clinical covariates, lipids and renal function. After additional adjustment for hsCRP, the *P* for trend remained statically significant. Results for hsCRP were comparable to those for GlycA; however, in the final model, when adjusted for GlycA, the *P* for trend was no longer significant.

There was a significant interaction between hsCRP and sex with cancer mortality (*P* for interaction 0.002, tested in a model with age and sex). Table S1 shows the sex-stratified analysis of hsCRP with cancer mortality. hsCRP was significantly associated with cancer mortality in men but not in women. The interaction term between GlycA and sex was also significant in a model with age and sex (*P* for interaction 0.045). In sex-stratified analyses, GlycA was found to be significantly associated with cancer mortality in men but not in women (Table S2).

Exploratory analyses between GlycA and hsCRP with lung cancer mortality (57 deaths) are presented in Table S3. GlycA was associated with lung cancer mortality in a crude model as well as after multivariable adjustments for clinical variables and hsCRP. The association between hsCRP and

Table 1 *Baseline characteristics according to sex-stratified quartiles of GlycA concentrations in 5526 participants of the PREVEND study*

	Quartiles of GlycA, μmol L^{-1}				
	1♂ ≤304♀ ≤313	2♂ 304–388♀313–352	3♂339–382♀ 353–394	4♂ >382♀ >394	*P*-value
Participants, *n*	1387	1386	1373	1380	
Age, years	49.1 ± 10.8	53.2 ± 12.0	55.4 ± 12.2	56.8 ± 12.1	<0.001
Female, *n* (%)	732 (52.8)	718 (51.8)	720 (52.4)	723 (52.4)	0.99
BMI, kg m^{-2}	24.7 ± 3.4	26.2 ± 3.7	27.3 ± 4.1	28.3 ± 4.9	<0.001
Smoking, *n* (%)					
Never	541 (39.0)	437 (31.5)	335 (24.4)	311 (22.5)	<0.001
Former	588 (42.4)	611 (44.0)	610 (44.4)	539 (39.1)	
Current	242 (17.4)	322 (23.2)	412 (30.0)	513 (37.2)	
Alcohol intake, *n* (%)					
<10 g d^{-1}	1339 (96.5)	1318 (95.1)	1297 (94.5)	1295 (93.8)	<0.001
>10 g d^{-1}	37 (2.7)	55 (4.0)	65 (4.7)	71 (5.1)	
Hypertension, *n* (%)	229 (16.5)	417 (30.1)	548 (39.9)	652 (47.2)	<0.001
DBP, mm Hg	70.4 ± 8.7	72.6 ± 8.8	74.0 ± 8.9	74.7 ± 8.9	<0.001
SBP, mm Hg	119.0 ± 15.7	124.5 ± 18.2	128.5 ± 19.7	131.3 ± 19.6	<0.001
History of CVD	35 (2.5)	81 (5.8)	98 (7.1)	135 (9.8)	<0.001
History of cancer	26 (1.9)	27 (1.9)	34 (2.5)	45 (3.3)	0.011
History of T2D, *n* (%)	34 (2.0)	69 (4.1)	134 (8.0)	182 (11.0)	<0.001
Lipid-lowering drug use, *n* (%)	59 (4.3)	111 (8.0)	172 (12.5)	227 (16.4)	<0.001
Blood pressure-lowering drug use, *n* (%)	135 (9.7)	264 (19.0)	372 (27.1)	459 (33.3)	<0.001
Use of glucose-lowering drugs, *n* (%)	8 (0.8)	28 (2.0)	59 (4.3)	80 (5.8)	<0.001
hsCRP, mg L^{-1}	0.53 [0.27–0.99]	1.00 [0.57–1.78]	1.78 [0.98–3.25]	3.80 [1.96–7.30]	<0.001
Glucose, mmol L^{-1}	4.7 ± 0.9	4.9 ± 0.9	5.1 ± 1.2	5.3 ± 1.5	<0.001
Total cholesterol, mmol L^{-1}	5.2 ± 1.0	5.4 ± 1.0	5.5 ± 1.1	5.6 ± 1.1	<0.001
HDL cholesterol, mmol L^{-1}	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	<0.001
Triglycerides, mmol L^{-1}	0.85 [0.64–1.18]	1.05 [0.79–1.44]	1.24 [0.90–1.72]	1.39 [1.03–1.87]	<0.001
eGFR (ml/min per 1.73 m^2)	90.9 ± 15.6	85.9 ± 17.4	83.4 ± 17.6	80.3 ± 19.7	<0.001
UAE, mg/24 h	7.2 [5.7–10.4]	7.8 [5.8–12.3]	8.5 [6.1–14.4]	9.5 [6.3–20.6]	<0.001

Data are expressed as mean ± SD, median [IQR] or proportion *n* (%). *P*-values are calculated by linear regression or chi-squared analysis.

BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFRcrea-cysC, estimated glomerular filtration rate based on creatinine–cystatin C equation; HDL cholesterol, high-density lipoprotein cholesterol; hsCRP; high-sensitive C-reactive protein; LDL cholesterol, low-density cholesterol; PREVEND, Prevention of Renal and Vascular End-stage Disease; SBP, systolic blood pressure; UAE, urinary albumin excretion.

Table 2 *Association between GlycA and hsCRP levels and all-cause mortality in 5526 participants (838 deaths) of the PREVEND study*

	Quartile 1	Quartile 2	*P*-value	Quartile 3	*P*-value	Quartile 4	*P*-value	*P* for trend[a]
GlycA								
Participants (*n*)	1362	1395		1363		1406		
Range, μmol L^{-1}	<309	≥309		≥346		≥388		
No. of deaths (%)	101 (7.4)	180 (12.9)		228 (16.7)		329 (23.4)		
Crude	Ref.	**1.77 [1..39–2.26]**	**<0.001**	**2.34 [1.86–2.96]**	**<0.001**	**3.37 [2.70–4.22]**	**<0.001**	**<0.001**
Model 1	Ref.	1.11 [0.87–1.42]	0.42	**1.37 [1.08–1.74]**	**0.01**	**1.78 [1.41–2.25]**	**<0.001**	**<0.001**
Model 2	Ref.	1.04 [0.81–1.33]	0.75	1.23 [0.96–1.56]	0.10	**1.56 [1.23–1.98]**	**<0.001**	**<0.001**
Model 3	Ref.	1.085 [0.84–1.39]	0.55	1.26 [0.99–1.62]	0.06	**1.65 [1.30–2.10]**	**<0.001**	**<0.001**
Model 4	Ref.	1.07 [0.84–1.38]	0.57	1.25 [0.98–1.60]	0.08	**1.58 [1.24–2.02]**	**<0.001**	**<0.001**
Model 5	Ref.	1.08 [0.84–1.39]	0.54	1.24 [0.97–1.58]	0.09	**1.51[1.19–1.93]**	**0.001**	**<0.001**
Model 6	Ref.	1.07 [0.83–1.37]	0.61	1.20 [0.93–1.55]	0.17	**1.43[1.09–1.87]**	**0.009**	**0.002**
hsCRP								
Participants (*n*)	1373	1388		1384		1381		
Range, mg L^{-1}	<0.62	≥0.62		≥1.36		≥3.08		
No. of deaths(%)	107 (7.8)	165 (11.9)		247 (17.8)		319 (23.1)		
Crude	Ref.	**1.53 [1.20–1.95]**	**0.001**	**2.37 [1.89–2.97]**	**<0.001**	**3.16 [2.54–3.93]**	**<0.001**	**<0.001**
Model 1	Ref.	0.93 [0.73–1.19]	0.57	1.24 [0.98–1.57]	0.08	**1.53 [1.22–1.93]**	**<0.001**	**<0.001**
Model 2	Ref.	0.95 [0.74–1.21]	0.66	1.19 [0.94–1.51]	0.14	**1.42 [1.13–1.80]**	**0.003**	**<0.001**
Model 3	Ref.	0.94 [0.73–1.20]	0.60	1.19 [0.93–1.51]	0.17	**1.36 [1.07–1.73]**	**0.011**	**0.01**
Model 33	Ref.	0.95 [0.74–1.22]	0.69	1.22 [0.96–1.55]	0.10	**1.45 [1.14–1.83]**	**0.002**	**<0.001**
Model 4	Ref.	0.95 [0.74–1.22]	0.69	1.22 [0.96–1.55]	0.10	**1.45 [1.14–1.83]**	**0.002**	**<0.001**
Model 5	Ref.	0.93 [0.72–1.19]	0.56	1.16 [0.91–1.47]	0.24	1.27 [1.00–1.62]	0.052	**0.005**
Model 6	Ref.	0.91 [0.71–1.16]	0.44	1.10 [0.86–1.41]	0.44	1.16 [0.88–1.51]	0.29	0.09

Hazard ratios were derived from Cox proportional hazards regression models.
Model 1: crude model + age, sex, BMI, alcohol intake (<10 g d^{-1} or >10 g d^{-1}) and smoking status (never, former current).
Model 2: model 1 + diabetes, systolic blood pressure, lipid-lowering drugs and anti-hypertensive medications.
Model 3: model 2 + total cholesterol, HDL cholesterol and triglycerides.
Model 4: Model 3 + history of CVD and history of cancer.
Model 5: Model 4 + $eGFR_{creatinine\ cystatin\ C}$ and UAE.
Model 6: Model 5 + hsCRP (for GlycA analyses) + GlycA (for hsCRP analyses).
Triglycerides, UAE and hsCRP were log transformed when used as a continuous variable in the analyses.
Abbreviations: *BMI,* body mass index*; HDL cholesterol,* high-density lipoprotein cholesterol; *CVD*, cardiovascular disease; *hsCRP,* high–sensitivity C-reactive protein; *UAE,* urinary albumin excretion; *PREVEND,* Prevention of Renal and Vascular End-stage Disease.
[a]Tests of trend across increasing quartiles were conducted by assigning the median for each quartile as its value and treating this as a continuous variable.
Values in bold are statistically significant (P<0.05).

lung cancer mortality was no longer significant after adjustment for age, sex, BMI, alcohol intake and smoking status (model 1). Exploratory analyses between GlycA and hsCRP with death attributable to gastrointestinal cancer (56 deaths) are displayed in Table S4. After multivariable adjustment, GlycA but not hsCRP was significantly associated with gastrointestinal cancer.

Meta-analysis of published studies

We identified six population-based prospective cohort studies that had reported associations between circulating GlycA and all-cause mortality risk (Table 5). Including the current study, the pooled analysis involved seven studies comprising 63 180 participants and 8153 all-cause mortality

Table 3 *Association between GlycA and hsCRP levels and cardiovascular mortality in 5526 participants (201 deaths) of the PREVEND study*

	Quartile 1	Quartile 2	*P*-value	Quartile 3	*P*-value	Quartile 4	*P*-value	*P* for trend[a]
GlycA								
Participants (*n*)	1362	1395		1363		1406		
Range, μmol L^{-1}	<309	≥309		≥346		≥388		
No. of deaths (%)	21 (1.5)	47 (3.4)		51 (3.7)		82 (5.8)		
Crude	Ref.	**2.23 [1.33–3.73]**	**0.002**	**2.53 [1.52–4.20]**	**<0.001**	**4.05 [2.51–6.55]**	**<0.001**	**<0.001**
Model 1	Ref.	1.24 [0.74–2.09]	0.41	1.25 [0.74–2.09]	0.41	**1.78 [1.09–2.93]**	**0.02**	**0.008**
Model 2	Ref.	1.08 [0.64–1.81]	0.78	0.96 [0.57–1.62]	0.88	1.32 [0.80–2.19]	0.28	0.15
Model 3	Ref.	1.13 [0.66–1.93]	0.65	0.98 [0.57–1.67]	0.93	1.39 [0.83–2.34]	0.21	0.13
Model 4	Ref.	1.08 [0.63–1.84]	0.79	0.93 [0.54–1.60]	0.80	1.26 [0.75–2.13]	0.39	0.26
Model 5	Ref.	1.06 [0.62–1.81]	0.83	0.90 [0.53–1.55]	0.72	1.14 [0.67–1.93]	0.64	0.56
Model 6	Ref.	1.04 [0.61–1.78]	0.89	0.86 [0.49–1.50]	0.60	1.05 [0.59–1.85]	0.88	0.85
hsCRP								
Participants (*n*)	1373	1388		1384		1381		
Range, mg L^{-1}	<0.62	≥0.62		≥1.36		≥3.08		
No. of deaths (%)	29 (2.1)	33 (2.4)		60 (4.3)		79 (5.7)		
Crude	Ref.	**1.13 [0.69–1.86]**	**0.63**	**2.12 [1.36–3.31]**	**0.001**	**2.89 [1.89–4.42]**	**<0.001**	**<0.001**
Model 1	Ref.	0.60 [0.36–0.99]	0.045	0.93 [0.58–1.47]	0.75	1.16 [0.74–1.81]	0.53	**0.019**
Model 2	Ref.	0.59 [0.36–0.98]	0.041	0.85 [0.54–1.34]	0.48	0.97 [0.62–1.53]	0.91	0.16
Model 3	Ref.	0.59 [0.35–0.98]	0.042	0.88 [0.55–1.40]	0.59	1.01 [0.64–1.61]	0.97	0.12
Model 4	Ref.	0.58 [0.35–0.97]	0.037	0.83 [0.52–1.34]	0.45	0.90 [0.56–1.44]	0.65	0.34
Model 5	Ref.	0.54 [0.32–0.91]	0.02	0.78 [0.48–1.25]	0.30	0.77 [0.47–1.25]	0.28	0.73
Model 6	Ref.	0.54 [0.32–0.91]	0.02	0.78 [0.48–1.26]	0.30	0.76 [0.45–1.30]	0.32	0.73

Hazard ratios were derived from Cox proportional hazards regression models.
Model 1: crude model + age, sex, BMI, alcohol intake (<10 g d^{-1} or >10 g d^{-1}) and smoking status (never, former current).
Model 2: model 1 + diabetes, systolic blood pressure, lipid-lowering drugs and anti-hypertensive medications.
Model 3: model 2 + total cholesterol, HDL cholesterol and triglycerides.
Model 4: Model 3 + history of CVD and history of cancer.
Model 5: Model 4 + $eGFR_{creatinine\ cystatin\ C}$ and UAE.
Model 6: Model 5 + hsCRP (for GlycA analyses) + GlycA (for hsCRP analyses).
Triglycerides, UAE and hsCRP were log transformed when used as a continuous variable in the analyses.
BMI, body mass index; HDL cholesterol, high-density lipoprotein cholesterol; CVD, cardiovascular disease; hsCRP, high–sensitivity C-reactive protein; UAE, urinary albumin excretion; PREVEND, Prevention of Renal and Vascular End-stage Disease.
[a]Tests of trend across increasing quartiles were conducted by assigning the median for each quartile as its value and treating this as a continuous variable.
Values in bold are statistically significant (P<0.05).

events. The pooled random effects multivariable-adjusted RR for all-cause mortality when comparing the top versus bottom quartiles of GlycA levels was 1.74 (95% CI: 1.40–2.17). Significant heterogeneity was noted (I^2 = 88%, 95% CI: 78–94; P < 0.001; Fig. 1). When studies with high CVD risk populations were excluded [18–20], the RR for all-cause mortality comparing extreme quartiles of GlycA was 1.37 (95% CI: 1.24–1.52). Heterogeneity was reduced to nonsignificance (I^2 = 8%, 95% CI: 0–86; P = 0.354). On exclusion of the study which comprised of only women, the RR for all-cause mortality comparing extreme quartiles of GlycA was 1.84 (95% CI: 1.52–2.23).

Table 4 *Association between GlycA and hsCRP levels and cancer mortality in 5526 participants (380 events) of the PREVEND study*

	Quartile 1	Quartile 2	*P*-value	Quartile 3	*P*-value	Quartile 4	*P*-value	*P* for trend[a]
GlycA								
Participants (*n*)	1362	1395		1363		1406		
Range, μmol L^{-1}	<309	≥309		≥346		≥388		
No. of deaths (%)	46 (3.4)	80 (5.7)		114 (8.4)		140 (10.0)		
Crude	Ref.	**1.73 [1.20–2.48]**	**0.003**	**2.56 [1.82–3.61]**	**<0.001**	**3.13 [2.25–4.37]**	**<0.001**	**<0.001**
Model 1	Ref.	1.14 [0.79–1.65]	0.49	**1.62 [1.14–2.30]**	**0.007**	**1.78 [1.26–2.53]**	**0.001**	**<0.001**
Model 2	Ref.	1.12 [0.78–1.62]	0.55	**1.57 [1.10–2.24]**	**0.013**	**1.72 [1.21–2.44]**	**0.002**	**<0.001**
Model 3	Ref.	1.17 [0.81–1.70]	0.41	**1.62 [1.13–2.32]**	**0.009**	**1.83 [1.28–2.62]**	**0.001**	**<0.001**
Model 4	Ref.	1.18 [0.82–1.72]	0.38	**1.64 [1.14–2.34]**	**0.007**	**1.81 [1.26–2.59]**	**0.001**	**<0.001**
Model 5	Ref.	1.20 [0.83–1.75]	0.33	**1.63 [1.14–2.34]**	**0.008**	**1.80 [1.26–2.59]**	**0.001**	**<0.001**
Model 6	Ref.	1.16 [0.79–1.68]	0.45	**1.49 [1.03–2.17]**	**0.036**	**1.55 [1.03–2.32]**	**0.034**	**0.023**
hsCRP								
Participants (*n*)	1373	1388		1384		1381		
Range, mg L^{-1}	<0.62	≥0.62		≥1.36		≥3.08		
No. of deaths (%)	38 (2.8)	84 (6.1)		119 (8.6)		139 (10.1)		
Crude	Ref.	**2.19 [1.49–3.21]**	**<0.001**	**3.20 [2.22–4.61]**	**<0.001**	**3.85 [2.69–5.51]**	**<0.001**	**<0.001**
Model 1	Ref.	1.46 [0.99–2.15]	0.056	**1.84 [1.26–2.68]**	**0.002**	**2.09 [1.43–3.05]**	**<0.001**	**0.001**
Model 2	Ref.	**1.48 [1.01–2.19]**	**0.047**	**1.84 [1.26–2.69]**	**0.002**	**2.06 [1.41–3.00]**	**<0.001**	**0.001**
Model 3	Ref.	**1.48 [1.00–2.19]**	**0.048**	**1.86 [1.27–2.73]**	**0.001**	**2.05 [1.40–3.01]**	**<0.001**	**0.002**
Model 4	Ref.	1.47 [0.99–2.17]	0.055	**1.83 [1.25–2.68]**	**0.002**	**1.98 [1.35–2.91]**	**0.001**	**0.004**
Model 5	Ref.	**1.48 [1.00–2.19]**	**0.049**	**1.82 [1.24–2.68]**	**0.002**	**1.96 [1.33–2.89]**	**0.001**	**0.006**
Model 6	Ref.	1.43 [0.97–2.12]	0.073	**1.71 [1.15–2.53]**	**0.007**	**1.71 [1.12–2.61]**	**0.014**	0.13

Hazard ratios were derived from Cox proportional hazards regression models.
Model 1: crude model + age, sex, BMI, alcohol intake (<10g d^{-1} or> 10 g d^{-1}) and smoking status (never, former current).
Model 2: model 1 + diabetes, systolic blood pressure, lipid-lowering drugs and anti-hypertensive medications.
Model 3: model 2 + total cholesterol, HDL cholesterol and triglycerides.
Model 4: Model 3 + history of CVD and history of cancer.
Model 5: Model 4 + $eGFR_{creatinine\ cystatin\ C}$ and UAE.
Model 6: Model 5 + hsCRP (for GlycA analyses) + GlycA (for hsCRP analyses).
Triglycerides, UAE and hsCRP were log transformed when used as a continuous variable in the analyses.
BMI, body mass index; CVD, cardiovascular disease; HDL cholesterol, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; PREVEND, Prevention of Renal and Vascular End-stage Disease; UAE, urinary albumin excretion.
[a]Tests of trend across increasing quartiles were conducted by assigning the median for each quartile as its value and treating this as a continuous variable.>
Values in bold are statistically significant (P<0.05).

Discussion

The present study demonstrates a significant association of GlycA with all-cause mortality, independent of established risk factors and potential confounders. Our pooled finding from the meta-analysis including 63 180 participants and 8153 deaths reinforces the validity and generalizability of the findings. The observed heterogeneity amongst these studies was explained by three studies reporting on high-risk populations. The positive association of hsCRP with all-cause mortality was attenuated to nonsignificance after adjustment for GlycA. GlycA and hsCRP were each not independently associated with cardiovascular mortality. In addition, sex-stratified analyses

Table 5 *Characteristics of prospective studies evaluating associations between GlycA and all-cause mortality*

Author, publication Year	Name of study	Location of study	Baseline year	Baseline age range	% Male	Duration of follow-up (years)	No. of participants	No. of deaths	Variables adjusted for
Current study	PREVEND	Netherlands	2001–2003	28–75	47.6	12.6	5527	838	Age, sex, body mass index, alcohol consumption (<10 g d^{-1} or >10 g d^{-1}), smoking status (never, former, current), diabetes, systolic blood pressure, lipid-lowering drugs, anti-hypertensive medications, history of CVD, history of cancer, $eGFR_{creatinine\ cystatin\ C}$, and urinary albumin excretion and hsCRP
Duprez, 2016	MESA	USA	2000–2002	45–84	47.0	12.1	6523	915	Age, race, sex and clinic, while the full model adds height, heart rate, systolic blood pressures, diastolic blood pressure, blood pressure-lowering medication, BMI, former and current smoking, diabetes, cholesterol lowering medication, total cholesterol, HDL cholesterol, triglycerides, low eGFR [<60 mL min^{-1} (1.73 m^2)$^{-1}$]
Lawler, 2016	WHS	USA	2005–2006	>45	0.0	20.5	27524	3523	Age, race, smoking (current or former), alcohol use (≥1 drink per day), history of hypertension, family history of myocardial infarction, body mass index, LDLc, HDLc, glycated haemoglobin and hsCRP.

Table 5 (*Continued*)

Author, publication Year	Name of study	Location of study	Baseline year	Baseline age range	% Male	Duration of follow-up (years)	No. of participants	No. of deaths	Variables adjusted for
Lawler, 2016	JUPITER	USA	2003–2006	Women ≥ 60Men ≥ 50	64.0	2.0	12527	278	Age, race, smoking (current or former), alcohol use (≥1 drink per day), history of hypertension, family history of coronary heart disease, body mass index, LDLc, HDLc, glycated haemoglobin and hsCRP.
McGarrah, 2017	CATHGEN	USA	2001–2011	>20	62.4	7.0	7617	2257	Age, sex, race, BMI, diabetes, hypertension, smoking, hyperlipidemia, and LDL-P, and additionally for presence of CAD and ejection fraction.
Otvos, 2017	AIM-HIGH trial	USA and Canada	2006–2010	≥45	85.8	1.0	2581 (1 year postbaseline)	102	Age, sex, diabetes history, and treatment assignment
Kettunen, 2018	ANGES	Finland	September 2002–March 2004	Not specified	64.0	12	881	240	Age, sex, albumin, VLDL-diameter, citrate, thrombocyte count, haemoglobin levels, left ventricular hypertrophy and ejection fraction
Total							63 180	8153	

ANGES: Angiography and Genes Study; AIM-HIGH trial: Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes; CATHGEN, Catheterization Genetics; JUPITER, Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin; PREVEND, Prevention of Renal and Vascular End-Stage Disease; MESA, Multi-Ethnic Study of Atherosclerosis; WHS, Women's Health Study.

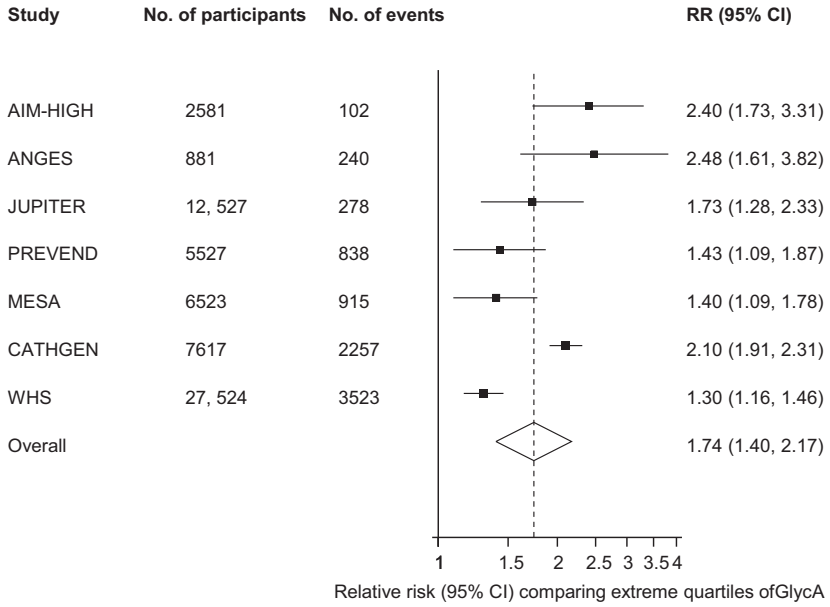

Fig. 1 *Relative risks for mortality comparing extreme quartiles of GlycA in published studies. ANGES: Angiography and Genes Study; AIM-HIGH trial: Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes; CATHGEN, Catheterization Genetics; CI, confidence intervals (bars); JUPITER, Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin; MESA, Multi-Ethnic Study of Atherosclerosis; PREVEND, Prevention of Renal and Vascular End-stage Disease; RR, relative risk; WHS, Women's health study.*

revealed that the positive association of GlycA and hsCRP with cancer mortality was only present in men, but not in women. The association of higher GlycA with increased total cancer mortality was in part attributable to an increased risk of lung and gastrointestinal cancer mortality.

GlycA represents a subset of acute-phase reactants, including α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin and transferrin [8]. Its composite nature likely has the advantage of giving more stability compared to a single and more variable marker such as hsCRP [8]. Based on the meta-analysis [11,18–21], individuals with the highest GlycA levels were found to have a 74% greater risk to die from any cause compared to individuals with the lowest GlycA category. Consistent with our findings, α1-acid glycoprotein, one of the major contributors to the GlycA signal, was found to be associated with all-cause mortality in two large cohorts in Estonia and Finland [28]. In line, Ritchie et al. [29] recently showed that of GlycA's constituent glycoproteins, α1-antitrypsin was the strongest predictor for future disease risk and mortality. Our analyses in the PREVEND study furthermore showed that hsCRP was also associated with all-cause mortality. Notably, however, significance was lost after adjustment for GlycA, suggesting that the association of GlycA with all-cause mortality was stronger.

The association of hsCRP with CVD has been firmly established in numerous studies [30,31]. Although we did find statistically significant trends of GlycA and hsCRP with CVD mortality in unadjusted models, these associations were abolished by controlling for age, sex and lifestyle factors. This may indicate that elevations in GlycA and hsCRP are nonspecific responses to environmental stimuli and may not be related directly or indirectly with the pathogenesis of cardiovascular mortality. In line with this, results from Mendelian randomization studies indicate that *CRP* variants do not independently confer increased CVD risk [32,33]. Moreover, whether CRP should be used to screen asymptomatic persons is still a matter of debate [34,35]. Notably, in the PREVEND study, we have shown before that GlycA and hsCRP were

associated with incident CVD, using a combined endpoint of CVD morbidity and mortality [9]. Overall, results of the current study, therefore, suggest that these associations are mainly driven by CVD morbidity. In our cohort, 24% of all deaths were attributable to cardiovascular causes, which is comparable with cardiovascular death rates from the entire Dutch population in 2016 [36]. This supports the idea that the PREVEND study is a representative reflection of the general Dutch population.

The current study showed that GlycA and hsCRP were positively associated with total cancer mortality in men and not in women. This finding is in agreement with the results of an earlier meta-analysis, including six studies comprising a total of 55 721 participants and 3180 deaths due to cancer, which observed an effect of elevated hsCRP on cancer-related mortality only in men [31]. The nonsignificant association in women may be attributable to lower statistical power or heterogeneity of different types of cancer. In addition, a cross-sectional study of postmenopausal women using hormone replacement therapy (HRT) showed increased CRP levels compared to women not taking HRT [37]. Comparable results were found in young adult women using low-dose oral contraceptives [38]. HRT and oral contraceptive use may cause elevated levels of both GlycA and hsCRP in women with relatively healthy lifestyles, which might attenuate the effect on cancer mortality risk. However, in our study, exclusion of 358 women on HRT therapy and oral contraceptives did not alter the results (data not shown). Further studies are needed to investigate the mechanisms behind the lack of statistical significance between systemic low-grade inflammation and cancer mortality in women. In addition, subgroup analysis results suggested that GlycA might also be predictive for lung cancer mortality. A similar observation was reported by Duprez et al. who found that GlycA was associated with lung cancer mortality in an analysis including 107 deaths [11]. Furthermore, in an exploratory analysis, we showed a significant association between GlycA and gastrointestinal cancer mortality. Interestingly, results of the WHS showed that GlycA was associated with colon cancer mortality in initially healthy women [16].

The strengths of our study include analyses of primary data as well as a meta-analysis of all available published cohorts on GlycA and all-cause mortality so far. Furthermore, the PREVEND study has measurement on comprehensive number of lifestyle and biological markers that enabled adequate adjustment for potential confounders. On the other hand, our study also has some limitations to consider. First, our findings were based on a single baseline measurement of hsCRP and GlycA. However, the study of Ritchie et al. [39] showed stable GlycA elevations for periods of up to a decade. Second, as an observational study, it does not allow for identification of underlying causes. Finally, our meta-analysis was based on study-level data and did not involve individual participant data, which might give more reliable risk estimates compared to study-level data.

In conclusion, in this prospective study involving both men and women, the relative risk for all-cause mortality increased significantly with each increasing quartile of baseline GlycA level. GlycA and hsCRP were not independently associated with cardiovascular mortality in this study. The association of GlycA and hsCRP with cancer mortality appears to be driven by men.

Conflict of interest statement

MAC is an employee of LabCorp.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

The GlycA measurements were performed by LipoScience/LabCorp (Morrisville, North Carolina, USA) at no cost. The help of prof. H.L. Hillege in the infrastructure of the PREVEND project is highly appreciated.

References

1 Kantor ED, Lampe JW, Kratz M, White E. Lifestyle factors and inflammation: associations by body mass index. *PLoS ONE* 2013; **8:** e67833.
2 Borodulin K, Laatikainen T, Salomaa V, Jousilahti P. Associations of leisure time physical activity, self-rated physical fitness, and estimated aerobic fitness with serum C-reactive protein among 3,803 adults. *Atherosclerosis* 2006; **185:** 381–7.
3 Wannamethee SG, Lowe GD, Shaper AG, Rumley A, Lennon L, Whincup PH. Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic

and inflammatory markers for cardiovascular disease. *Eur Heart J* 2005; **26:** 1765–73.
4 Ajani UA, Ford ES, Mokdad AH. Dietary fiber and C-reactive protein: findings from national health and nutrition examination survey data. *J Nutr* 2004; **134:** 1181–5.
5 Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003; **107:** 499–511.
6 Duncan BB, Schmidt MI, Pankow JS, et al. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 2003; **52:** 1799–805.
7 Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002; **420:** 860–7.
8 Otvos JD, Shalaurova I, Wolak-Dinsmore J, et al. GlycA: a composite nuclear magnetic resonance biomarker of systemic inflammation. *Clin Chem* 2015; **61:** 714–23.
9 Gruppen EG, Riphagen IJ, Connelly MA, Otvos JD, Bakker SJ, Dullaart RP. GlycA, a pro-inflammatory glycoprotein biomarker, and incident cardiovascular disease: relationship with C-reactive protein and renal function. *PLoS ONE* 2015; **10:** e0139057.
10 Dullaart RP, Gruppen EG, Connelly MA, Otvos JD, Lefrandt JD. GlycA, a biomarker of inflammatory glycoproteins, is more closely related to the leptin/adiponectin ratio than to glucose tolerance status. *Clin Biochem* 2015; **48:** 811–4.
11 Duprez DA, Otvos J, Sanchez OA, Mackey RH, Tracy R, Jacobs Jr DR. Comparison of the predictive value of GlycA and other biomarkers of inflammation for total death, incident cardiovascular events, noncardiovascular and noncancer inflammatory-related events, and total cancer events. *Clin Chem* 2016; **62:** 1020–31.
12 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.
13 Akinkuolie AO, Pradhan AD, Buring JE, Ridker PM, Mora S. Novel protein glycan side-chain biomarker and risk of incident type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol* 2015; **35:** 1544–50.
14 Connelly MA, Gruppen EG, Wolak-Dinsmore J, et al. GlycA, a marker of acute phase glycoproteins, and the risk of incident type 2 diabetes mellitus: PREVEND study. *Clin Chim Acta* 2016; **452:** 10–7.
15 Akinkuolie AO, Glynn RJ, Padmanabhan L, Ridker PM, Mora S. Circulating N-linked glycoprotein side-chain biomarker, rosuvastatin therapy, and incident cardiovascular disease: an analysis from the JUPITER trial. *J Am Heart Assoc* 2016; **5.** https://doi.org/10.1161/JAHA.116.003822
16 Chandler PD, Akinkuolie AO, Tobias DK, et al. Association of N-linked glycoprotein acetyls and colorectal cancer incidence and mortality. *PLoS ONE* 2016; **11:** e0165615.
17 Gruppen EG, Connelly MA, Sluiter WJ, Bakker SJ, Dullaart RP. Higher plasma GlycA, a novel pro-inflammatory glycoprotein biomarker, is associated with reduced life expectancy: The PREVEND study. *Clin Chim Acta* 2018; **488:** 7–12.
18 McGarrah RW, Kelly JP, Craig DM, et al. A novel protein glycan-derived inflammation biomarker independently predicts cardiovascular disease and modifies the association of HDL subclasses with mortality. *Clin Chem* 2017; **63:** 288–96.
19 Otvos JD, Guyton JR, Connelly MA, et al. Relations of GlycA and lipoprotein particle subspecies with cardiovascular events and mortality: a post hoc analysis of the AIM-HIGH trial. *J Clin Lipidol* 2018; **12:** 348–55.
20 Kettunen Johannes, Ritchie Scott C, Anufrieva Olga, et al. Biomarker glycoprotein acetyls is associated with the risk of a wide spectrum of incident diseases and stratifies mortality risk in angiography patients. *Circulation* 2018; **11.** https://doi.org/10.1161/CIRCGEN.118.002234
21 Lawler PR, Akinkuolie AO, Chandler PD, et al. Circulating N-linked glycoprotein acetyls and longitudinal mortality risk. *Circ Res* 2016; **118:** 1106–15.
22 Hillege HL, Janssen W, Bak A, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. *J Intern Med* 2001; **249:** 519–26.
23 Casparie M, Tiebosch A, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol* 2007; **29:** 19–24.
24 Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012; **367:** 20–9.
25 Matyus SP, Braun PJ, Wolak-Dinsmore J, et al. NMR measurement of LDL particle number using the Vantera® Clinical Analyzer. *Clin Biochem* 2014; **47:** 203–10.
26 Corsetti JP, Bakker SJ, Sparks CE, Dullaart RP. Apolipoprotein A-II influences apolipoprotein E-linked cardiovascular disease risk in women with high levels of HDL cholesterol and C-reactive protein. *PLoS ONE* 2012; **7:** e39110.
27 Selvin S. *Statistical Analysis of Epidemiologic Data*. Oxford, UK: Oxford University Press, 2004.
28 Fischer K, Kettunen J, Würtz P, et al. Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. *PLoS Medicine* 2014; **11:** e1001606.
29 Ritchie SC, Kettunen J, Brozynska M, et al. Elevated alpha-1 antitrypsin is a major component of GlycA-associated risk for future morbidity and mortality. *bioRxiv* 2018.
30 Collaboration Emerging Risk Factors. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet* 2010; **375:** 132–40.
31 Li Y, Zhong X, Cheng G, et al. Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: a meta-analysis. *Atherosclerosis* 2017; **259:** 75–82.
32 Elliott P, Chambers JC, Zhang W, et al. Genetic loci associated with C-reactive protein levels and risk of coronary heart disease. *JAMA* 2009; **302:** 37–48.
33 Zacho J, Tybjærg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med* 2008; **359:** 1897–908.
34 Pepys MB. CRP or not CRP? That is the question. *Arterioscler Thromb Vasc Biol* 2005; **25:** 1091–4.
35 Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med* 2004; **350:** 1387–97.
36 Bots M, Buddeke J, Koopman C, Vaartjes I, Visseren F. Harten vaatziekten in nederland 2017. *Cijfers of leefstijl, risicofactoren, ziekte en sterfte*, 2017.

37 Ridker PM, Hennekens CH, Rifai N, Buring JE, Manson JE. Hormone replacement therapy and increased plasma concentration of C-reactive protein. *Circulation* 1999; **100:** 713–6.
38 Dreon DM, Slavin JL, Phinney SD. Oral contraceptive use and increased plasma concentration of C-reactive protein. *Life Sci* 2003; **73:** 1245–52.
39 Ritchie SC, Würtz P, Nath AP, et al. The biomarker GlycA is associated with chronic inflammation and predicts long-term risk of severe infection. *Cell Syst* 2015; **1:** 293–301.

Correspondence: Eke G. Gruppen, Department of Nephrology and Endocrinology, University Medical Center Groningen, University of Groningen, P.O. Box 30.001, Groningen 9700 RB, The Netherlands. (fax: 0503619392; e-mail: e.g.gruppen@umcg.nl).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Relative risks for mortality comparing extreme quartiles of GlycA in published studies.

Table S1. Sex-stratified analyses between hsCRP and cancer mortality in 5526 participants (380 events) of the PREVEND study.

Table S2. Sex-stratified analyses between GlycA and cancer mortality in 5526 participants (380 events) of the PREVEND study.

Table S3. Association between GlycA and hsCRP levels and lung cancer mortality in 5526 participants (57 events) of the PREVEND study.

Table S4. Association between GlycA and hsCRP levels and gastrointestinal cancer mortality in 5526 participants (56 events) of the PREVEND study.

