



# Serum Galectin-3 and Subsequent Risk of Coronary Heart Disease in Subjects With Childhood-Onset Type 1 Diabetes: A Cohort Study

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## OBJECTIVE

To study whether serum galectin-3 and other biomarkers of inflammation predict coronary heart disease (CHD) in subjects with long-standing childhood-onset type 1 diabetes.

## RESEARCH DESIGN AND METHODS

A population-based nationwide cohort of 299 subjects with type 1 diabetes diagnosed in Norway at <15 years of age during 1973–1982 was examined in 2002–2003 at a mean age of 33 years (range 21–44), with mean diabetes duration of 24 years (range 19–30). Subjects were followed through 31 December 2017 for their first CHD event registered by a hospitalization or cause of death using nationwide registries. Stored serum samples were available for 296 subjects and analyzed for interleukin-6 (IL-6), IL-6 receptor, IL-18, hs-CRP, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), galectin-3, and high-sensitivity troponin T. Adjusted hazard ratios (aHRs) for CHD per SD increase in biomarker were estimated using Cox regression.

## RESULTS

Of 295 subjects, 40 (13.6%) had a documented CHD event during a mean follow-up of 14.4 years (range 0.5–16). IL-6 (aHR 1.32 [95% CI 1.07–1.63]), galectin-3 (aHR 1.44 [95% CI 1.09–1.80]), and TIMP-1 (aHR 1.37 [95% CI 1.04–1.81]) were significant predictors of CHD after adjustment for conventional risk factors.

## CONCLUSIONS

Galectin-3 was significantly associated with future CHD in subjects with type 1 diabetes, and if the results are replicated in larger studies, it may aid in prediction together with conventional risk factors for CHD.

Subjects with type 1 diabetes have increased risk of premature death, with cardiovascular disease as a considerable contributor (1,2). They have increased risk of acute myocardial infarction, heart failure, and cerebral stroke (3). The pathogenesis of vascular complications in type 1 diabetes is complex. The risk of coronary heart disease (CHD) is currently predicted to some extent by focusing on conventional risk factors, including hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) and diabetes duration (4,5), but despite that, subjects with type 1 diabetes have increased risk of CHD (4,6).

Heier et al. (7) reported that young subjects with type 1 diabetes had increased levels of several inflammatory markers compared with matched healthy control

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subjects. Biomarkers of inflammation have shown some predictive value for CHD in individuals both with and without diabetes (8–10) and may offer insight into pathophysiological mechanisms. Several studies report increased inflammatory markers in the circulation to be associated with general atherosclerosis and cardiovascular disease, such as CRP, interleukin-6 (IL-6), IL-6 receptor (IL-6R), IL-18, matrix metalloproteinases (MMPs), and tissue inhibitor of metalloproteinase (TIMP) (11–16).

A more novel biomarker is galectin-3, a member of the lectin family of proteins that bind carbohydrates, found both in the circulation and different intracellular locations in many cell types. The potential role of galectin-3 in the development of chronic and acute heart failure in subjects with or without diabetes has recently gained attention (17–19). In the American Heart Association's guidelines for management of heart failure, galectin-3 is also mentioned as an emerging biomarker, having both predictive and prognostic value for patients with heart failure, but larger studies are needed before taking it into clinical use (20).

Galectin-3 is involved in several processes, such as fibrogenesis, inflammation, remodeling, and tissue repair (21,22). It may have both pro- and anti-inflammatory actions, depending on the target (23). A study on galectin-3 knockout mice showed reduced cardiac fibrosis and left ventricular dysfunction (24); another study in mice found galectin-3 to be associated with plaque formation and atherosclerosis (25).

There are few studies among subjects with childhood-onset type 1 diabetes with long-term follow-up assessing CHD. The aim of this study was to investigate whether selected biomarkers reflecting inflammation, fibrosis, and myocardial injury are associated with risk of CHD in people with long-standing childhood-onset type 1 diabetes.

## RESEARCH DESIGN AND METHODS

### Participants and Design

All newly diagnosed subjects with type 1 diabetes in Norway <15 years of age during 1973–1982 were initially identified retrospectively with a high degree of ascertainment ( $n = 1,906$ , all Caucasian) (26). A nationwide, random sample ( $n = 355$ ) of these subjects was invited to a

standardized clinical examination in 2002–2003 at their local hospital, including blood and urine sampling (27), defined as baseline for the current analyses. The examined cohort consisted of 299 subjects with type 1 diabetes eligible for our study. One had a CHD event before baseline, and three lacked blood samples, leaving 295 in our study cohort for analysis (Fig. 1). The participants were followed prospectively for end points from the time of examination in 2002–2003 and until the end of 2017. A full description of the cohort is provided in a previous article (27).

### Outcome

The outcome was incident CHD event, defined as the first hospitalization with a primary or secondary diagnosis of CHD or death with CHD as the underlying cause. Case subjects with a documented incident with ICD-10 I20–I25, including subcategories, were included. The codes include CHD (mainly unstable and stable angina, acute myocardial infarction, and chronic ischemic heart disease).

### Ethics Statement

All subjects gave written informed consent at baseline, and the study was approved by the Regional Committee for Medical and Health Research Ethics (2017/138; Oslo, Norway).

### Laboratory Analyses at Baseline 2002–2003

All blood samples were left to coagulate for 30 min before being centrifuged for 15 min and immediately frozen at  $-80^{\circ}\text{C}$ . As samples were collected from all over Norway, they were transported to Ullevål University Hospital on dry ice and kept frozen and stored at  $-80^{\circ}\text{C}$ .

HbA<sub>1c</sub> was measured by high-performance liquid chromatography (VARIANT; Bio-Rad Laboratories, Richmond, CA); the intra-assay coefficient of variation (CV) was <3%. It was measured in percent and converted to millimoles per mole by using the link <http://www.ngsp.org/convert1.asp>. Lipids were measured with standard enzymatic methods and urinary albumin concentration with immunoturbidimetry. Albumin excretion was calculated on the basis of an average of two overnight timed urine samples. Albuminuria was defined as either microalbuminuria (30–299 mg/L) or macroalbuminuria ( $\geq 300$  mg/L).

### Laboratory Analyses of Baseline Biomarkers in 2018

The stored serum samples from baseline were analyzed for the inflammatory markers IL-6, IL-6R, IL-18, hs-CRP, MMP-9, TIMP-1, galectin-3, and high-sensitivity troponin T (hs-TNT).

Circulating levels of IL-6, IL-6R, MMP-9, TIMP-1, and galectin-3 were measured by ELISA Quantikine kit (Bio-Techne; R&D Systems, Abingdon, U.K.) and IL-18 by ELISA (Medical & Biological Laboratories Co., Ltd, Nagoya, Japan). hs-CRP was determined by an ELISA (DRG Instruments GmbH, Marburg, Germany) with a detection limit of 0.1 mg/L. In our laboratory, the interassay CVs were: IL-6, 2.9%; IL-6R, 5.4%; IL-18, 4.2%; hs-CRP, 5.6%; TIMP-1, 3.1%; MMP-9, 5.9%; and galectin-3, 5.1%.

TNT is a well-established specific biomarker for myocardial injury and is used in clinical practice when evaluating acute coronary syndrome. hs-TNT was analyzed using Cobas 800 and e801 from Roche Diagnostics. The detection level for hs-TNT was 3 ng/L, lower level of quantification was 5 ng/L, and the CV was 10% at 3.83 ng/L.

### Linkage to National Registries

Information on CHD events for all subjects was obtained by individual linkage to nationwide hospitalization data (1994–2014) from the Cardiovascular Disease in Norway (CVDNOR) project (28) and the Norwegian Patient Registry (29). Data on underlying cause of death or emigration were linked from the Norwegian Cause of Death Registry. All linkage was carried out

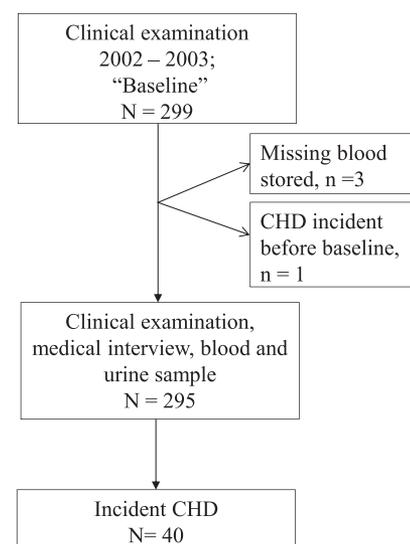


Figure 1—Overview of the eligible cohort.

using the personal identification number unique to every Norwegian resident. Data from the Norwegian Patient Registry and the Norwegian Cause of Death Registry have been used in this publication. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by these registries is intended or should be inferred.

### Other Covariates

The examination in 2002–2003 included medical history, obtained from interview and available hospital records, blood pressure, collection of overnight timed urine, venous blood samples, and fundus photography (27). The medical history interview included use of antihypertensive medications, lipid-lowering medication, and smoking habits. Blood pressure was measured twice with the patient in the sitting position after 10 min of rest, using the same mercury sphygmomanometer for all of the subjects. The mean of two readings was used in the analyses and modeled in the Cox regression per 10 mmHg. The presence and severity of diabetes retinopathy were assessed from 45° retinal photographs obtained with a wide-angle camera and scored centrally, without knowledge of the subject's identity, by an ophthalmologist (30). Two out of 295 subjects used an insulin pump at baseline, and the rest used an insulin pen, in which the majority of the patients had basal insulin twice a day combined with fast-acting insulin at meals.

### Statistical Analyses

Cox regression models were used to estimate hazard ratios (HRs) for the association between biomarkers and time to an incident CHD event. Follow-up time was counted from the baseline examination in 2002–2003 and failure as the date of the first event with CHD documented in our linked registries. The cohort was followed, to the first CHD event, death, emigration, or end of follow-up on 31 December 2017, whichever occurred first.

To limit the number of covariates to adjust for in our main model with inflammatory markers, we used a stepwise backward selection approach to select “conventional CHD risk factors” (sex, age at baseline, BMI in kilograms per meter squared, systolic blood pressure, albuminuria, smoking [yes/no], use of

antihypertensive medication [yes/no], HbA<sub>1c</sub>, cholesterol, HDLs, LDLs, and triglycerides) based on *P* values and retained variables with *P* < 0.05 as covariates to adjust for in our models with inflammatory markers. Three covariates were significant: HbA<sub>1c</sub>, albuminuria, and systolic blood pressure. We then added all eight biomarkers to the three covariates mentioned and again used the same approach as above.

To assess whether our main results were robust toward other variable-selection strategies, we also selected covariates to adjust for using an alternative approach. In this study, we assessed the measured conventional CHD risk factors for unadjusted and adjusted association with CHD and also for association with inflammatory biomarkers and selected the covariates most strongly predicting CHD (see Supplementary Material, “Approach to selection of covariates for adjusted analyses” for more details on the rationale for this approach). This alternative approach led to selection of the same set of covariates and thus supports the robustness of our model selection strategy.

BMI, systolic blood pressure, age at baseline, and HbA<sub>1c</sub> were included as continuous variables. Systolic blood pressure was presented as a continuous variable, but in units of 10 mmHg. Albuminuria, use of antihypertensive medication, smoking, and sex are included as binary variables. Significance level was set as *P* < 0.05. BMI, systolic blood pressure, and HbA<sub>1c</sub> were normally distributed; the other covariates were binary.

Complete covariate data were available from all subjects, except a few with missing urine (and thus albuminuria) and BMI. For the seven subjects with missing urine, we imputed the mean albumin concentration from the remaining subjects with the same sex and birth year and used these values in the analyses of six subjects. One subject was already on dialysis and thus had macroalbuminuria. One subject had missing weight, and one was missing height. We imputed mean weight and height from the remaining subjects with the same sex, weight, or height, respectively, and birth year  $\pm 3$  years and used that for the analysis.

*z* scores were constructed for the biomarkers by subtracting the mean and dividing by the SD. The HRs are therefore per SD change in biomarker.

We also assessed the inflammatory biomarkers' predictive ability, singly or in combination, by estimating the area under the receiver operating characteristic (ROC) curve (AUC). We compared ROC curves, with and without HbA<sub>1c</sub> and galectin-3, based on the set of predictions from the logistic regression model (Fig. 2). The curve plots two parameters, true-positive rate (sensitivity) and false-positive rate (1 – specificity), to measure the ability to predict CHD by the combination of covariates.

All analyses were done in Stata software version 15 (StataCorp LLC, College Station, TX).

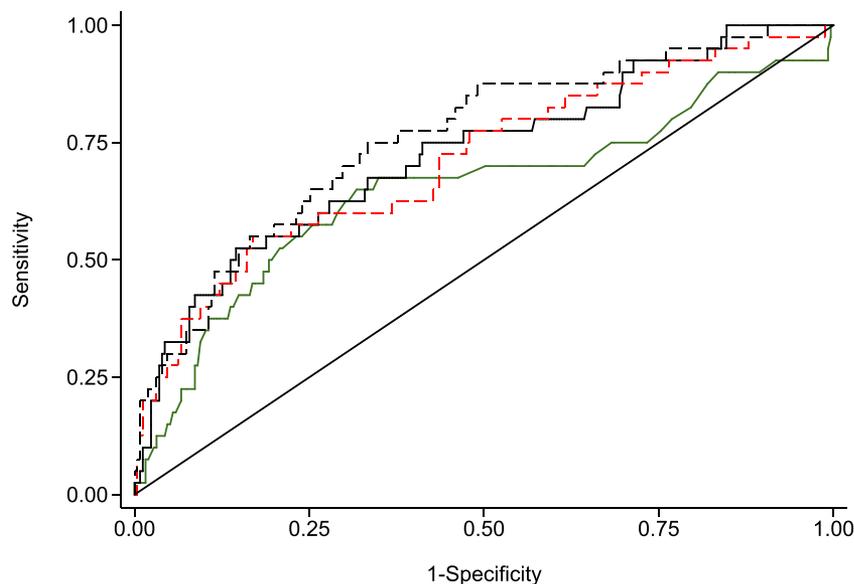
### Data and Resource Availability

All data are accessible upon application and approval from the Regional Committee for Medical and Health Research Ethics in Norway, Norwegian Childhood Diabetes Registry, CVDNOR, the Norwegian Patient Registry, and Norwegian Cause of Death Registry.

### RESULTS

At baseline, mean age was 32.7 years (range 21–44), mean diabetes duration was 24.3 years (range 19–30), mean HbA<sub>1c</sub> was 8.2% (66 mmol/mol) (range 4.9–13.7), 19.7% had micro- or macroalbuminuria, and 33.9% had hypertension (Table 1). Subjects who later suffered a CHD event tended to have a less favorable risk factor profile than those with no CHD event (Table 1). In unadjusted models assessing time to CHD event, only some of the conventional CHD risk factors such as smoking and systolic blood pressure were associated with CHD, but not age, BMI, and lipid levels (Supplementary Table 1). Despite comparable duration of diabetes, the group who later suffered a CHD event had numerically higher proportions with retinopathy and albuminuria and higher levels of HbA<sub>1c</sub> (Table 1).

There were modest correlations between most pairs of biomarkers (Supplementary Fig. 2). The strongest correlation was between IL-6 and hs-CRP (*r* = 0.35) and between MMP-9 and galectin-3 (*r* = 0.33) (Supplementary Fig. 2). Smoking, serum triglycerides, and albuminuria were the covariates associated with higher levels of several biomarkers, and there were no significant sex differences in biomarkers, except hs-CRP and IL-18 (Supplementary Table 3).



**Figure 2**—ROC curves showing predictions from logistic regression model with risk factors and galectin-3. Risk factors included: systolic blood pressure, albuminuria, and HbA<sub>1c</sub> (%). Green solid line: risk factors excluded HbA<sub>1c</sub>, AUC 0.65 (95% CI 0.54–0.78); red dashed line: risk factors excluded HbA<sub>1c</sub> plus galectin-3, AUC 0.71 (95% CI 0.62–0.81); black solid line: risk factors, AUC 0.72 (95% CI 0.63–0.82); and black dashed line: risk factors plus galectin-3, AUC 0.76 (95% CI 0.68–0.84).

Follow-up time for the total cohort was 14.4 years (range 0.5–16). Out of 295 subjects, 40 (13.6%) had their first CHD event during a mean follow-up of 9.4 years (range 0.5–15.7). Mean age at first CHD event was 44 years (range 30–58). Out of 40 subjects with an event, 2 of the subjects had a fatal event.

**Table 1**—Characteristics of the study cohort of Norwegian subjects with type 1 diabetes at the baseline examination in 2002–2003

	Total (N = 295)	CHD event, N = 40	No event, N = 255
Women, N (%)	136 (46)	15 (38)	121 (47)
Age, years, mean (range)	32.7 (20.9–44.0)	34.6 (21.8–43.6)	32.4 (20.9–44.0)
Duration of diabetes, years, mean (range)	24.3 (19.3–29.9)	25.0 (19.8–29.9)	24.2 (19.3–29.9)
HbA <sub>1c</sub> , %	8.2 (1.2)	8.8 (1.2)	8.0 (1.2)
HbA <sub>1c</sub> , mmol/mol	66 (10)	73 (10)	64 (10)
BMI, kg/m <sup>2</sup>	26 (3.8)	25 (3.6)	26 (3.8)
Blood pressure, mmHg			
Systolic	126 (14)	130 (18)	125 (13)
Diastolic	79 (10)	82 (10)	79 (10)
Hypertension*, N (%)	100 (33.9)	22 (55.0)	78 (30.6)
BP-lowering medication, N (%)	47 (15.9)	14 (35.0)	33 (12.9)
Lipid-lowering medication, N (%)	17 (5.8)	8 (20.0)	9 (3.5)
Normoalbuminuria, N (%)	237 (80.3)	23 (57.5)	214 (83.9)
Albuminuria (>30 mg/L), N (%)	58 (19.7)	17 (42.5)	41 (16.1)
Total cholesterol, mmol/L	5.3 (1.0)	5.5 (1.1)	5.3 (1.0)
LDL cholesterol, mmol/L	3.2 (0.8)	3.3 (0.8)	3.2 (0.9)
HDL cholesterol, mmol/L	1.6 (0.5)	1.5 (0.4)	1.6 (0.5)
Triglycerides, mmol/L	1.3 (0.9)	1.5 (1.0)	1.3 (0.8)
Current smoking, N (%)	119 (40.3)	24 (60.0)	95 (37.3)
Retinopathy†, N (%)	166 (56.3)	29 (72.5)	137 (53.7)

Data are mean (SD) unless otherwise indicated. BP, blood pressure. \*Systolic blood pressure  $\geq 140$  mmHg and/or diastolic blood pressure  $\geq 90$  mmHg and/or blood pressure-lowering medication. †Any form of retinopathy (30).

### Inflammatory Markers and Prediction of CHD

When all biomarkers were adjusted independently with the conventional risk factors (HbA<sub>1c</sub>, albuminuria, and systolic blood pressure) from backward selection approach, IL-6, galectin-3, and TIMP-1 were significant associated with a future event of CHD. Adjusted HRs (aHRs) were 1.32 (95% CI 1.07–1.63), 1.44 (95% CI 1.18–1.77), and 1.37 (95% CI 1.04–1.81) respectively, per SD increase (Table 2). Only galectin-3 significantly predicted CHD with an aHR of 1.43 (95% CI 1.17–1.74;  $P < 0.001$ ), per SD increase, using stepwise backward selection with all eight biomarkers included with conventional risk factors as mentioned above.

The area under the ROC curve based on predictions from logistic regression with HbA<sub>1c</sub> only was 0.68 (95% CI 0.60–0.77), similar to that for galectin-3 alone, 0.68 (95% CI 0.58–0.77). The area increased to 0.74 (95% CI 0.66–0.82) after adding galectin-3 to the model with HbA<sub>1c</sub>. When all three significant conventional risk factors were added to galectin-3, the AUC increased to 0.76 (95% CI 0.68–0.84). Galectin-3 seems to have a contribution to risk prediction similar to that of HbA<sub>1c</sub> on top of conventional risk factors, but the added value in addition to HbA<sub>1c</sub> seems marginal based on evaluation of the ROC-AUC (Fig. 2).

To further address potential small bias in the ROC-AUC, we used fivefold cross-validation implemented in the STATA package *cvauroc*. The area under the ROC after fivefold cross-validation for conventional risk factors, including HbA<sub>1c</sub>, was 0.72 (bootstrapped bias-corrected 95% CI 0.60–0.81). When adding galectin-3 to the model, the score was 0.75 (bootstrapped bias-corrected 95% CI 0.65–0.84).

When assessing the change in estimate (HR) after each observation one by one, we found few or no influential points. The most influential single observation was in hs-TNT (dfbeta  $> 0.1$ ). The rest had dfbeta values in the range  $-0.06$  to 0.06.

### Robustness Analyses

In response to a reviewer comment, we performed a subanalysis in which the group was divided in two: diabetes diagnosis before or at 9 years of age ( $n = 154$ ) versus

**Table 2—Association between biomarkers and subsequent risk of CHD in subjects with type 1 diabetes**

Variable	Mean (SD)	Unadjusted HR per SD* (95% CI)	P value	Adjusted HR per SD* (95% CI)	P value
IL-6 (pg/mL)	2.09 (1.98)	1.46 (1.19–1.79)	<0.01	1.32 (1.07–1.63)	0.01
CHD event	3.03 (3.00)				
No CHD event	1.95 (1.73)				
IL-6R (pg/mL)	45,493 (10,798)	1.47 (1.09–1.98)	0.01	1.29 (0.96–1.73)	0.09
CHD event	49,347 (11,452)				
No CHD event	44,889 (10,588)				
IL-18 (pg/mL)	313 (130)	1.13 (0.86–1.49)	0.38	1.11 (0.81–1.52)	0.52
CHD event	328 (118)				
No CHD event	311 (132)				
hs-CRP (mg/L)	4.07 (6.40)	1.27 (1.02–1.57)	0.03	1.22 (0.97–1.55)	0.09
CHD event	5.79 (9.64)				
No CHD event	3.80 (5.71)				
TIMP-1 (ng/mL)	203 (42)	1.69 (1.29–2.20)	<0.01	1.37 (1.04–1.81)	0.03
CHD event	226 (59)				
No CHD event	199 (37)				
MMP-9 (ng/mL)	631 (291)	1.00 (1.00–1.00)	0.56	1.05 (0.77–1.42)	0.75
CHD event	656 (304)				
No CHD event	627 (290)				
Galectin-3 (ng/mL)	8.20 (2.65)	1.40 (1.19–1.64)	<0.001	1.44 (1.18–1.77)	<0.001
CHD event	9.81 (3.43)				
No CHD event	7.91 (2.42)				
hs-TNT (ng/L)	4.0 (4.8)	1.30 (1.09–1.55)	0.003	1.23 (0.98–1.55)	0.08
CHD event	5.8 (8.1)				
No CHD event	3.8 (4.0)				

\*HR per SD and adjusted for systolic blood pressure, albuminuria, and HbA<sub>1c</sub> (%).

later ( $n = 141$ ). The highest age group had 24 incidents versus 16 in the lower age group. There was a significant association with galectin-3 with the highest age group, but the difference between the two groups was not significant.

Subanalyses were done after restricting the CHD end-point definition to include only acute myocardial infarction (ICD-10 code I21–I22;  $n = 25$  with end point). This gave generally very similar results to those for our primary end-point definition, but IL-6 and TIMP-1 were no longer statistically significant. Galectin-3 became borderline nonsignificant, with a  $P$  value of 0.05 and aHR reduced from 1.44 to 1.33 (Supplementary Table 4).

There were 10 subjects with hs-CRP values  $>20$  mg/L, of whom 2 had a CHD event. To rule out possibility of potentially intercurrent infection in those patients, we excluded the 10 subjects and did a sensitivity analysis with  $z$  scores, both with and without log-transforming the data. The association remained similar and nonsignificant (Supplementary Table 5).

The frequency distribution of many biomarkers was right skewed (Supplementary Fig. 1), and to assess whether it influenced the results, we log-transformed

the biomarkers before the analyses (Supplementary Table 2). The log transformation did not make any essential change in the interpretation of the data; hence, the model with  $z$  scores without log transformation was chosen when presenting associations between the biomarkers and future events of CHD.

Based on earlier studies and well-established predictors (5), we then chose some conventional risk factors to include in the adjusted analyses: BMI (in kilograms per meter squared), age, sex, use of antihypertensive drugs (yes/no), and smoking (yes/no) (Supplementary Material, “Approach to selection of covariates for adjusted analyses”). The HRs were approximately the same, but hs-CRP changed to be borderline significant (aHR 1.34 [95% CI 1.04–1.71]), while TIMP-1 changed to borderline nonsignificant (aHR 1.26 [95% CI 0.94–1.69]) (Supplementary Table 6).

We also analyzed data for subjects with diabetic ketoacidosis (DKA) during the previous year for baseline examination. Three subjects had one incident each of DKA. None of these had any documented CHD and hence did not affect analysis or specifically galectin-3.

## CONCLUSIONS

We report in a longitudinal study of subjects with type 1 diabetes that IL-6, TIMP-1, and especially galectin-3 were significantly associated with future CHD, after adjusting for conventional risk factors.

### Strength and Limitations

An important strength of our study is the nationwide prospective design and long follow-up. The clinical characteristics in our study contain detailed information at baseline of the subjects, as they were all recorded and examined by the same doctor (T.S.) (27). All blood samples both at baseline and in 2018 were analyzed at the same laboratory at Oslo University Hospital. The outcome biomarkers for the present investigation were analyzed within a defined time period, using the same lot numbers of the respective kits used. Another strength is the possibility to link each individual by means of their unique individual identification number to other nationwide registries. The limitations of our study are the low number of subjects and lack of repeated measures of biomarkers and risk factors, including any information on DKA or severe hypoglycemic events during the

follow-up. As our study is observational, we cannot rule out the possibility that there are unmeasured confounders. We have only measured free IL-18; in addition to that, IL-18-binding protein levels could have been of interest, but small amounts of blood sample limited the number of markers that could be analyzed.

DKA is a severe acute complication of type 1 diabetes and has a potentially fatal outcome. Myocardial infarction may in rare cases cause DKA, but is reported to be one of the most common precipitations of death in patients with DKA. In addition to that, increased levels of troponin I during an episode of DKA are linked to major cardiac events, as well as after the episode of DKA, in patients without primary coronary syndrome (31). In our cohort, there were three patients admitted to the hospital a year prior to baseline examination, and the information is based on self-report. The number of events with DKA is too small to give any interpretable data. It would have been interesting to have data prior to CHD incidents documented and see the association between DKA and our outcome.

### Galectin-3

We are not aware of any previous published studies of galectin-3 and future CHD in subjects with type 1 diabetes. A few other studies are nevertheless of relevance. In a recent Spanish study in which researchers followed subjects (mean age >60 years) with coronary artery disease, subjects with type 2 diabetes had higher values of galectin-3 compared with those without. Out of several studied markers, galectin-3 was the only one significantly associated with a future acute ischemic event (32). A potential effect of galectin-3 on the risk of CHD in subjects with type 1 diabetes, consistent with findings in other studies, associates galectin-3 with cardiac malfunction (18,24,33,34).

The marker is found in mice and humans, expressed in atherosclerotic lesions (25). We are unable to point out the pathophysiology of galectin-3, and the exact molecular mechanism linking galectin-3 to CHD is unknown, but some insight may be gained from animal models. Pejnovic et al. (35) reports that galectin-3-deficient mice had higher body weight and fasting glycemia, suggesting that the presence of galectin-3 would have prevented these phenotypes. In

contrast, galectin-3 is involved in plaque development and atherosclerosis (25). Galectin-3 may play a protective role in acute inflammation and translate into fibrogenesis and scarring in chronic inflammation (36).

In our study, galectin-3 remained significant and aHR nearly unchanged (from unadjusted) when adjusted for the conventional risk factors in our model. We find that galectin-3 associated with future CHD events in individuals with type 1 diabetes and hence implicates galectin-3 in CHD development. We can speculate whether high galectin-3 levels also indicate higher atherosclerotic lesions in patients with type 1 diabetes and thus later result in thromboembolic incidents in the coronary arteries. It is also possible that the increased levels of galectin-3 are a result of underlying, asymptomatic CHD in these patients.

### Systemic Low-Grade Inflammation, hs-CRP, and IL-6

Atherosclerosis is currently considered a chronic inflammatory disease and becomes clinically manifest when it causes thrombosis and, generally, vascular disease (37,38). Furthermore, low-grade chronic inflammation is commonly found with aging (8,39). hs-CRP showed non-significant association with the statistical method stepwise backward model. When adding other well-known conventional risk factors, hs-CRP became statistically significant with minor changes in aHR, generally consistent with previous studies in people with or without type 1 diabetes (9,10).

We also found a significant association of the inflammatory biomarker IL-6 with future risk for CHD. A cross-sectional study by Schram et al. (9) showed, for example, independent significant association among CRP, IL-6, and tumor necrosis factor- $\alpha$  levels with cardiovascular disease, retinopathy, and albuminuria. The role of IL-6 in type 1 diabetes is discussed, but is reported to be elevated years before onset of type 2 diabetes (40). Having in mind the present findings in our study, one hypothesis could be that the association is through insulin insensitivity, which the subjects with type 1 diabetes can develop with time.

### MMP and its Inhibitor

Vascular complications are, in subjects with type 1 diabetes, also seen as part of

advanced glycation end products. This, in turn, can lead to changes in extracellular remodeling and induce production of several MMPs and TIMP-1 (12). In our study, we found no association with MMP-9, but a borderline significant association with TIMP-1, which became nonsignificant when adjusted for additional covariates in robustness analyses. This correlation is in harmony with previous studies (7,11,12).

### Potential Implications of the Findings

In order for clinical care of subjects with type 1 diabetes to be of maximum benefit, early recognition of risk factors is important for prevention of cardiovascular disease. It might be time to focus on such biomarkers in the risk assessment for CHD and to search for treatment that can reduce inflammation.

### Conclusion

In this prospective study on type 1 diabetes, we find a significant association of galectin-3 with future CHD. The molecular mechanisms involved remain unclear. If our findings are replicated, galectin-3 may represent a novel finding in multivariable risk prediction for CHD.

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**Author Contributions.** M.S. wrote the first draft of the manuscript and organized the data. M.S. and G.T. analyzed the data. L.C.S. supervised the analysis and interpretation of data. T.S. and G.J. initiated the study. T.S. collected the clinical data. M.S., I.S., T.S., and G.J. planned and conceptualized the project. I.A. provided end-point definition. All authors critically reviewed, contributed to discussion, and approved the final version of the manuscript. M.S. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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