Supplemental material to:

Maryam Saeed et al. « Serum galectin-3 and subsequent risk of coronary heart disease in subjects with childhood-onset type 1 diabetes. A cohort study »

Content

Approach to selection of covariates for adjusted analyses

While our primary approach to selection of covariates to adjust for in the final model was backward selection from a "full model" including lipid profile, sex, age at examination time, smoking (yes/no), BMI kg/m^2, albuminuria, use of antihypertensive medication (yes/no), HbA1c, systolic blood pressure , we wanted to assess the robustness of our results towards using other approaches. No single, simple approach to variable selection works optimally in all settings, so our alternative approach was as follows. We had available data on most of traditional risk factors for coronary heart disease (CHD), here referred to as covariates. We planned a priori a strategy for selection of the factors to include in our adjusted analyses in the primary analyses and in sensitivity analyses. Because we expected a limited number of patients with outcome (CHD), we wanted to limit the number of confounders to some extent. Furthermore, we attempted to take into account several of the different guiding principles that can be applied when selecting adjustment factors in epidemiological analysis (Rothman, Greenland et al. 2008). An old rule of thumb says that in analyses one should ideally have 10 or more subjects with outcome (events) per covariate in the model, but subsequent studies have shown that this rule can be relaxed to at least half of that number, and even fewer for sensitivity analyses (Vittinghoff and McCulloch 2007). With an a priori expected 30-50 cases of CHD in our study, we expected that approximately 8-10 covariates in the model would be more than sufficient. Categorical variables with few subjects in at least one category substantiate potential problems of sparse data, so we decided to include continuous variables as such (not categorizing them), and to include smoking and albuminuria as binary, rather than divided into finer categories. (In sensitivity analyses, we investigated and confirmed linearity assumptions for continuous variables by testing squared terms, and found that adjusting for albuminuria in 3 categories, normal, micro- and macroalbuminuria, did not substantially influence our main results).

Briefly, a confounder predicts both exposure and outcome. However, in settings with measurement errors and unobserved confounding factors, which is inevitable in human observational studies, it is safest to focus on (causal) predictors of the outcome rather than predictors of exposure (Pearl 2011). The magnitude of the statistical association of covariates and biomarkers are shown in Supplemental Table S2, and that of covariates and CHD are shown in Supplemental Table S1. Furthermore, a confounder (that should be controlled) should not be a mediator, meaning it is not on the causal path between exposure and outcome (Rothman, Greenland et al. 2008). Ideally, one should know the true causal structure among all variables of relevance, but this is impossible in practice. We cannot rule out with certainty that some of the traditional risk factors for CHD are influenced to some extent by our biomarkers (measured at the same time in our study). We nevertheless decided that we wanted to adjust our primary analyses for traditional CHD risk factors, acknowledging the fact that some variables may be partial mediators, and that adjusted analyses should be interpreted as "independent" of potential mediation. We also reasoned that it would be most interesting to see the association between biomarkers and CHD after adjustment for some potential mediators, such as obesity (as indicated by BMI). For consistency and simplicity, we decided a priori to adjust for the same set of core covariates for each of our eight biomarkers, rather than selecting a separate set of covariates for each biomarker.

While correlated covariates in the same model can create problems of severely inflated variance (collinearity) if they are highly correlated with each other, we decided to include only one of a set of related variables such as systolic- and diastolic blood pressure, regardless of whether the correlation was too weak to cause severely inflated variance.

Based on the above guiding principles and some substance matter judgements, but without assessing the influence of covariate selection on significance of the biomarker CHD associations, we ended up with a core set of covariates as shown in the main text methods and results (and footnote of main Table 2).

Finally, we decided a priori to run sensitivity analyses where we adjusted for additional covariates, both one by one, and all in the same model. We assessed the change in aHR for association between biomarker and CHD, as well as the variance (CI) and confirmed that results were robust to different choices of covariates.

Supplemental references

Pearl, J. (2011). "Invited commentary: understanding bias amplification." Am J Epidemiol 174(11): 1223-1227; discussion pg 1228-1229.

Rothman, K. J., et al. (2008). Modern epidemiology. Philadelphia, Lippincott, Williams & Wilkins.

Vittinghoff, E. and C. E. McCulloch (2007). "Relaxing the rule of ten events per variable in logistic and Cox regression." Am J Epidemiol 165(6): 710-718.

Supplemental tables

Table S1. Associations between conventional cardiovascular disease risk factors and subsequent risk of coronary heart disease (CHD) in subjects with type 1 diabetes

Variable	Unadjusted HR*, 95% CI	Adjusted‡ HR, 95% CI	P-value§
Female	0.67, (0.35 – 1.27)	0.93, (0.44 – 1.98)	0.85
Smoking at baseline (yes/no)	2.36, (1.25 – 4.44)	1.52, (0.75 – 3.07)	0.25
Age (per year)	1.06, (0.99 – 1.14)	1.07, (0.99 – 1.16)	0.09
Systolic blood pressure (per 10 mm/ Hg)	1.25, (1.01 – 1.55)	1.37, (1.07 – 1.76)	0.02
Antihypertensive medication (yes/no)	2.95, (1.54 – 5.65)	1.29, (0.56 – 2.71)	0.61
BMI (per kg/m^2)	0.95, (0.86 – 1.04)	0.93, (0.83 – 1.04)	0.20
Total cholesterol (per mmol/L)	1.12, (0.84 – 1.50)	0.81, (0.30 – 2.20)	0.68
LDL-cholesterol (per mmol/L)	1.18, (0.83 – 1.67)	1.08, (0.34 – 3.43)	0.90
HDL-cholesterol (per mmol/L)	0.61, (0.27 – 1.38)	0.90, (0.40 – 2.05)	0.80
Triglycerides (per mmol/L)	1.16, (0.88 – 1.53)	1.06, (0.58 – 1.93)	0.86
HbA1c (%)	1.50, (1.22 – 1.85)	1.52, (1.14 – 2.03)	<0.01
Any albuminuria at baseline§	3.41, (1.82 – 6.38)	2.15, (0.99 – 4.70)	0.06
-Microalbuminuria	2.53 , (1.20 – 5.33)	1.87, (0.80 – 4.38)	0.15
-Macroalbuminuria	6.91, (3.08 – 15.47)	3.36, (1.11 – 10.12)	0.03

*Hazard ratio, †Confidence interval, ‡Adjusted for all the variables listed above with albuminuria as a binary variable, §Macroalbuminuria: albumin concentration in urine >= 300 mg/L, microalbuminuria 30-299 mg/L, (any) albuminuria >=30 mg/L. §P-value from adjusted model.

Table S2. Association between biomarkers and subsequent risk of CHD in subjects with type 1 diabetes, with log transformed biomarkers*

Variable	Unadjusted HR per SD*, 95% CI	P-value	Adjusted HR per SD*, 95% CI	P-value
IL-6	1.63, (1.24 – 2.14)	<0.001	1.47, (1.11 – 1.94)	0.006
IL-6R	1.50, (1.08 – 2.08)	0.016	1.32, (0.96 – 1.82)	0.08
IL-18	1.18, (0.86 – 1.63)	0.31	1.13, (0.82 – 1.57)	0.45
Hs-CRP	1.30, (0.95 – 1.78)	0.97	1.18, (0.85 – 1.65)	0.31
TIMP1	1.68, (1.22 – 2.30)	0.001	1.34, (0.97 – 1.85)	0.08
MMP-9	0.93, (0.66 – 1.29)	0.65	0.92, (0.68 – 1.26)	0.61
Galectin-3	1.70, (1.33 – 2.18)	<0.001	1.62, (1.22 – 2.15)	0.001
Hs-TNT	1.37, (1.08 – 1.75)	0.01	1.16, (0.90 – 1.51)	0.25

*Hazard ratio per standard deviation (SD) in natural log-transformed levels, and adjusted for systolic blood pressure, albuminuria, HbA1c (%).
Abbreviations: IL-18: Interleukin-18; IL-6**:** Interleukin-6; IL-6R: Interleukin-6 receptor; hsCRP: High sensitivity-C-reactive protein; MMP9: Matrix metalloproteinase-9; TIMP-1: TIMP Metallopeptidase inhibitor 1 (a tissue inhibitor of metalloproteinases); hsTNT: High sensitivity troponin-T.

Table S3. Association of covariates with biomarkers in subjects with type 1

	IL-6	IL-6R	IL-18	Hs-CRP	MMP-9	TIMP-1	Galectin-3	Hs-TNT
Female **(vs male)**	0.19 (-0.04,0.42)	-0.10 (-0.33, 0.14)	**-0.09** (-0.32,0.14)	**0.41** (0.18,0.64)	0.13 (-0.10,0.36)	-0.06 (-0.30,0.17)	-0.03 (-0.26,0.20)	-0.36 (-0.59,0.14)
Smoking **(yes/no)**	0.16 (-0.08,0.39)	0.24 (0.00,0.47)	0.34 (0.11,0..57)	-0.16 (-0.40,0.08)	**0.42** (0.19,0.65)	**0.28** (0.05,0.51)	**0.34** (0.11,0.57)	**0.39** (0.16,0.62)
Age **(per year)**	0.02 (-0.004,0.04)	-0.01 (-0.03,0.02)	0.002 (-0.21,0.25)	-0.01 (-0.03,0.01)	-0.01 (-0.03,0.02)	0.01 (-0.02,0.03)	0.01 (-0.02,0.03)	0.002 (-0.02,0.03)
Systolic blood pressure **(per 10 mm Hg)**	-0.03 (-0.11, 0.06)	0.07 (-0.02,0.15)	-0.06 (-0.14,0.02)	0.03 (-0.05,0.11)	0.02 (-0.06,0.11)	0.08 (-0.002,0.16)	0.02 (-0.07,0.10)	0.004 (-0.08,0.09)
BMI **(per kg/m^2)**	0.02 (-0.01,0.05)	0.01 (-0.02,0.04)	-0.004 (-0.03,0.03)	**0.06** (0.03,0.09)	0.0 (-0.004,0.06)	0.01 (-0.02,0.04)	-0.004 (-0.03,0.03)	-0.01 (-0.04,0.02)
Total cholesterol † **(per mmol/L)**	-0.02 (-0.13,0.09)	0.09 (-0.03,0.20)	0.01 (-0.10,0.12)	**0.13** (0.02,0.25)	-0.01 (-0.12,0.11)	**0.21** (0.11,0.32)	0.12 (-0.005,0.23)	-0.08 (-0.19,0.04)
LDL-cholesterol **(per mmol/L)**	0.01 (-0.13,0.14)	0.13 (-0.01,0.27)	0.05 (-0.09,0.19)	0.10 (-0.04,0.24)	-0.03 (-0.17,0.11)	0.24 (0.11,0.38)	0.09 (-0.06,0.23)	-0.03 (-0.17,0.11)
HDL- cholesterol **(per mmol/L)**	**-0.33** (-0.55, -0.10)	-0.12 (-0.35,0.11)	**-0.30** (-0.53, -0.06)	0.01 (-0.23,0.25)	-0.24 (-0.47, -0.01)	-0.07 (-0.30,0.16)	**-0.30** (-0.53, -0.07)	**-0.40** (-0.63, -0.18)
Triglycerides **(per mmol/L)**	0.09 (-0.04,0.22)	**0.17** (0.04,0.30)	0.10 (-0.04,0.23)	**0.28** (0.13,0.42)	**0.20** (0.07,0.33)	**0.14** (0.01,0.27)	**0.33** (0.21,0.46)	0.11 (-0.03,0.24)
Albuminuria **(>30mg/L)**	**0.40** (0.13,0.70)	0.28 (0.01,0.57)	0.24 (-0.04,0.53)	0.15 (-0.15,0.45)	0.16 (-0.14,0.44)	**0.63** (0.35,0.91)	**0.49** (0.21,0.77)	**0.35** (0.06,0.64)
HbA1c **(per %-point)**	0.09 (-0.01,0.18)	0.03 (-0.07,0.12)	0.05 (-0.04,0.15)	0.00 (-0.10,0.10)	0.02 (-0.07,0.12)	**0.14** (0.04,0.23)	0.08 (-0.02,0.17)	0.08 (-0.01, 0.17)

* Data are simple linear regression coefficients (95% CI) with z-score transformed biomarkers at dependent variable. Regression coefficients significantly different from zero are marked in bold. Abbreviations: IL-18: Interleukin-18; IL-6**:** Interleukin-6; IL-6R: Interleukin-6 receptor; hsCRP: High sensitivity-C-reactive protein; MMP9: Matrix metalloproteinase-9; TIMP-1: TIMP Metallopeptidase inhibitor 1 (a tissue inhibitor of metalloproteinases); hsTNT: High sensitivity troponin-T. †Missing data on total cholesterol n=2, LDL-cholesterol n=7, HDL-cholesterol n=2, triglycerides n=3

Table S4. Sensitivity of results on association between inflammatory biomarkers on future CHD in 296 patients with type 1 diabetes, after limiting the outcome definition*†

			CHD; primary outcome definition*. N = 40		CHD; limited outcome definition†. N = 25	
Variable	**Median (25,75 p)**	**Mean (SD)**	**Adjusted HR‡, 95% CI**	**P-value**	**Adjusted HR‡, 95% CI**	**P-value**
IL-6 (pg/mL)	1.43 (1.04, 2.30)	2.10 (1.98)	1.32, (1.07 – 1.63)	0.01	1.19 (0.88 – 1.62)	0.27
IL-6R (pg/mL)	44575 (36183, 52766)	45509 (10783)	1.29, (0.96 – 1.73)	0.09	1.22 (0.85 – 1.75)	0.28
IL-18 (pg/mL)	319 (223, 370)	313 (130)	1.11, (0.81 – 1.52)	0.52	1.04 (0.70 – 1.55)	0.86
hsCRP (mg/L)§	1.95 (0.90, 4.16)	4.06 (6.4)	1.22, (0.97 – 1.55)	0.09	1.21 (0.95 – 1.54)	0.12
TIMP-1 (ng/mL)	197 (174, 226)	203 (43)	1.37, (1.04 – 1.81)	0.03	1.38 (0.96 – 1.97)	0.08
MMP-9 (ng/mL)	578 (425, 780)	630 (291)	1.05, (0.77 – 1.42)	0.75	1.29 (0.92 – 1.79)	0.14
Galectin-3 (ng/mL)	7.71 (6.64, 9.18)	8.20 (2.69)	1.44, (1.18 – 1.77)	<0.001	1.33 (1.00 – 1.78)	0.05
hsTNT (ng/L)	2.50 (2.5, 2.5)	4.3 (6.2)	1.23, (0.98 – 1.55)	0.08	1.25 (0.97 – 1.62)	0.09

* Primary definition: ICD-10; I20-I25 and ICD-9; 410-414, † Limited definition: ICD-10; I20-I22 and ICD-9; 410-411. ‡ Hazard ratio per standard deviation increase in biomarker, and adjusted for systolic blood pressure at baseline, albuminuria at baseline and HbA1c at baseline (%) (all quantitative variables entered as continuous in Cox regression). Abbreviations: IL-18: Interleukin-18; IL-6**:** Interleukin-6; IL-6R: Interleukin-6 receptor; hsCRP: High sensitivity-C-reactive protein; MMP-9: Matrix metalloproteinase-9; TIMP-1: TIMP Metallopeptidase inhibitor 1 (a tissue inhibitor of metalloproteinases); hsTNT: High sensitivity troponin-T.
§Values >20 mg/L are excluded.

Table S5. Sensitivity analyses excluding subjects with hs-CRP values >20mg/L

	Unadjusted HR*; 95% CI	**P-value**	**Adjusted HR†, 95% CI**	**P-value**
Variable				
Hs-CRP without log-transformation	1.22 (0.92 – 1.60)	0.17	1.16 (0.86 – 1.57)	0.33
Hs-CRP with log-transformation	1.25 (0.91 – 1.74)	0.17	1.14 (0.80 – 1.61)	0.47

*Unadjusted hazard ratio per standard deviation increase. † Hazard ratio per standard deviation increase adjusted for sex, age at baseline, BMI at baseline, smoking (yes/no), systolic blood pressure at baseline, antihypertensive medication at baseline (yes/no), albuminuria at baseline, HbA1c at baseline (% (mmol/mol)).

Table S6. Association between biomarkers and subsequent risk of coronary heart disease (CHD) in subjects with type 1 diabetes, after adding well-known conventional risk factors.

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	1.29, (1.03 – 1.60)	0.02
CHD event	3.03 (3.00)				
No CHD event	1.95 (1.73)				
IL-6R (pg/mL)	45493 (10798)	1.47, (1.09 – 1.98)	0.01	1.28, (0.94 – 1.73)	0.11
CHD event	49347 (11452)				
No CHD event	44889 (10588)				
IL-18 (pg/mL)	313 (130)	1.13 , (0.86 – 1.49)	0.38	1.06, (0.75 – 1.48)	0.76
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	1.34, (1.04 – 1.71)	0.02
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	1.26, (0.94 – 1.69)	0.12
CHD event	226 (59)				
No CHD event	199 (37)				
MMP-9 (ng/mL)	631 (291)	1.09, (0.81 – 1.47)	0.56	0.98, (0.71 – 1.37)	0.92
CHD event	656 (304)				
No CHD event	627 (290)				
Galectin-3 (ng/mL)	8.20 (2.65)	1.40, (1.19 – 1.64)	<0.01	1.40, (1.09 – 1.80)	<0.01
CHD event	9.81 (3.43)				
No CHD event	7.91 (2.42)				
Hs-TNT (ng/L)	4.0 (4.8)	1.30, (1.10 – 1.55)	<0.01	1.16, (0.90 – 1.49)	0.26
CHD event	5.8 (8.1)				
No CHD event	3.8 (4.0)				

*Hazard ratio per standard deviation (SD) and adjusted for; sex, age at baseline, BMI, smoking (yes/no), systolic blood pressure, albuminuria, antihypertensive medication (yes/no), HbA1c (%).
Abbreviations: IL-18: Interleukin-18; IL-6**:** Interleukin-6; IL-6R: Interleukin-6 receptor; hsCRP: High sensitivity-C-reactive protein; MMP9: Matrix metalloproteinase-9; TIMP-1: TIMP Metallopeptidase inhibitor 1 (a tissue inhibitor of metalloproteinases); hsTNT: High sensitivity troponin-T.

Supplemental figures

Figure S1. The distribution of biomarkers in our study.

Vertical marks on x-axis represents 25, 50 and 75 percentiles.

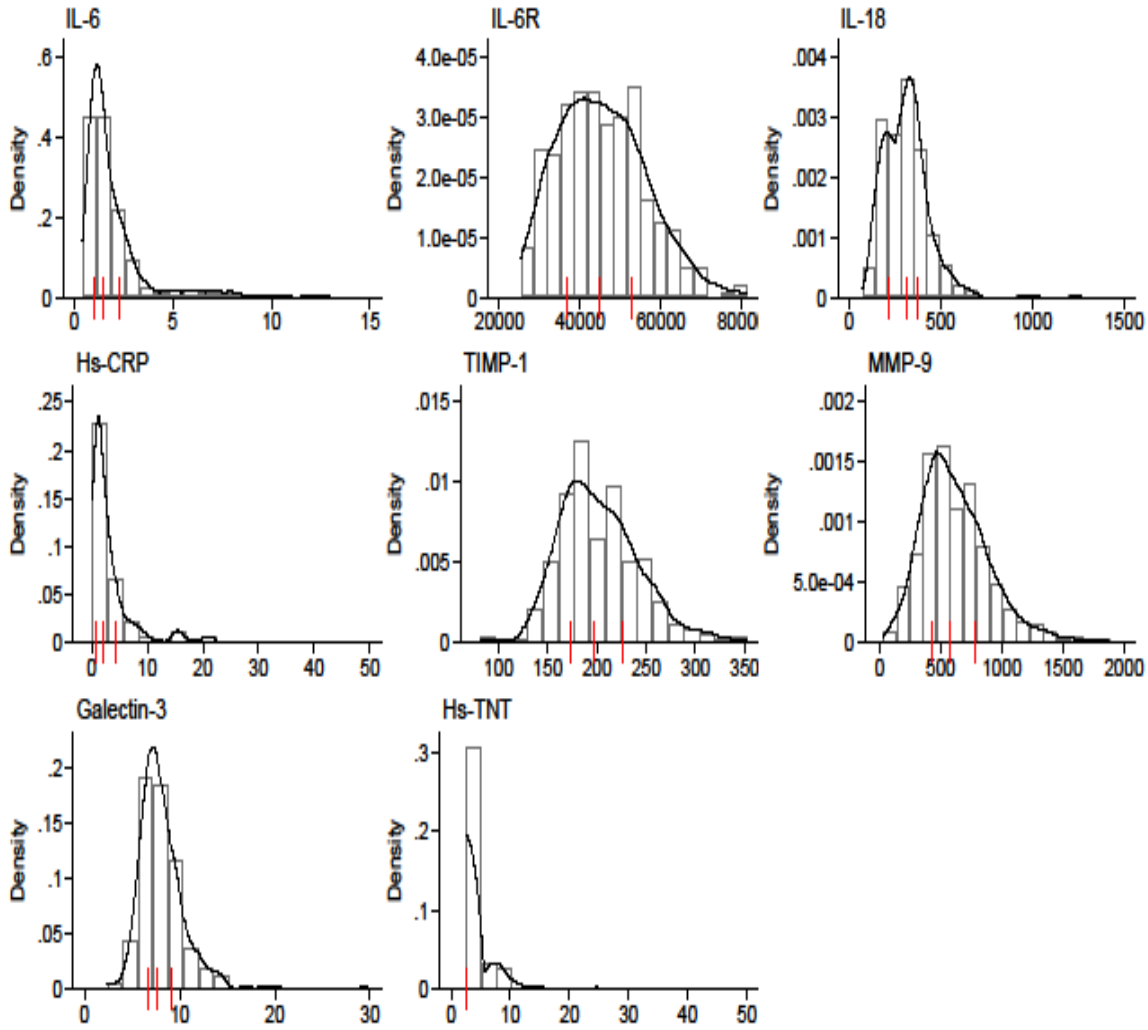

Figure S2. The spearman correlation between the different biomarkers using z-scores