Biomarkers and prediction models for type 2 diabetes and diabetes related outcomes

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Chapter 10

Plasma procalcitonin and risk of type 2 diabetes in the general population

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Diabetologia. 2011;54:2463-5

To the Editor

Chronic low-grade inflammation is a key feature of the pathophysiology of obesity, insulin resistance and type 2 diabetes (T2D)[1]. *In vitro* studies have found that parenchymal cells of tissues, including adipocytes secrete procalcitonin in response to stimulation by activated macrophages[2]. Since obesity is associated with increased presence of activated macrophages in adipose tissue, a similar scenario may play a role *in vivo*[2, 3]. In an initial study, we found that procalcitonin is associated with insulin resistance and all components of the metabolic syndrome[3]. Our current aim is to test the prospective association of plasma procalcitonin with incident T2D in the general population, and to compare its predictive value with that of high sensitivity C-reactive protein (hs-CRP).

The study population was obtained from the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, Groningen, the Netherlands. Details of the study design, recruitment, and measurements have been published elsewhere[3, 4]. Of 8,592 participants in this cohort, procalcitonin was missing in 677 individuals. Further exclusion was for, 295 individuals with diabetes at baseline and 1,002 individuals with missing data on covariates or follow-up data on development of diabetes. The current analyses were performed on 6,618 non-diabetic participants with complete data. In baseline samples, plasma procalcitonin was measured by a novel commercially available immuno-luminometric assay (B.R.A.H.M.S PCT sensitive LIA, BRAHMS GmbH, Hennigsdorf,Germany). Assays were performed in EDTA-plasma aliquots that had been stored frozen at -80°C, without prior thawing and re-freezing. The intraassay CV was 6% at 0.1 ng/ml and 8% at 0.03 ng/ml. The functional assay sensitivity, defined as the lowest concentration to be determined with an interassay CV of 20% was 0.007 ng/ml and lowest detection limit 0.006 ng/ml. The assay technique for procalcitonin and other biomarkers has been described previously[3, 5].

Incident T2D was ascertained if 1 or more available conditions were met: self-report of physician diagnosis; fasting plasma glucose of ≥7.0 mmol/l; random sample plasma glucose ≥11.1 mmol/l; use of antidiabetic agents according to a central pharmacy-registration.

We applied logistic regression models to test the associations of procalcitonin and hs-CRP with incident diabetes. To assess added value of procalcitonin and hs-CRP, we examined improvement of diabetes prediction in terms of C statistic, a measure of discrimination, and integrated discrimination improvement (IDI), a measure of reclassification. A p value of 0.05 or less from two-sided tests was considered statistically significant. The statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL) and R version 2.11.0 (Vienna,Austria) (http://cran.r-project.org/).

The median (interquartile range[IQR]) of plasma procalcitonin was 0.016 (0.013-0.020) ng/ml; men had a higher level than women. Anthropometric and clinical characteristics are summarized in Table S1. Participants with highern

procalcitonin were older, more obese and had higher hs-CRP (correlation coefficient = 0.24; p<0.001) (Figure S1).

During mean (SD) follow-up for 7.6 (0.8) years, 385 participants developed T2D. Median (IQR) procalcitonin levels were 0.019 (0.015-0.023) and 0.016 (0.013-0.019) ng/ml in incident cases and non-cases respectively (p<0.001). Odds ratios (ORs) for incident T2D by quartiles of procalcitonin and hs-CRP are presented in Table 1. A graded increase in risk was observed in crude analysis, after adjustment for age, sex, smoking and alcohol intake (model 2), and additional adjustment for hypertension and parental history of diabetes (model 3) (p<0.001). In model 4, with further adjustment for BMI and waist circumference, the OR for incident T2D in the fourth quartile of procalcitonin was 1.74 (95%CI, 1.13-2.68) compared with the lowest quartile (p=0.008). The association of hs-CRP lost significance with this further adjustment (Table 1). In a secondary analysis, the association of procalcitonin with incident T2D remained significant after adjustment for HDL-cholesterol (OR 1.25; 95%CI,1.01-1.53; p=0.04).

C-statistic improved from 0.78 (0.76-0.81) to 0.79 (0.77-0.81) (p=0.05) and IDI was 0.0025 (p=0.02) after adding procalcitonin to a clinical prediction model including sex, smoking, waist circumference, hypertension and family history of diabetes[6]. We observed no improvement in c statistic (p=0.23) or IDI (p=0.35) when hs-CRP was added instead of procalcitonin.

We found plasma procalcitonin to be an independent predictor of incident T2D in the general population. Particularly, plasma procalcitonin was more strongly associated with incident T2D than hs-CRP after accounting for adiposity.

The link between obesity and diabetes is mediated through both low-grade inflammation and non-inflammatory processes[1, 7]. In our data, adjustment for adiposity attenuated the association of procalcitonin with T2D risk. This supports obesity partly contributes to this association. Another part might be explained by adipocyte dysfunction, other inflammatory conditions or lipid markers rather than adipose tissue mass[3]. In line with prior evidence[7], the association between hs-CRP and T2D materially lost significance after accounting for adiposity. This suggests that procalcitonin as a pro-inflammatory predictor of T2D may be more independent of obesity than hs-CRP.

Our findings suggest that the calcitonin-related system may play a role in the pathophysiology of diabetes. Experimental studies have demonstrated biological activity of procalcitonin on calcitonin receptor family complexes, affecting vascular tone, insulin sensitivity and insulin secretion by the pancreatic beta-cells[2, 8].

Some limitations of this study should be noted. While our study only recruited Caucasians in the Netherlands, it is unclear if our findings would be replicable in others. Another limitation was for the excluded individuals with missing data or without confirmed fasting blood sampling. However, only numerically small differences in baseline characteristics were found between those who included in the study and the excluded individuals. Moreover, we had no data on other inflammatory markers such as interleukin-6 or new diabetes risk factors such as

Table 1. Odds ratios for incident type 2 diabetes by quartiles of plasma procalcitonin and hs-CRP (n= 6,618)

	Quartiles of plasma procalcitonin, ng/ml, [a] OR (95% CI)					Per Unit Increase in Log [b]	
	1 (≤0.012)	2 (0.013-0.015)	3 (0.016-0.019)	4 (≥0.020)	*P* value for Trend		*P* value
Incidence rate, per 1000 person-years	3.0	6.0	7.2	13.7			
Crude analysis	1.00	2.00 (1.31-3.05)	2.41 (1.59-3.65)	4.80 (3.23-7.12)	<0.001	1.86 (1.58-2.19)	<0.001
Model 1	1.00	1.70 (1.11-2. 60)	1.67 (1.09-2.56)	2.91 (1.92-4.42)	<0.001	1.50 (1.26-1.79)	<0.001
Model 2	1.00	1.68 (1.10-2.57)	1.64 (1.07-2.52)	2. 85 (1.88-4.34)	<0.001	1.50 (1.26-1.79)	<0.001
Model 3	1.00	1.64 (1.07-2.52)	1.58 (1.03-2.43)	2.59 (1.70-3.96)	<0.001	1.45 (1.21-1.72)	<0.001
Model 4	1.00	1.45 (0.93-2.24)	1.18 (0.76-1.83)	1.74 (1.13-2.68)	0.008	1.32 (1.09-1.60)	0.004
	Quartiles of hs-CRP, mg/l, OR (95% CI)						
	1 (≤0.54)	2 (0.54-1.18)	3 (1.19-2.76)	4 (≥2.77)			
Incidence rate, per 1000 person-years	2.6	5.7	9.5	12.0			
Crude analysis [c]	1.00	2.22 (1.45-3.40)	3.79 (2.55-5.64)	4.83 (3.27-7.13)	<0.001	1.38 (1.29-1.48)	<0.001
Model 1	1.00	1.83 (1.19-2.81)	2.79 (1.86-4.18)	3.55 (2.38-5.29)	<0.001	1.30 (1.21-1.40)	<0.001
Model 2	1.00	1.79 (1.16-2.75)	2.68 (1.78-4.02)	3.33 (2.22-4.98)	<0.001	1.28 (1.19-1.38)	<0.001
Model 3	1.00	1.66 (1.08-2.56)	2.38 (1.58-3.58)	2.91 (1.94-4.39)	<0.001	1.25 (1.16-1.35)	<0.001
Model 4	1.00	1.21 (0.78-1.88)	1.44 (0.94-2.19)	1.42 (0.92-2.19)	0.26	1.06 (0.97-1.16)	0.16

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; CI, confidence interval.

[a] Plasma procalcitonin levels were measured within low range of <0.1 ng/ml.

[b] Odds ratios expressed per unit increase in log2-transformed level of procalcitonin and hs-CRP.

[c] Analyses based on a sample of participants with data on hs-CRP (n= 6,393[361 incident cases]).

Model 1 is adjusted for age and sex.

Model 2 is adjusted for variables in model 1 plus alcohol use and smoking status.

Model 3 is adjusted for variables in model 2 plus hypertension and parental history of diabetes.

Model 4 is adjusted for variables in model 3 plus body mass index and waist circumference

gamma glutamyl transferase to compare their predictive value for T2D with that of procalcitonin.

In conclusion, plasma procalcitonin levels are associated with incident T2D independent of common diabetes risk factors. Our findings may be considered an opening for further studies on a potential role of the calcitonin-related system in the pathophysiology of diabetes.

Acknowledgments

This work was supported by the Netherlands Heart Foundation, Dutch Diabetes Research Foundation and Dutch Kidney Foundation. This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine (www.ctmm.nl); project PREDICCt (grant 01C-104-07).

Duality of interest statement: Dr Struck is an employee of BRAHMS GmbH, a company which manufactures the procalcitonin assay and holds patent rights on procalcitonin. The present study was not financed by BRAHMS GmbH. No other author has anything to declare. None of the study sponsors had a role in study design; in data collection, analysis, or interpretation; in writing the report; or in the decision to submit for publication.

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Appendix

Table S1. Baseline characteristics of participants for the whole population and according to quartiles of plasma procalcitonin

Characteristic	Whole population	Procalcitonin Quartiles, ng/ml [a,b]			
		(≤0.012)	(0.013-0.015)	(0.016-0.019)	(≥0.020)
No. of participants	6618	1282	1902	1776	1658
Age, y	48.3±12.3	43.2±10.2	46.3±11.4	50.1±12.4	52.8±12.8
Men, no. (%)	3154 (47.8)	284 (22.2)	687 (36.1)	1006 (56.6)	1195 (72.1)
Weight, kg	77.7±14.0	71.5±12.1	75.0±13.1	79.8±13.8	83.2±14.1
Height, cm	173.1±9.4	171.0±8.6	172.3±9.1	173.9±9.7	174.9±9.3
BMI, kg/m^2	25.9±4.1	24.4±3.6	25.2±4.0	26.4±4.1	27.2±4.2
Waist circumference, cm	87.6±12.8	80.6±11.2	84.8±11.8	89.8±12.1	94.0±12.1
Systolic blood pressure, mmHg	123.4±18.9	115.7±16.5	120.9±18.2	125.2±18.4	130.2±19.5
Diastolic blood pressure, mmHg	71.4±9.6	67.9±9.0	70.2±9.3	72.1±9.1	74.7±9.7
Total Cholesterol, mmol/l	5.6±1.1	5.3±1.0	5.5±1.1	5.7±1.1	5.8±1.1
HDL cholesterol, mmol/l	1.3±0.4	1.5±0.4	1.4±0.4	1.3±0.4	1.2±0.3
Triglyceride, mmol/l	1.1 (0.8-1.6)	0.9 (0.7-1.2)	1.0 (0.8-1.4)	1.2 (0.9-1.7)	1.4 (1.0-2.1)
Glucose, mmol/l	4.7±0.6	4.5±0.6	4.6±0.6	4.8±0.6	4.9±0.7
Insulin, pmol/l	46.8 (33-70.2)	39.0 (29.4-52.2)	43.8 (31.2-61.2)	49.8 (34.8-73.8)	60.0 (39.9-91.2)
HOMA-IR	1.6 (1.1-2.5)	1.3 (0.9-1.8)	1.5 (1.0-2.1)	1.7 (1.2-2.7)	2.2 (1.4-3.4)
Tobacco smoking, no. (%)					
Never	1979 (30.0)	419 (32.7)	600 (31.5)	521 (29.3)	449 (27.0)
Ex-smoker	2384 (36.1)	439 (34.2)	626 (32.9)	646 (36.4)	679 (41.0)
Current smoker	2239 (33.9)	424 (33.1)	676 (35.5)	609 (34.3)	530 (32.0)
Alcohol use, no. (%)					
Never	1611 (24.4)	298 (23.2)	453 (23.8)	461 (26.0)	404 (24.4)
1 to 4 drinks per month	1024 (15.5)	229 (17.9)	299 (15.7)	253 (14.2)	245 (14.8)
2 to 7 drinks per week	2306 (35.0)	467 (36.4)	704 (37.4)	609 (34.3)	532 (32.1)
1 to 3 drinks per day	1326 (20.1)	247 (19.3)	358 (18.8)	364 (20.5)	361 (21.8)
≥ 4 drinks per day	330 (5.0)	41 (3.2)	88 (4.6)	89 (5.0)	116 (7.0)
Urine albumin excretion, mg/24h	9.2 (6.3-16.0)	8.1 (5.9-12.7)	8.6 (6.1-14.2)	9.2 (6.3-16.1)	11.2 (7.1-22.6)
hs-CRP, mg/L	1.2 (0.5-2.8)	0.8 (0.3-2.1)	1.0 (0.4-2.3)	1.2 (0.6-2.8)	1.8 (0.9-4.0)

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance, hs-CRP, high sensitivity C-reactive protein. Data are percent, mean±SD or median (interquartile range).

[a] All *P* values were <0.001 for the comparison across procalcitonin quartiles using χ^2 test (categorical data), spearman rank correlation (ordinal data), and ANOVA or Kruskal-Wallis (continuous data).
[b] From included sample, 6,523 to 6,606 participants had complete data on other biomarkers.

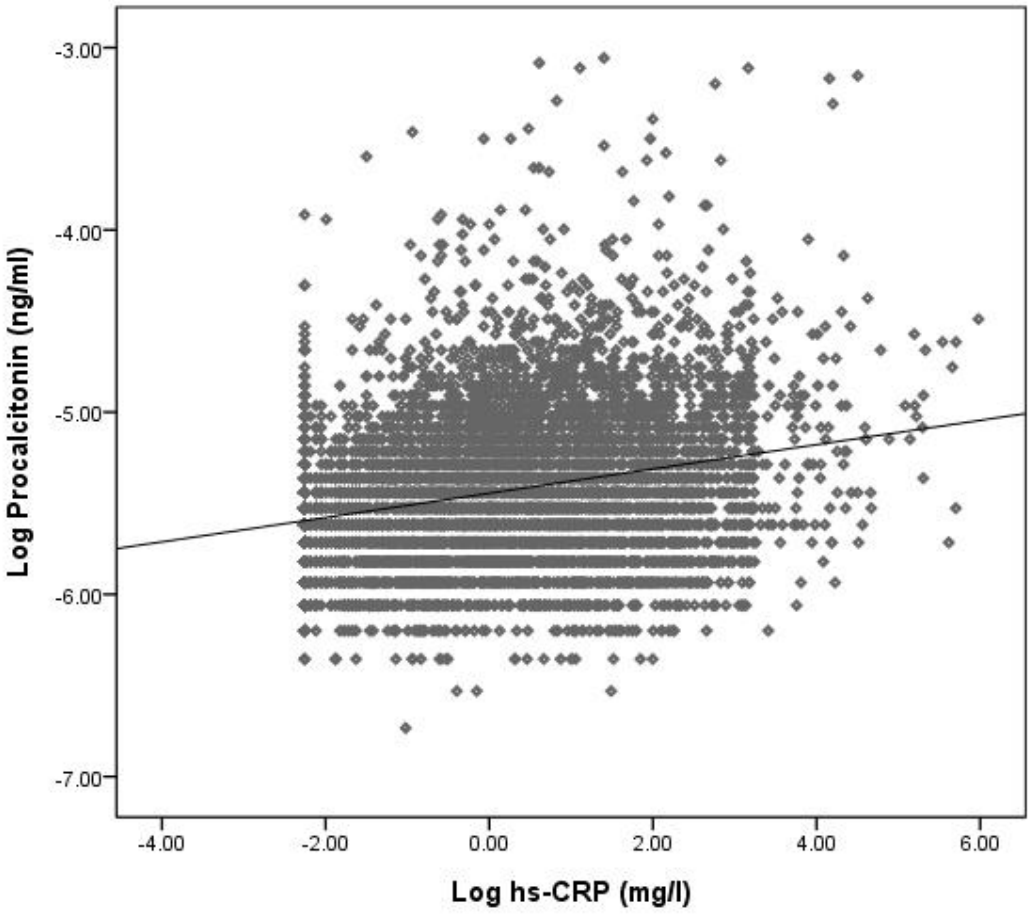

Figure S1. Association between $\log_2$ high-sensitivity C-reactive protein and $\log_2$ procalcitonin, (β= 0.24, *P* <0.001)