Profile of the Immune and Inflammatory Response in Individuals with Prediabetes and Type 2 Diabetes

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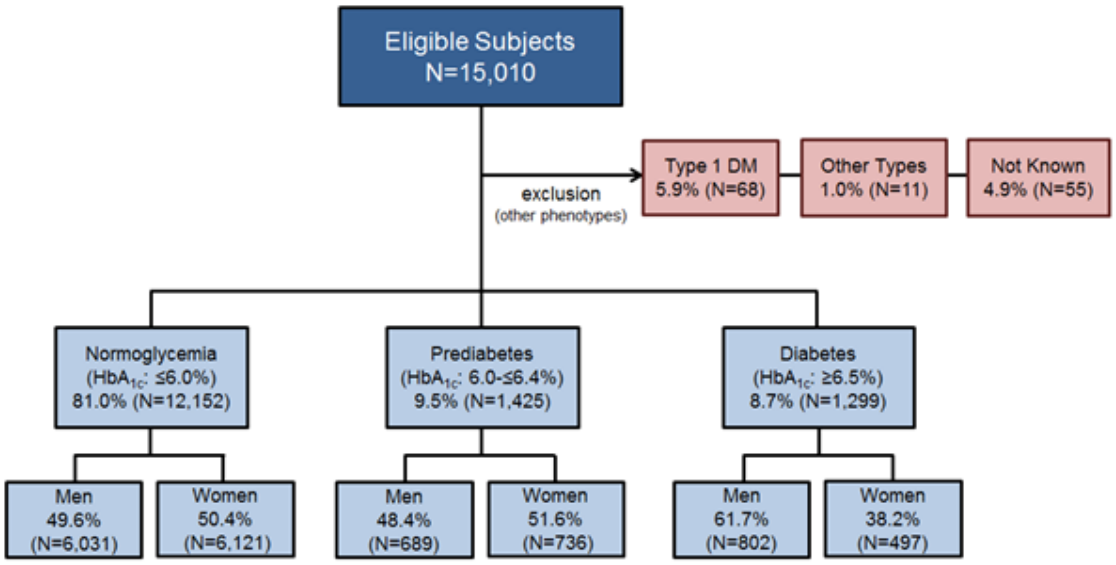
Definitions of Cardiovascular Risk Factors and Comorbidities

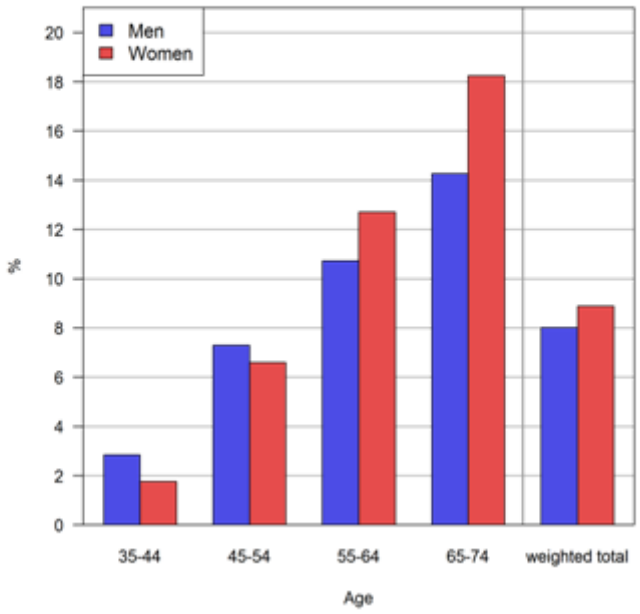
Height was measured (±0.1 cm) as the maximum distance to the uppermost position on the head from heels, with the individual standing barefoot. Body weight was measured (±0.1 kg) using a digital weighing scale. Waist circumference was measured midway between the iliac crest and the lower rib margin at the end of normal expiration. All anthropometric parameters were measured by trained nurses. Body-mass index (BMI) and weight to height ratio (WHtR) were calculated. Blood pressure was measured in triplicates on the left upper arm after resting for at least 5 minutes in seated position. For evaluation, the average values of the three systolic (SBP) and diastolic (DBP) blood pressure measurements were used. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg at rest obtained as the mean of the second and third measurement, or by taking any antihypertensive drugs within the last 2 weeks. Dyslipidemia was defined as diagnosis of dyslipidemia made by a physician, a LDL/HDL-ratio of >3.5 or triglycerides ≥150 mg/dL. Data of cardiovascular disease were collected in a computer-assisted personal interview and questionnaires. Patients were classified as having a cardiovascular event based on the medical history or diagnosis of cardiac arterial disease (CAD), myocardial infarction (MI), stroke, peripheral artery disease (PAD), arterial fibrillation (AF) or chronic heart failure (CHF). In general, all examinations were performed according to standard operating procedures by certified medical technical assistants.

Supplementary Figure S1. Flow diagram for the study sample in the present analysis. Blue colored boxes indicate an inclusion and red colored an exclusion of subjects from the study population. All included subjects were successfully investigated for HbA_{1c} concentration.

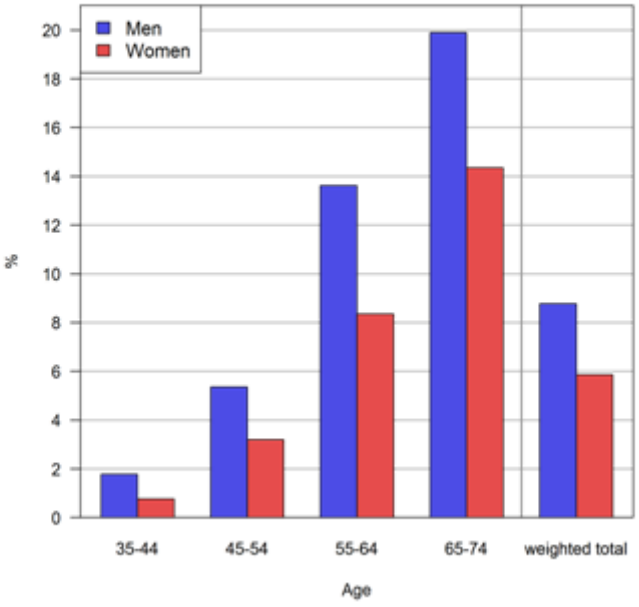


Supplementary Figure S2. Distribution of Prediabetes (A) and diabetes (B) according to age in the GHS sample.

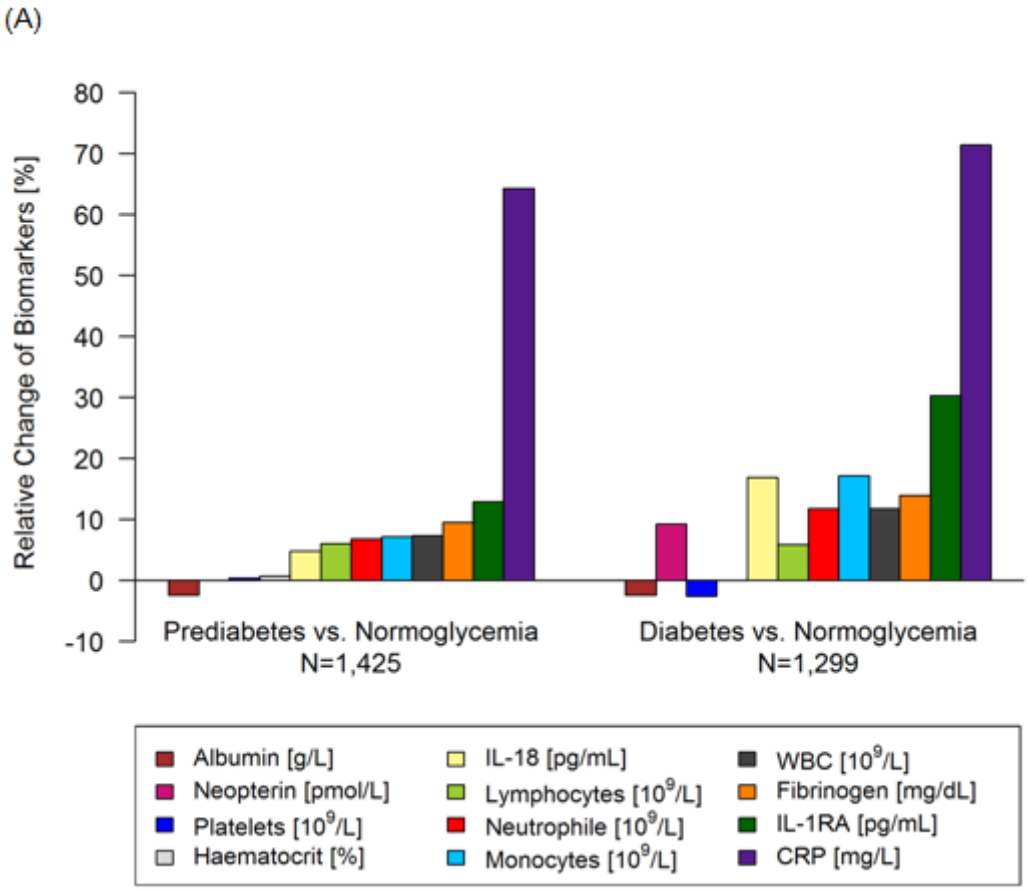
(A)



(B)

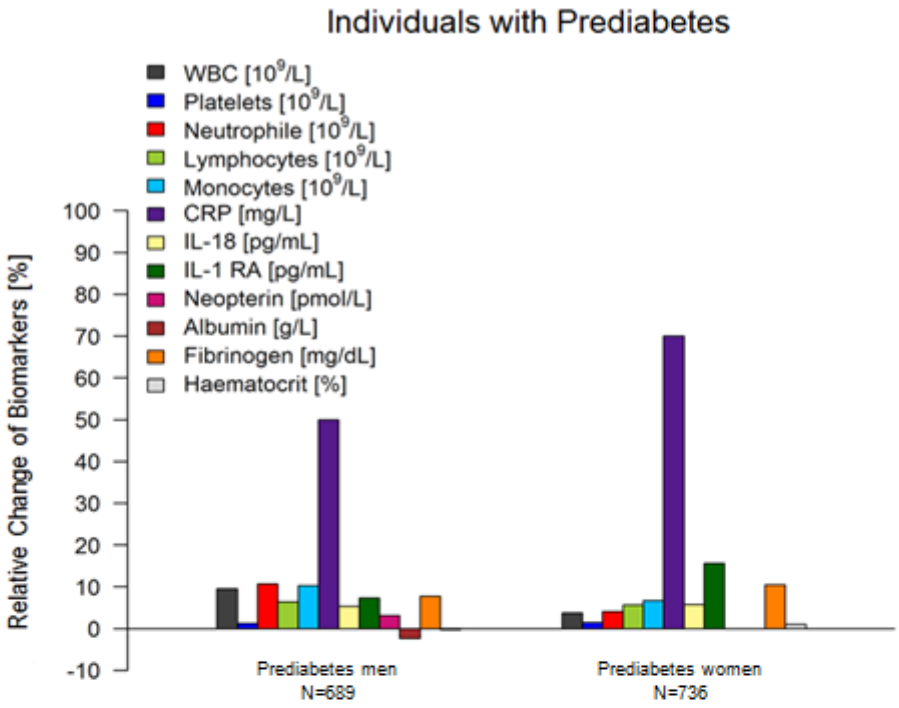


Supplementary Figure S3. Relative change of inflammatory and immune markers in prediabetes and diabetes compared to normoglycemia. Relative change of the concentration of inflammatory and immune biomarkers in subjects with prediabetes and T2D diseased subjects compared to normoglycemic study participants (A). Sex-specific relative change of inflammatory and immune markers in individuals with prediabetes (B) and diabetes (C) compared to normoglycemia.

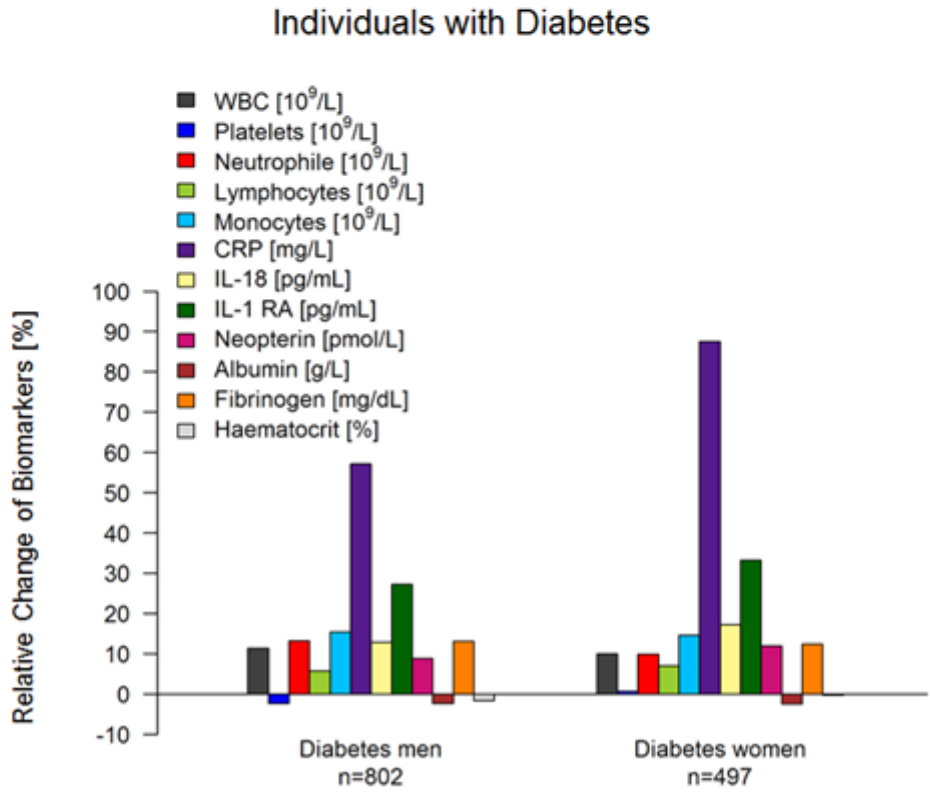


SUPPLEMENTARY DATA

(B)



(C)



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Correlation analysis between biomarkers

When focusing on correlations between inflammatory and immune biomarkers in subjects affected with T2D, strong positive correlations were detected between fibrinogen and CRP (r=0.53), and moderate correlations between IL-1RA and WBC (r=0.41), IL-1RA and CRP (r=0.36), and IL-1RA and granulocytes (r=0.36). A moderate inverse correlation was observed between albumin and CRP (r=0.31). With respect to the associations in prediabetic individuals, strong positive correlations were detected between fibrinogen and CRP (r=0.53), and moderate correlations between IL-1RA and WBC (r=0.39), IL-1RA and granulocytes (r=0.38), IL-1RA and monocytes (r=0.33), and IL-1RA and CRP (r=0.30). Comparable data were observed in individuals with normal HbA_{1c} concentrations, i.e. a strong positive correlation was detected between fibrinogen and CRP (r=0.54), moderate correlations between IL-1RA and WBC (r=0.41), IL-1RA and granulocytes (r=0.42), and IL-1RA and CRP (r=0.34); moderate negative correlation was observed between albumin and CRP (r=0.24). Furthermore, WBC correlated with granulocytes, lymphocytes and monocytes in the diabetic and prediabetic cohort and individuals with normal HbA_{1c} concentration, due to the fact, that granulocyte, lymphocyte and monocyte numbers were calculated according from the WBC count.
Markers of inflammation were correlated with cardiovascular risk factors in individuals with T2D. Moderate correlations were found between IL-1RA and BMI (r=0.42), as well as CRP and BMI (r=0.31). This was comparable in subjects with prediabetes and the normoglycemic group (prediabetes: CRP and BMI, r=0.38; IL-1RA and BMI, r=0.38, normoglycemic group: CRP and BMI, r=0.38; IL-1RA and BMI, r=0.32).

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Supplementary Table S1. Specific medical treatment of patients having T2D.

ATC Code	Therapy	%
A10	Drugs used in Diabetes	87.0
A10A	Insulin and analogues	28.4
A10B	Blood glucose lowering drugd excl. Insulins	72.2
A10AB	Insulins and analogues for injection, fast-acting	20.6
A10AC	Insulins and analogues for injection, intermediate-acting	13.1
A10AD	Insulins and analogues for injection, intermediate- or long-acting combined with fast-acting	2.4
A10AE	Insulins and analogues for injection, long-acting	8.6
A10BA	Biguanides	56.5
A10BB	Sulfonamides, urea derivatives	17.6
A10BD	Combinations of oral blood glucose lowering drugs	5.8
A10BF	Alpha glucosidase inhibitors	1.1
A10BG	Thiazolidinediones	3.6
A10BH	Dipeptidyl peptidase 4 (DPP-4) inhibitors	2.9
A10BX	Other blood glucose lowering drugs, excl. insulins	3.8

Supplementary Table S2. Concentrations of inflammatory and immune biomarkers according to presence of cardiovascular disease within the subgroup with prediabetes (A) and diabetes (B). Data are presented as median (Q1, Q3).

(A)

Variable	Prediabetes without CVD (68.0%, N=1,106)	Prediabetes with CVD (32.0%, N=273)	*P*
WBC [10^9/L]	7.20 (6.08/8.54)	7.50 (6.09/8.92)	0.043
Granulocytes [10^9/L]	277.0 (239.0/329.8)	252.0 (218.0/298.0)	< 0.0001
Lymphocytes [10^9/L]	61.99±8.27	63.57±8.42	0.0055
Monocytes [10^9/L]	27.36±7.56	25.28±7.43	< 0.0001
Platelets [10^9/L]	5.80 (5.00/6.80)	6.10 (5.20/7.30)	0.0038
CRP [mg/L]	2.30 (1.10/4.30)	2.50 (1.20/4.70)	0.13
IL-18 [pg/mL]	230 (180/295)	237 (184/336)	0.42
IL-1RA [pg/mL]	347.6 (266.1/480.8)	362.3 (282.5/517.0)	0.19
Neopterin [pmol/L]	5.34 (4.70/6.13)	5.96 (4.81/7.10)	0.028
Albumin [g/L]	41.0±3.1	40.7±2.7	0.12
Fibrinogen [mg/dL]	340.0 (295.0/403.0)	364.0 (308.0/443.0)	< 0.0001
Haematocrit [%]	42.0±3.3	42.3±3.6	0.25

(B)

Variable	T2D without CVD (68.0%, N=860)	T2D with CVD (32.0%, N=378)	*P*
WBC [10^9/L]	7.43 (6.22/8.65)	7.90 (6.70/9.19)	< 0.0001
Granulocytes [10^9/L]	267.0 (221.0/311.0)	253.0 (215.0/304.6)	0.055
Lymphocytes [10^9/L]	63.5 (58.1/68.5)	65.0 (58.5/70.2)	0.014
Monocytes [10^9/L]	25.80 (21.30/30.41)	24.35 (18.84/29.60)	0.00047
Platelets [10^9/L]	6.00 (5.10/7.00)	6.20 (5.34/7.26)	0.018
CRP [mg/L]	2.40 (1.20/5.00)	2.55 (1.40/5.50)	0.094
IL-18 [pg/mL]	262 (203/339)	248 (206/329)	0.78
IL-1RA [pg/mL]	385 (283/527)	448 (323/598)	0.0082
Neopterin [pmol/L]	5.70 (4.90/6.70)	6.60 (5.60/8.39)	< 0.0001
Albumin [g/L]	41.0 (39.0/43.0)	41.0 (39.0/43.0)	0.0032
Fibrinogen [mg/dL]	349.0 (302.0/406.0)	383.0 (329.0/452.0)	< 0.0001
Haematocrit [%]	41.9 (39.6/44.4)	41.8 (39.6/44.1)	0.24

Supplementary Table S3. Frequency of cardiovascular disease according to diabetes duration and treatment.

Duration of T2D	Frequency of CVD (%/N)
<1 year	8.8% (26)
1-2 years	6.8% (20)
2-5 years	21.7% (64)
5-10 years	30.2% (89)
>10 years	32.5% (96)
Treatment of Diabetes	Frequency of CVD (%/N)
No treatment	3.3% (12)
Dietary only	3.9% (14)
NIDDM	39.2% (142)
IDDM	30.1% (109)

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Supplementary Table S4. Concentration of inflammatory and immune biomarkers according to disease treatment.

Variable	No therapy/Dietary	NIDDM	IDDM	*P* for trend	*P* (NIDDM vs. IDDM)
Size [N]	109	563	358		
WBC [10^9/L]	7.2 (6.1/8.7)	7.5 (6.3/8.7)	7.8 (6.5/9.2)	0.00076	0.0084
Granulocytes [10^9/L]	4.52 (3.76/5.57)	4.76 (3.83/5.74)	5.05 (4.03/6.06)	0.0017	0.012
Lymphocytes [10^9/L]	1.79 (1.43/2.26)	1.83 (1.47/2.29)	1.86 (1.46/2.30)	0.62	0.92
Monocytes [10^9/L]	0.43 (0.33/0.52)	0.45 (0.36/0.53)	0.48 (0.38/0.58)	0.00055	0.0067
Platelets [10^9/L]	249.5 (216.4/305.2)	261.0 (217.0/310.8)	259.0 (217.0/300.6)	0.78	0.47
CRP [mg/L]	2.4 (1.2/5.2)	2.1 (1.1/4.5)	2.6 (1.4/5.4)	0.032	0.0022
IL-1RA [pg/mL]	346 (237/553)	400 (286/505)	398 (297/580)	<0.0001	0.27
IL-18 [pg/mL]	284 (205/342)	263 (199/343)	249 (198/339)	<0.0001	0.83
Neopterin [pmol/L]	5.9 (5.1/7.9)	5.7 (4.9/7.1)	6.4 (5.3/8.2)	<0.0001	0.0058
Albumin [g/L]	41.0 (39.0/43.0)	41.0 (39.0/43.0)	41.0 (39.0/43.0)	0.0089	0.0027
Fibrinogen [mg/dL]	367 (317/423)	347 (298/404)	373 (323/441)	0.0015	<0.0001
Haematokrit [%]	41.5 (39.6/43.9)	41.4 (39.2/43.7)	42.0 (39.7/44.2)	0.055	0.041

Variables are presented as median with 25^{th} and 75^{th} percentile.

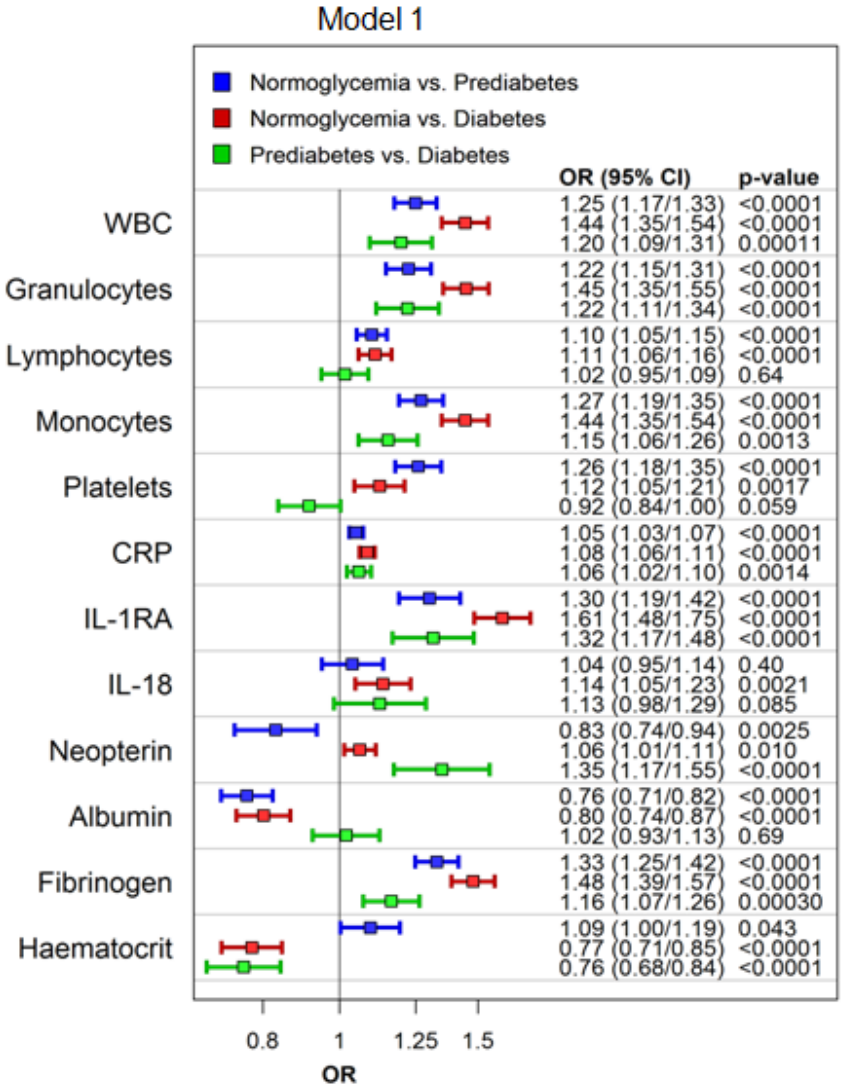
Supplementary Table S5. Concentration of inflammatory and immune biomarkers according to diabetes disease duration.

	Duration of DM	Mean (SD)	Median (Q1/Q3)
WBC [10^9/L]	≤1 year	7.80 (2.06)	7.63 (6.17/9.18)
	1-2 years	7.90 (2.38)	7.54 (6.23/8.70)
	2-5 years	7.97 (2.05)	7.66 (6.50/9.14)
	5-10 years	7.76 (2.09)	7.53 (6.29/8.68)
	> 10 years	7.84 (2.12)	7.56 (6.33/8.96)
Platelets [10^9/L]	≤1 year	266 (64.5)	258 (226/305)
	1-2 years	255 (76.6)	244 (198/306)
	2-5 years	278 (90.4)	270 (224/317)
	5-10 years	259 (64.8)	254 (215/302)
	> 10 years	262 (68.3)	258 (215/302)
Granulocytes [10^9/L]	≤1 year	62.7 (7.67)	62.6 (57.5/68.3)
	1-2 years	63.6 (7.76)	63.0 (58.5/68.5)
	2-5 years	63.8 (8.01)	64.1 (58.2/69.5)
	5-10 years	64.3 (7.81)	64.2 (59.7/69.6)
	> 10 years	65.4 (7.78)	66.0 (60.1/71.0)
Lymphocytes [10^9/L]	≤1 year	26.6 (6.90)	26.7 (22.4/31.5)
	1-2 years	25.7 (7.08)	25.9 (20.5/29.7)
	2-5 years	25.5 (7.24)	25.4 (19.9/30.2)
	5-10 years	24.9 (6.80)	24.9 (20.5/29.2)
	> 10 years	23.6 (7.12)	22.7 (18.4/28.4)
Monocytes [10^9/L]	≤1 year	6.20 (1.70)	6.00 (5.10/7.00)
	1-2 years	6.10 (1.26)	6.00 (5.14/6.96)
	2-5 years	5.98 (1.58)	5.70 (5.00/6.60)
	5-10 years	6.25 (1.55)	6.05 (5.20/7.10)
	> 10 years	6.22 (1.61)	5.90 (5.20/7.18)
CRP [mg/L]	≤1 year	4.79 (6.91)	2.60 (1.30/5.00)
	1-2 years	4.85 (8.69)	2.50 (1.40/5.19)
	2-5 years	4.16 (8.56)	2.30 (1.12/4.68)
	5-10 years	4.90 (12.2)	2.40 (1.20/5.10)
	> 10 years	4.39 (7.06)	2.20 (1.10/5.00)
IL-18 [pg/mL]	≤1 year	268 (100.0)	258 (196/326)
	1-2 years	274 (104)	258 (189/332)
	2-5 years	294 (113)	285 (219/355)
	5-10 years	283 (110)	256 (200/350)
	> 10 years	289 (120)	268 (208/350)
IL-1RA [pg/mL]	≤1 year	445 (223)	409 (298/513)
	1-2 years	514 (311)	424 (346/550)
	2-5 years	450 (201)	428 (282/539)
	5-10 years	458 (259)	401 (297/561)
	> 10 years	441 (222)	367 (287/545)
Neopterin [pmol/L]	≤1 year	6.25 (2.15)	5.66 (4.60/6.97)
	1-2 years	7.22 (4.60)	5.73 (5.22/7.08)

	2-5 years	6.13 (2.05)	5.80 (5.00/6.83)
	5-10 years	6.98 (3.53)	6.27 (5.21/7.69)
	> 10 years	9.47 (15.3)	6.60 (5.50/8.55)
Albumin [g/L]	≤1 year	40.9 (3.18)	41.0 (39.0/43.0)
	1-2 years	41.0 (3.07)	41.0 (39.0/43.0)
	2-5 years	41.1 (3.00)	41.0 (39.0/43.0)
	5-10 years	41.0 (3.17)	41.0 (39.0/43.0)
	> 10 years	40.6 (3.38)	41.0 (39.0/43.0)
Fibrinogen [mg/dL]	≤1 year	384 (96.4)	375 (316/432)
	1-2 years	361 (79.4)	355 (307/405)
	2-5 years	364 (90.4)	354 (302/410)
	5-10 years	377 (101)	361 (309/424)
	> 10 years	384 (104)	365 (319/428)
Haematokrit [%]	≤1 year	42.1 (3.58)	42.2 (40.1/44.3)
	1-2 years	41.4 (3.09)	41.4 (38.9/44.0)
	2-5 years	41.6 (3.45)	41.7 (39.8/43.6)
	5-10 years	41.5 (3.70)	41.4 (39.4/44.0)
	> 10 years	41.4 (3.75)	41.6 (39.0/44.0)

SUPPLEMENTARY DATA

Supplementary Figure S4. Impact of inflammatory and immune biomarkers on the presence of prediabetes and diabetes. A logistic model with diabetes or prediabetes vs normoglycemia and diabetes vs prediabetes as dependent variable was calculated for each biomarker. Model 1 was adjusted for sex and age, Model 2 additionally for cardiovascular risk factors, including waist-to-height ratio, hypertension, smoking, dyslipidaemia, and comorbidities including coronary artery disease, myocardial infarction, stroke, peripheral artery disease, atrial fibrillation, and chronic obstructive pulmonary disease as well as arthritis, hemostatic disorder, autoimmune disease and acute infection.



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