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Inflammatory biomarkers as prognostic tools for diabetic retinopathy progression: a prospective study

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ABSTRACT

Aims: This study aimed to evaluate the prognostic potential of circulating inflammatory biomarkers, specifically hsCRP, IL-6, TNF-α, and CD163, for predicting diabetic retinopathy (DR) in adults with recently diagnosed type 2 diabetes.
Methods: A prospective cohort of 3,363 individuals from the Danish Centre for Strategic Research in Type 2 Diabetes was followed prospectively (median follow-up of nine years). Baseline concentrations of four biomarkers were measured, and DR outcomes were assessed. Logistic regressions were used to evaluate associations with DR presence at baseline, while Cox regressions were used to analyze the development and progression of DR, adjusting for potential confounders, including HbA1c.
Results: No associations were observed between baseline concentrations of inflammatory biomarkers and DR presence, development, or progression over time. When comparing the highest quartile to the lowest quartile for a given biomarker in adjusted models, the hazard ratios (95% CI) for the development or progression of DR were: hsCRP 0.76 (0.55–1.03), IL-6 0.84 (0.62–1.14), TNF-α 1.03 (0.76–1.40), and CD163 0.84 (0.62–1.14).
Conclusions: Our results indicate that systemic inflammatory biomarkers may not serve as reliable predictors for DR in early-stage type 2 diabetes. These findings contrast with prior cross-sectional studies suggesting a role for systemic low-grade inflammation in DR development.
Inflammatory biomarkers.
Diabetic retinopathy.

1. Background

Diabetic retinopathy (DR) is a serious microvascular complication of diabetes and a leading cause of visual impairment globally.[1,2] The main risk factors for the development of DR include diabetes duration, chronic hyperglycemia, and hypertension. [3,4] Among these, chronic hyperglycemia is considered the primary driver of DR, and intensive glycemic control has been shown to reduce its development and progression. [5] Additional risk factors encompass dyslipidemia, and the presence of other microvascular complications, such as diabetic nephropathy and neuropathy. [6–10] While these risk factors, particularly diabetes duration and glycemic control, are strong predictors of DR development and severity, predicting the onset or rate of progression of DR in an individual remains a clinical challenge. This challenge is further compounded by scarce resources and capacity constraints in providing regular retinal screening for all patients with diabetes in many countries. [11,12] Identifying factors associated with the onset of DR might prove a fruitful avenue in furthering our understanding of early

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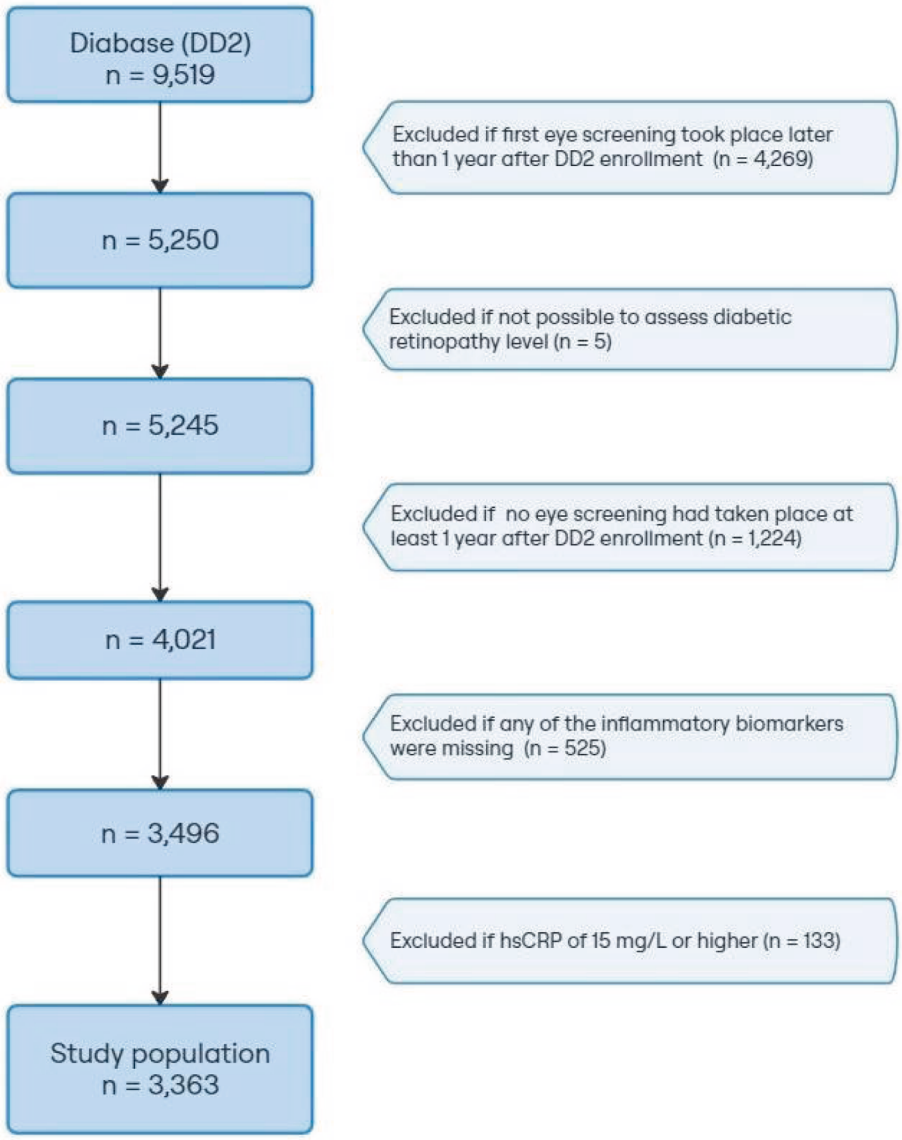
detection of DR, enabling development of predictive risk models for DR and thereby the ability to offer individualized and cost-effective screenings programs. [11,12] Inflammatory processes are increasingly recognized as critical contributors to DR pathogenesis, [13–15] and elevated levels of inflammatory markers, as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α), have been observed in ocular vitreous and aqueous humor samples from diabetic patients with DR. [16,17] In addition, several cross-sectional studies have investigated the clinical potential of circulating biomarkers to detect sight-threatening DR. Thus, as summarized in a *meta*-analysis, associations between circulating biomarkers of inflammation, including high sensitive CRP (hsCRP), IL-6, and TNF-α and the presence or severity of DR in patients with type 2 diabetes have been observed. [18] Of these markers, TNF-α showed the most consistent association, with levels increasing across all DR stages, while hsCRP and IL-6 showed potential for monitoring disease progression. Another inflammatory marker of interest is soluble Cluster of Differentiation 163 (CD163), a receptor protein expressed on macrophages and monocytes, which can be measured in plasma. [19] Elevated levels of soluble CD163 have recently been associated with DR progression during a 12-years follow-up period of 270 individuals with type 1 diabetes, independent of other risk factors. [20] Although these findings highlight the clinical potential of inflammatory biomarkers as predictors of DR, most studies have been either small scale or cross-sectional. The prognostic potential of circulating biomarkers of systemic low-grade inflammation for predicting DR development or progression of DR in patients with type 2 diabetes remains less explored.

In this study, we investigate associations between selected circulating inflammatory biomarkers, namely hsCRP, IL-6, TNF-α, and soluble CD163, and DR presence, development, and progression in patients with recently diagnosed type 2 diabetes. Specifically, we hypothesize



Notes: The figure shows the construction of our population, as defined by our exclusion criteria.

Fig. 1. Sample Construction. *Notes*: The figure shows the construction of our population, as defined by our exclusion criteria.

that: Markers of systemic low-grade inflammation are independently associated with the presence of DR. Moreover, that these biomarkers are also associated with development and progression of DR, even after controlling for known risk factors, including diabetes duration, HbA1c, blood pressure, eGFR, urine albumin-creatinine ratio (UACR), and antidiabetic treatment.

2. Methods

2.1. Data

This section details our sample construction, as well as our definition of exposure, outcomes, and the various covariates we adjust for.

2.1.1. Sample construction

The study cohort comprised of individuals with recently diagnosed type 2 diabetes enrolled in the ongoing Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort and biobank. [21] The cohort comprised 11,381 individuals included between 2010 and 2021, [22] each with a unique personal identification number, which was used for data linkage.

We retrieved data from 42,732 eye-screening visits, spread amongst 9,519 unique individuals. Of these, we only included individuals with at least one eye screening taking place no later than one year after DD2 enrollment (5,250 unique individuals). Of these, we removed visits where it was not possible to assess DR in any eye, which left us with 5,245 unique individuals. We next removed individuals with no eye assessment after one year of enrollment into DD2, which left us with 4,021 individuals.

Since our focus is on measuring associations between biomarkers at baseline and state and progression of DR, we only kept individuals with measures of all four biomarkers, which left us with 3,496 individuals. Lastly, we excluded individuals with baseline hsCRP above 15 mg/L, which left us with a final sample size of 3,363 individuals. Fig. 1 illustrates our sample construction.

As baseline eye measurements, we used the closest eye screening date to DD2 enrollment, determined based on proximity, whether occurring before or after enrollment. Follow-up began one year after DD2 enrollment and continued until the final screening episode, the occurrence of the study endpoint, death, or emigration.

2.1.2. Exposure (inflammatory biomarkers)

Inflammatory biomarkers were measured using blood samples collected at DD2 enrollment and stored at −80°C until analysis. [23] Plasma IL-6 and TNF-α were quantified using the Meso Scale Discovery technique with validated V-plex immunoassays (Meso Scale Diagnostics, Rockville, MD, USA). The analytical coefficients of variation were 12.3% for IL-6 and 14.4% for TNF-α, respectively. The lower limit of quantification (LLOQ) for IL-6 and TNF-α were 0.63 ng/L and 0.69 ng/L, respectively. Additional details for IL-6 and TNF-α have been partially presented before, [22] but not in relation to DR.

Soluble CD163 plasma concentrations were measured using an electro-chemoluminescence based-ELISA assays on a MESO Quickplex SQ 120 instrument (MSD, Gaithersburg, USA). Soluble CD163 was analyzed with a U-plex assay, as part of a custom made 7-plex assay. The analytical coefficient of variation was 16.9% and the LLOQ was 524 ng/L.

Serum hsCRP concentrations were measured using an in-house time-resolved immunofluorometric assay, as previously described, [24] and additional details have been partially presented before, [22,25] but not in relation to DR. In our primary analyses, we treated each of the four biomarkers as categorical variables, each with three levels representing the bottom 25% (<Q1), middle 25%-75% (Q1-Q3), and top 25% (>Q3).

2.1.3. Outcomes (DR)

We obtained DR outcomes from the Danish Registry of Diabetic Retinopathy (DiaBase). DiaBase has collected data nationwide since 2013, with retrospective data available from August 2007. [26] DR screening was conducted by practicing ophthalmologist or at designated hospitals following national guidelines, [27] and a validation study by Thykjær et al. showed high agreement between graders. [28] Study outcomes were (1) presence of DR in one or both eyes at baseline, (2) development of any DR (regardless of whether in one or both eyes), *conditional on no DR at baseline*, (3) progression of DR, defined by a one-level worsening in both eyes *or* a two-level worsening in (at least) one eye, and (4) a composite measure capturing development and progression of DR in one or both eyes. Our primary endpoint was the composite measure. Eye related health included screening visit dates, historical records of eye surgeries, method of examination, visual acuity measurements, and assessments of retinal and maculopathy status. We included patients regardless of whether we had access to eye data for one or both of their eyes. For patients for whom we had access to data on both eyes, we define DR as the presence of DR in one or both of their eyes; these individuals did not contribute with two observations. We assessed DR status using the International Clinical Diabetic Retinopathy Severity Scale, [29] which categorizes DR into five levels: (0) No DR; (1) mild non-proliferative DR (NPDR), characterized by microaneurysms and/or dot hemorrhages only; (2) moderate NPDR, characterized by more than microaneurysms and/or dot hemorrhages but less severe than Level 3; (3) severe NPDR, defined as > 20 intraretinal hemorrhages in all four quadrants, or intraretinal microvascular abnormalities in at least one quadrant, but not proliferative; (4) proliferative DR (PDR), defined by neovascularization (active or treated) or vitreous/preretinal hemorrhage. For follow-up analyses, eyes were excluded if PDR was present at baseline. As our focus was on patients, such an individual could still be included, provided she only had PDR in one eye at baseline.

2.1.4. Covariates

The DD2 database provided data on traditional cardiovascular disease (CVD) risk factors, biomarker measurements, and pharmacological treatments, as reported by physicians and patients at enrollment.

Baseline and longitudinal data on DR risk factors and outcomes were retrieved from the DD2 cohort, DiaBase, which is part of the Danish Clinical Quality Program, National Clinical Registries, and relevant Danish national registries detailed in Supplementary Table 1. From the registries, we obtained demographic information (age, sex), diabetes and lifestyle characteristics (diabetes duration, HbA1c, smoking status, alcohol consumption, waist-hip ratio), whether the patient had any previous CVD (defined as a prior diagnosis of myocardial infarction, stroke, atrial fibrillation, heart failure, or peripheral arterial disease), renal function (eGFR, UACR), blood pressure (systolic, diastolic), lipids (total cholesterol, HDL, LDL, triglycerides), and medication (antidiabetic, antihypertensive, lipid lowering). See Table 1 for further details.

2.2. Statistics

2.2.1. Baseline characteristics

Individuals with and without any DR at baseline were compared for similarities/differences. For numeric variables, medians alongside Q1 and Q3 were reported, as well as the number of observations with missing data, and differences were tested using Mann-Whitney U tests. For categorical variables, sample sizes of each category were reported, as well as the share each category represented, and differences were tested using χ^2 tests.

2.2.2. Baseline associations

Logistic regression was used to evaluate possible associations between inflammatory biomarkers and the presence of DR at baseline. Regression models were fitted for each of the four biomarkers, using the given biomarker as a categorical variable as described in *2.1.2 Exposure (inflammatory biomarkers)*. Two sets of these four regressions were estimated, one unadjusted and one where the model adjusted for

Table 1
Summary statistics by presence of any DR at baseline.

	No DR at baseline (n = 3,091)	DR at baseline (n = 272)	P-value
Demographics			
Female, n (%)	1293 (41.83%)	84 (30.88%)	<0.005
Age (years), median (Q1; Q3)	61.33 (52.62; 68.19)	60.43 (51.9; 68.08)	0.37
Diabetes			
Diabetes duration (years), median (Q1; Q3)	1.01 (0.21; 3.10)	1.82 (0.37; 4.16)	<0.005
HbA1c (mmol/mol), median (Q1; Q3)	48 (43; 54)	52 (46; 59)	<0.005
HbA1c (%), median (Q1; Q3)	6.5 (6.1; 7.1)	6.9 (6.4; 7.5)	<0.005
Lifestyle			
Smoking			0.37
Current, n (%)	404 (16.52%)	45 (20.18%)	
Former, n (%)	876 (35.83%)	75 (33.63%)	
Never, n (%)	1165 (47.65%)	103 (46.19%)	
Alcohol consumption above guidelines, n (%)*	184 (5.97%)	19 (7.01%)	0.58
Waist-hip ratio, median (Q1; Q3)	0.98 (0.92; 1.04)	1.00 (0.94; 1.05)	0.01
Previous cardiovascular disease			
Previous cardiovascular disease†	828 (26.79%)	93 (34.19%)	0.01
Renal function			
eGFR (mL/min/1.73 m^2) below 60, n (%)	209 (6.76%)	15 (5.51%)	0.62
UACR‡ (mg/g) above 30, n (%)	44 (1.42%)	10 (3.68%)	0.01
Blood pressure			
Systolic BP (mmHg), median (Q1; Q3)	130 (124; 140)	133 (126; 140)	0.07
Diastolic BP (mmHg), median (Q1; Q3)	80 (75; 86)	80 (75; 86)	0.27
Lipids			
Total cholesterol (mmol/L), median (Q1; Q3)	4.20 (3.60; 4.90)	4.00 (3.40; 4.80)	0.02
HDL (mmol/L), median (Q1; Q3)	1.20 (0.99; 1.40)	1.20 (0.99; 1.40)	0.94
LDL (mmol/L), median (Q1; Q3)	2.10 (1.60; 2.70)	2.00 (1.65; 2.60)	0.26
Triglycerides (mmol/L), median (Q1; Q3)	1.70 (1.20; 2.42)	1.54 (1.10; 2.26)	0.04
Medication			
Antidiabetic medication			<0.005
Oral and insulin, n (%)	225 (7.28%)	53 (19.49%)	
None, n (%)	316 (10.22%)	22 (8.09%)	
Oral, n (%)	2550 (82.5%)	197 (72.43%)	
Antihypertensive medication (excl. loop), n (%)	2154 (69.69%)	195 (71.69%)	0.53
Lipid lowering medication, n (%)	2247 (72.69%)	189 (69.49%)	0.29
Biomarkers			
hsCRP (mg/L), median (Q1; Q3)	1.83 (0.78; 3.97)	1.66 (0.68; 3.58)	0.27
IL-6 (ng/L), median (Q1; Q3)	1.15 (0.77; 1.69)	1.12 (0.76; 1.70)	0.91
TNF-α (ng/L), median (Q1; Q3)	0.98 (0.81; 1.22)	1.02 (0.80; 1.22)	0.78
CD163 (mg/L), median (Q1; Q3)	0.80 (0.59; 1.08)	0.80 (0.57; 1.08)	0.99

Notes: The table shows medians (alongside Q1 and Q3) for numeric variables, and counts (alongside shares) for categorical variables, stratified by presence of any DR at baseline. P-values are based on Mann-Whitney U tests for numeric and χ^2 tests for categorical variables, comparing patients with/without any presence of DR at baseline. *High alcohol consumption was defined as > 14 alcoholic drinks/week for women and 21 alcoholic drinks/week for men. †Previous CVD was defined as a prior diagnosis of myocardial infarction, stroke, atrial fibrillation, heart failure, or peripheral arterial disease. ‡UACR: Urine albumin-creatinine ratio.

demographic information, diabetes and lifestyle characteristics, whether the patient had any previous CVD, renal function, blood pressure, lipids, and medication (see *2.1.4 Covariates* for additional details). Results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs).

2.2.3. Development and progression of DR

Associations between inflammatory biomarkers and development or progression of DR were estimated using Cox regression. Cox regressions were estimated with outcome variables measuring (1) DR incident, (2) the progression of DR, or (3) a composite outcome, where an event was defined as either of the two other outcomes. For all outcomes, regression models were fitted for each of the four biomarkers, using the given biomarker as a categorical variable as described in *2.1.2 Exposure (inflammatory biomarkers)*. Two sets of these 12 regressions were estimated, one unadjusted and one adjusted for demographic information, diabetes and lifestyle characteristics, whether the patient had any previous CVD, renal function, blood pressure, lipids, and medication (see *2.1.4 Covariates* for additional details). We expressed results as hazard ratios (HRs) with 95% CIs.

2.2.4. Imputation

The sample construction strategy ensured that exposures and outcomes were always observed, but the adjusted regressions were hindered by a relatively large share of missingness. To this end, imputed missing control variables were used, estimated using multiple imputation by chained equations (MICE) to maximize statistical power and minimize selection bias. Imputations were based on outcome variables, exposure variables, and other control variables. A total of 20 complete datasets were generated, with missing values sampled from the predictive distribution based on observed data. The MICE approach assumes that data is missing at random, in the sense that we can fully account for data's missingness through our *other* variables and missingness thus only depends on observed information. Columns with missing data are computed using Gibbs sampling, using all other columns as predictors. Since these predictor columns may themselves contain missing values, the most recently computed imputations are used for those predictions.

Supplementary Fig. 1 shows the empirical distributions of all the numerical control variables we imputed for our real sample as well as two imputed samples, illustrating that the actual and imputed distributions closely align.

2.2.5. Sensitivity analyses

A sample where individuals with baseline hsCRP above 10 mg/L were excluded and a sample which included all individuals regardless of baseline hsCRP were constructed (to more conservatively drop individuals with any acute inflammation or infection), and the sensitivity of the results to the definition of these criteria were assessed. Additionally, we tested baseline associations using log-values of our biomarkers rather than the categorical specifications.

2.2.6. Heterogeneity analyses

We tested whether age, sex, diabetes duration at baseline, or HbA1c levels modified the associations between inflammatory biomarkers and development/progression of DR by interaction analyses, using our combined outcome and interacting the heterogeneity variable with the log value of our biomarkers in Cox regressions, adjusting for potential confounders.

2.2.7. Statistical program

All analyses were conducted in R version 4.3.3 (R Development Core Team (2022) R: a language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria).

2.3. Ethics

The study was approved by the Danish National Committee on Biomedical Research Ethics (record number S-20100082), the Danish Data Protection Agency (record number 2008–58-0035) has approved the DD2 project, and the study was conducted in concordance with the Helsinki Declaration II.

3. Results

3.1. Descriptive statistics

At baseline, DR was present in 272 (8.1%) of the 3,363 individuals. Of the individuals with DR at baseline, 214 (6.4% of the study population) had mild (level 1), 48 (1.4% of the study population) moderate DR (level 2), and 10 (0.3% of the study population) had severe to proliferative DR (levels 3–4). At follow-up, DR was present in 581 (17.3%) individuals. Of the individuals with DR at follow-up, 441 (13.1% of the study population) had mild, 126 (3.7% of the study population) moderate DR, and 14 (0.4% of the study population) severe to proliferative DR.

Table 1 shows summary statistics, stratified by the presence of any level of DR at baseline. Patients with DR at baseline were more likely to be male, had longer diabetes durations, had higher HbA1c levels, were more likely to have had previous CVD, and received more antidiabetic medication at enrollment. There were no significant differences in terms of the circulating levels of IL-6. TNF-α, hsCRP, or CD163 between patients with and without DR at baseline.

Supplementary Table 2 in the appendix shows the equivalent of Table 1, now stratified into three groups (no DR, mild DR, and moderate to severe DR at baseline).

3.2. Baseline associations

Table 2 shows associations between biomarker levels and the presence of any DR at baseline, from univariate and multivariate logistic regressions.

We observed no significant differences in the prevalence of any DR at baseline between any of our inflammatory groups regardless of whether we controlled for potential confounders. Most of the estimated ORs were close to one, and all with 95% CIs that included one.

Supplementary Table 3 shows results equivalent to those of Table 2, but with inflammatory biomarkers included as continuous variables (log transformed) rather than categorized. All estimated ORs were close to one, and none was even marginally significant. When excluding individuals with baseline hsCRP above 10 mg/L or when including all individuals regardless of baseline hsCRP, results remained unchanged.

3.3. Development and progression of DR

The cohort was followed from 2010 to 2023, with a median follow up of nine years. During this period, 442 individuals developed DR or had progression of DR. Table 3 shows associations between biomarker levels and (1) the development of any DR after baseline (*Panel A*), (2) the progression of DR (*Panel B*), and (3) our combined outcome (*Panel C*), assessed using Cox regression. As the reference group, we used individuals in the lowest quartile of the given biomarker.

We observed no significant differences in the combined endpoint of development/progression of DR between any of our inflammatory groups regardless of whether we adjusted for potential confounders. Most of the estimated HRs were close to one, and all CIs included one. When excluding individuals with baseline hsCRP above 10 mg/L or when including all individuals regardless of baseline hsCRP, results remained unchanged.

When looking at development and progression separately, results remained largely unchanged, with most HRs close to one, with the possible exception of individuals in the middle two quarters of hsCRP being less likely to progress in DR score (Panel B, unadjusted model, HR = 0.71 [0.53–0.96]), but this result disappeared when controlling for potential confounders.

Supplementary Table 4 shows results where we interact the log value of our biomarkers with age, diabetes duration, HbA1c, and sex using adjusted models (See *2.2.6 Heterogeneity analyses* for details). Neither age, diabetes duration, nor HbA1c levels significantly modified the associations between inflammatory markers and incidence or progression of DR. For sex, HRs were lower for males than females for IL-6 and TNF-α (when estimating separate adjusted models for each sex, rather than

Table 2
Associations between biomarkers and DR at baseline.

	Unadjusted			Adjusted		
Independent variable	**OR**	**95% CI**	**P-value**	**OR**	**95% CI**	**P-value**
hsCRP 25–75%	0.90	0.67–1.21	0.46	0.88	0.63–1.25	0.48
hsCRP > 75%	0.76	0.53–1.08	0.13	0.70	0.45–1.10	0.12
IL-6 25–75%	0.99	0.73–1.35	0.94	0.87	0.61–1.25	0.46
IL-6 > 75%	1.02	0.72–1.45	0.91	0.89	0.59–1.35	0.59
TNF-α 25–75%	0.92	0.69–1.25	0.59	0.96	0.68–1.37	0.84
TNF-α > 75%	0.98	0.69–1.38	0.90	1.07	0.70–1.64	0.74
CD163 25–75%	0.95	0.71–1.30	0.76	1.08	0.76–1.53	0.68
CD163 > 75%	0.97	0.68–1.37	0.86	1.01	0.67–1.53	0.96

Notes: The table shows logistic regression results from eight regressions (four biomarkers and two specifications). Outcome is DR at baseline. Reference groups defined as the lowest quartile of the given biomarker. The adjusted regressions control for demographic information (age, sex), diabetes and lifestyle characteristics (diabetes duration, HbA1c, smoking status, alcohol consumption, waist-hip ratio), whether the patient had any previous CVD (defined as a prior diagnosis of myocardial infarction, stroke, atrial fibrillation, heart failure, or peripheral arterial disease), renal function (eGFR, UACR), blood pressure (systolic, diastolic), lipids (total cholesterol, HDL, LDL, triglycerides), and medication (antidiabetic, antihypertensive, lipid lowering).

Table 3
Associations between biomarkers and development or progression of DR.

	Unadjusted			Adjusted		
Independent variable	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
Panel A: Any DR						
hsCRP 25–75%	0.82	0.65–1.04	0.10	0.82	0.62–1.07	0.14
hsCRP > 75%	0.97	0.74–1.27	0.82	0.83	0.60–1.14	0.25
IL-6 25–75%	0.95	0.75–1.21	0.69	0.98	0.75–1.29	0.91
IL-6 > 75%	0.94	0.71–1.24	0.66	0.94	0.68–1.29	0.69
TNF-α 25–75%	0.93	0.74–1.17	0.52	1.04	0.80–1.34	0.79
TNF-α > 75%	1.02	0.77–1.34	0.90	1.18	0.85–1.63	0.32
CD163 25–75%	0.99	0.78–1.24	0.90	0.94	0.72–1.21	0.62
CD163 > 75%	0.96	0.73–1.28	0.80	0.89	0.65–1.22	0.46
Panel B: DR progression						
hsCRP 25–75%	0.71	0.53–0.96	0.03	0.76	0.55–1.07	0.12
hsCRP > 75%	0.81	0.57–1.15	0.24	0.76	0.50–1.14	0.18
IL-6 25–75%	1.01	0.74–1.37	0.96	1.07	0.76–1.51	0.68
IL-6 > 75%	0.94	0.65–1.34	0.72	0.88	0.58–1.35	0.56
TNF-α 25–75%	0.84	0.63–1.13	0.26	0.93	0.68–1.29	0.68
TNF-α > 75%	0.95	0.67–1.35	0.79	1.00	0.66–1.52	0.99
CD163 25–75%	0.94	0.70–1.25	0.66	0.95	0.69–1.31	0.75
CD163 > 75%	0.88	0.61–1.26	0.48	0.72	0.48–1.09	0.12
Panel C: Combined outcome						
hsCRP 25–75%	0.81	0.65–1.01	0.06	0.83	0.64–1.06	0.14
hsCRP > 75%	0.88	0.68–1.14	0.32	0.76	0.55–1.03	0.08
IL-6 25–75%	0.93	0.74–1.16	0.52	0.94	0.73–1.21	0.63
IL-6 > 75%	0.87	0.67–1.13	0.30	0.84	0.62–1.14	0.27
TNF-α 25–75%	0.89	0.72–1.10	0.29	0.97	0.76–1.24	0.81
TNF-α > 75%	0.95	0.73–1.24	0.71	1.03	0.76–1.40	0.84
CD163 25–75%	0.98	0.78–1.22	0.83	0.96	0.75–1.22	0.73
CD163 > 75%	0.94	0.72–1.22	0.62	0.84	0.62–1.14	0.26

Notes: The table shows association between the four biomarkers and DR. Outcomes are development of any DR (*Panel A*), progression of DR (*Panel B*), and our combined outcome (*Panel C*). Reference groups defined as the lowest quartile of the given biomarker. The adjusted regressions control for demographic information (age, sex), diabetes and lifestyle characteristics (diabetes duration, HbA1c, smoking status, alcohol consumption, waist-hip ratio), whether the patient had any previous CVD (defined as a prior diagnosis of myocardial infarction, stroke, atrial fibrillation, heart failure, or peripheral arterial disease), renal function (eGFR, UACR), blood pressure (systolic, diastolic), lipids (total cholesterol, HDL, LDL, triglycerides), and medication (antidiabetic, antihypertensive, lipid lowering).

including the interaction term, none of our estimates are significantly different from one, with the smallest p-value being 0.12).

4. Discussion

In this large prospective cohort study of 3,363 individuals with recently diagnosed type 2 diabetes, we found no associations between baseline circulating levels of inflammatory biomarkers (hsCRP, IL-6, TNF-α, and soluble CD163) and the presence, development, or progression of DR. These findings are in contrast with prior studies reporting associations between systemic inflammatory biomarkers and DR. A *meta*-analysis from 2021 based on 144 cross-sectional studies including both participants with type 1 and type 2 diabetes concluded that circulating levels of hsCRP, IL-6, and TNF-α were positively associated with DR presence or severity, with TNF-α showing the most robust association across all DR stages. [18] Similarly, another *meta*-analysis of cross-sectional studies from 2022, comparing patients with PDR to patients with NPDR, found elevated levels of inflammatory biomarkers, including IL-6 and TNF-α, in both the vitreous and serum from patients with PDR. [30] Higher levels of soluble CD163, a marker of macrophage activation, has also been associated with long-term DR progression in patients with type 1 diabetes, [20] and in a cross-sectional study, higher soluble CD163 levels were associated with microvascular complications in patients with type 1 or type 2 diabetes. [31].

Collectively, these studies suggest that increased levels of circulating inflammatory biomarkers are associated with the presence of DR, aligning with known local involvement of low-grade inflammation and endothelial activation in the pathogenesis of DR. [13–16] However, systemic low grade inflammation may more strongly reflect acute phases of DR development or ongoing disease activity and might be less relevant as a long-term predictor in patients with recently diagnosed type 2 diabetes. Many previous studies exploring hsCRP's association with DR employed case-control or cross-sectional designs, with a *meta*-analysis from 2015 concluding that CRP is correlated with severity of DR. [30] However, results are conflicting. A study investigating the association between plasma levels of hsCRP and DR in long-term type 1 diabetes patients found that patients with higher levels of hsCRP were more likely to have PDR in an age- and sex-adjusted model, although this disappeared when adding additional covariates. [32] Likewise, in the EURODIAB study, [33] the association between CRP and DR decreased after adjusting for body mass index (BMI).

More recent studies have also failed to confirm an association between circulating inflammatory biomarkers and DR. The TODAY study, which followed young patients with type 2 diabetes for 2–6.5 years, found no associations between inflammatory biomarkers, including hsCRP, IL-6, and TNF-α, and DR. [34] Similarly, a study in adults with type 2 diabetes reported no associations between circulating inflammatory biomarkers and presence of DR; notably, IL-6 levels were slightly lower in patients with DR than in those without. [35].

4.1. Potential explanations for discrepancies

The mixed findings in the literature may be attributed to several factors including study populations, disease stages, and methodologies. Our study focused on individuals with recently diagnosed type 2 diabetes, where the presence of DR was relatively rare and levels of DR at baseline when present were low, which may limit the detectability of systemic inflammation as a predictive factor. The role of systemic inflammation in DR might differ in patients with longer diabetes duration or more advanced disease stages. Moreover, many previous studies relied on cross-sectional or case-control study designs, which may limit generalizability. For example, the *meta*-analysis by Storti et al. [18] included a large number of predominantly case-control studies. For TNF-α alone, 65 studies were included of which 58 were case-control studies and with over half of the patients with type 2 diabetes originating from Asia, making the overall population in this *meta*-analysis considerably different from our study population. This, combined with limited information on diagnostic criteria for DR staging, may account for differences. Similarly, in the *meta*-analysis by Song et al. [30] investigating hsCRP in patients with type 2 diabetes, the control group comprised both healthy individuals *and* patients with type 2 diabetes (but without DR). Inclusion of healthy individuals in the control group thereby contributed to lower levels of hsCRP, likely influencing the result of the study.

In contrast to many previous studies, our prospective design allowed assessment of biomarker levels before DR developed or progressed. Cross-sectional studies may reflect reverse causation – capturing inflammation as a consequence rather than a predictor of microvascular disease. Furthermore, participants in our study had short diabetes duration, with most DR cases being mild. The role of systemic inflammatory biomarkers in DR may become more important in more advanced stages of type 2 diabetes. As mentioned, the TODAY study, also in early-stage type 2 diabetes patients, similarly found no association between systemic inflammatory markers and DR. [34] Additionally, we adjusted for a wide range of confounders including glycemic control, blood pressure, and renal function – factors that may partly explain positive associations observed in less rigorously controlled studies.

Variations in sample timing relative to disease progression may also contribute to inconsistent findings. Systemic biomarkers measured at a single point of time may not fully capture the dynamic nature of inflammation in patients with DR. In addition, some variation may be due to a range of confounding factors not taken into account in the various studies.

4.2. Systemic versus local inflammations

Systemic biomarkers like hsCRP and TNF-α may not fully capture local inflammatory activity within the retinal microenvironment. DR pathophysiology involves complex processes, including oxidative stress, endothelial dysfunction, and localized retinal inflammation. Evidence suggests that local production of proinflammatory cytokines within the eye may play a more significant role in DR development than systemic inflammation. [36,37] In early stages of DR, inflammation may be confined to the retina and not reflected in circulating biomarker levels. By contrast, systemic markers may rise only in more advanced disease, where inflammation becomes generalized. This compartmentalization could explain the lack of systemic signal in our early-stage cohort. Future studies utilizing retinal-specific biomarkers or advanced imaging modalities might provide deeper insights into DR progression.

4.3. Strengths and limitations

The strengths of our study include its large sample size, prospective design, and the use of national registries, ensuring high-quality data. The near-complete and long-term follow-up enabled robust assessments of biomarker associations with DR incidence and progression. Additionally, DR diagnoses were conducted by practicing ophthalmologist or at designated hospitals following national guidelines, [27] enhancing the reliability of our findings. Further, a recent validation study showed high agreement between graders. [28].

However, limitations should be acknowledged. First, inflammatory biomarkers were measured only at baseline, precluding analysis of temporal changes in inflammation. Repeated measurements might offer insight into dynamic inflammatory processes. Second, while we adjusted for some key confounders, unmeasured factors such as diet and physical activity may have influenced our results. Third, our findings may not generalize to populations with longer diabetes duration or more advanced DR, where inflammation may play a larger role. Fourth, not all individuals in our database received any eye examinations following our study start (one year after enrollment into DD2), thus excluding those individuals from our analyses. These individuals may be systematically

different, e.g., in terms of their socioeconomic background; see the discussion in Thykjær et al. [38] To investigate this, Supplementary Table 5 reports summary statistics for included patients and for patients excluded due to lack of any follow-up eye examination. Characteristics were broadly comparable between included and excluded participants. However, excluded participants had a slightly more adverse cardiometabolic risk profile, including less frequent use of lipid-lowering therapy, higher blood pressure, and higher levels of inflammatory biomarkers. We therefore cannot exclude that individuals at higher risk of retinopathy progression were underrepresented in the analytical sample, potentially attenuating observed associations between inflammatory markers and retinopathy outcomes. Fifth, since patients are not continuously screened, our measure of development or progression of DR represents a delayed measurement. Time inevitably passes between a change in a patient's DR status and an eye assessment. This may make it harder for us to measure relationships between our biomarkers and DR development and progression. Finally, since eye measurements do not occur exactly at DD2 enrollment, our baseline measurements no not coincide with the timing of eye biomarker measurements. However, almost all patients have a baseline eye measurement within one year of the date of their DD2 enrollment (see Supplementary Fig. 2), and the median number of days between DD2 enrollment and the closest eye measurement is 32 days (meaning 32 days *after* DD2 enrollment; the first quarter is 71 days *prior* to DD2 enrollment and the third quarter is 146.5 days *after* DD2 enrollment).

5. Conclusions

To conclude, this large prospective cohort study found no associations between baseline levels of circulating systemic inflammatory biomarkers (hsCRP, IL-6, TNF-α, and soluble CD163) and the presence, development, or progression of DR in individuals with recently diagnosed type 2 diabetes. Our findings challenge the assumption that systemic low-grade inflammation, as measured by these biomarkers, is central to early DR pathogenesis and suggests that these systemic inflammatory markers may not be reliable for assessing DR risk in individuals with early-stage type 2 diabetes. Future research should explore the role of local retinal inflammation and other mechanisms in DR pathophysiology to identify more reliable biomarkers to enhance early detection and enable individualized treatment strategies for DR.

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Ethics approval and consent to participate.

The study was approved by the Danish National Committee on Biomedical Research Ethics (record number S-20100082), the Danish Data Protection Agency (record number 2008–58-0035) has approved the DD2 project, and the study was conducted in concordance with the Helsinki Declaration II.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2026.113195.

References

[1] Sofizadeh S, Eeg-Olofsson K, Lind M. Prevalence and risk factors for diabetic retinopathy at diagnosis of type 2 diabetes: an observational study of 77,681 patients from the Swedish National Diabetes Registry. BMJ Open Diabetes Res Care 2024;12(3):e003976. https://doi.org/10.1136/bmjdrc-2023-003976.

[2] Grauslund J, Pedersen FN, Andersen N, et al. Presence and development of diabetic retinopathy in 153,238 patients with type 2 diabetes in the danish Registry of Diabetic Retinopathy. Acta Ophthalmol 2023;101(2):207–14. https://doi.org/10.1111/aos.15012.

[3] Zhang L, Krzentowski G, Albert A, Lefebvre PJ. Risk of developing retinopathy in Diabetes Control and Complications Trial type 1 diabetic patients with good or poor metabolic control. Diabetes Care 2001;24(7):1275–9. https://doi.org/10.2337/diacare.24.7.1275.

[4] Perais J, Agarwal R, Evans JR, et al. Prognostic factors for the development and progression of proliferative diabetic retinopathy in people with diabetic retinopathy. Cochrane Database Syst Rev 2023;2:CD013775. https://doi.org/10.1002/14651858.CD013775.pub2.

[5] UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352 (9131):837-853. Doi: 10.1016/S0140-6736(98)07019-6.

[6] Kohner EM. Diabetic retinopathy and high blood pressure: defining the risk. Am J Hypertens 1997;10(6 Pt 2):181S–S183. https://doi.org/10.1016/S0895-7061(97)00073-1.

[7] Chung YR, Park SW, Choi SY, et al. Association of statin use and hypertriglyceridemia with diabetic macular edema in patients with type 2 diabetes and diabetic retinopathy. Cardiovasc Diabetol 2017;16(1):4. https://doi.org/10.1186/s12933-016-0480-7.

[8] Estacio RO, McFarling E, Biggerstaff S, et al. Overt albuminuria predicts diabetic retinopathy in Hispanics with NIDDM. Am J Kidney Dis 1998;31(6):947–53. https://doi.org/10.1053/ajkd.1998.v31.pm9631840.

[9] Wang W, Ji Q, Ran X, et al. Prevalence and risk factors of diabetic peripheral neuropathy: a population-based cross-sectional study in China. Diabetes Metab Res Rev 2023;39(8):e3702.

[10] Yamada K, Ohsugi M, Ito Y, et al. Retrospective database study on risk factors for diabetic retinopathy and diabetic kidney disease in Japanese patients with diabetes mellitus. J Diabetes Investig 2024;15(1):123–30. https://doi.org/10.1111/jdi.14341.

[11] Vujosevic S, Aldington SJ, Silva P, et al. Screening for diabetic retinopathy: new perspectives and challenges. Lancet Diabetes Endocrinol 2020;8(4):337–47. https://doi.org/10.1016/S2213-8587(19)30411-5.

[12] Abou Taha A, Dinesen S, Vergmann AS, Grauslund J. Present and future screening programs for diabetic retinopathy: a narrative review. Int J Retina Vitreous 2024;10(1):14. https://doi.org/10.1186/s40942-024-00534-8.

[13] Antonetti DA, Silva PS, Stitt AW. Current understanding of the molecular and cellular pathology of diabetic retinopathy. Nat Rev Endocrinol 2021;17(4):195–206. https://doi.org/10.1038/s41574-020-00451-4.

[14] Morya AK, Ramesh PV, Nishant P, et al. Diabetic retinopathy: a review on its pathophysiology and novel treatment modalities. World J Methodol 2024;14(4):95–881. https://doi.org/10.5662/wjm.v14.i4.95.

[15] Srejovic JV, Muric MD, Jakovljevic VL, et al. Molecular and cellular mechanisms involved in the pathophysiology of retinal vascular disease—interplay between inflammation and oxidative stress. Int J Mol Sci 2024;25(21):11850. https://doi.org/10.3390/ijms252111850.

[16] Rübsam A, Parikh S, Fort PE. Role of inflammation in diabetic retinopathy. Int J Mol Sci 2018;19(4):942. https://doi.org/10.3390/ijms19040942.

[17] Wu F, Lamy R, Ma D, et al. Correlation of aqueous, vitreous, and plasma cytokine levels in patients with proliferative diabetic retinopathy. Invest Ophthalmol Vis Sci 2020;61(2):26. https://doi.org/10.1167/iovs.61.2.26.

[18] Storti F, Pulley J, Kuner P, et al. Circulating biomarkers of inflammation and endothelial activation in diabetic retinopathy. Transl Vis Sci Technol 2021;10(12):8. https://doi.org/10.1167/tvst.10.12.8.
[19] Semnani-Azad Z, Blanco Mejia S, Connelly PW, et al. The association of soluble CD163, a novel biomarker of macrophage activation, with type 2 diabetes mellitus and its underlying physiological disorders: a systematic review. Obes Rev 2021;22(9):e13257. https://doi.org/10.1111/obr.13257.
[20] Hector S, Thulesius HO, Landin-Olsson M, et al. Soluble CD163 and glycated haemoglobin were independently associated with the progression of diabetic retinopathy in adult patients with type 1 diabetes. BMJ Open Ophthalmol 2023;8(1):e001314. https://doi.org/10.1136/bmjophth-2023-001314.
[21] Kristensen FP, Nicolaisen SK, Nielsen JS, et al. The danish Centre for Strategic Research in Type 2 Diabetes (DD2) Project Cohort and Biobank from 2010 through 2023—a cohort profile update. Clin Epidemiol 2024;16:641–56. https://doi.org/10.2147/CLEP.S392393.
[22] Domazet SL, Olesen TB, Stidsen JV, et al. Low-grade inflammation in persons with recently diagnosed type 2 diabetes: the role of abdominal adiposity and putative mediators. Diabetes Obes Metab 2024;26(6):2092–101. https://doi.org/10.1111/dom.15514.
[23] Christensen H, Nielsen JS, Sørensen KM, et al. New national biobank of the danish center for strategic research on type 2 diabetes (DD2). Clin Epidemiol 2012;4:37–42. https://doi.org/10.2147/CLEP.S33042.
[24] Holt CB, Østergaard JA, Thiel S, et al. Circulating lectin pathway proteins do not predict short-term cardiac outcomes after myocardial infarction. Clin Exp Immunol 2019;198(1):94–100. https://doi.org/10.1111/cei.13315.
[25] Gedebjerg A, Bjerre M, Kjaergaard AD, et al. CRP, C-peptide, and risk of first-time cardiovascular events and mortality in early type 2 diabetes: a danish cohort study. Diabetes Care 2023;46(5):1037–45. https://doi.org/10.2337/dc22-1234.
[26] Andersen N, Hjortdal JØ, Schielke KC, et al. The danish registry of diabetic retinopathy. Clin Epidemiol 2016;8:613–9. https://doi.org/10.2147/CLEP.S113650.
[27] Grauslund J, Andersen N, Andresen J, et al. Evidence-based danish guidelines for screening of diabetic retinopathy. Acta Ophthalmol 2018;96(8):763–9. https://doi.org/10.1111/aos.13936.
[28] Thykjær AS, Stokholm L, Kawasaki R, et al. Inter grader agreement in the danish screening program for diabetic retinopathy. Invest Ophthalmol Vis Sci 2023;101(7):783–8. https://doi.org/10.1111/aos.15667.
[29] Wilkinson CP, Ferris 3rd FL, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 2003;110(9):1677–82. https://doi.org/10.1016/S0161-6420(03)00873-1.
[30] Song J, Chen S, Liu X, et al. Relationship between C-reactive protein level and diabetic retinopathy: a systematic review and meta-analysis. PLoS One 2015;10(12):e0144406. https://doi.org/10.1371/journal.pone.0144406.
[31] Siwan E, Parry SN, Williams KH, et al. Circulating soluble CD163 as a potential biomarker of diabetes complications. J Diabetes Complications 2023;37(8):108525. https://doi.org/10.1016/j.jdiacomp.2022.108525.
[32] Laursen JVN, Hoffmann SS, Green A, et al. Associations between diabetic retinopathy and plasma levels of high-sensitive C-reactive protein or von Willebrand factor in long-term type 1 diabetic patients. Curr Eye Res 2013;38(1):174–9. https://doi.org/10.3109/02713683.2012.733048.
[33] Schram MT, Chaturvedi N, Schalkwijk CG, et al. EURODIAB prospective Complications Study Group. Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes—the EURODIAB prospective Complications Study. Diabetologia 2005;48:370–8. https://doi.org/10.1007/s00125-004-1628-8.
[34] Levitsky LL, Drews KL, Haymond M, et al. TODAY Study Group. the obesity paradox: retinopathy, obesity, and circulating risk markers in youth with type 2 diabetes in the TODAY study. J Diabetes Complications 2022;36(11):108259. https://doi.org/10.1016/j.jdiacomp.2022.108259.
[35] Chen X, Zhang X, Gong Z, et al. The link between diabetic retinal and renal microvasculopathy is associated with dyslipidemia and upregulated circulating level of cytokines. Front Public Health 2023;10:1040319. https://doi.org/10.3389/fpubh.2022.1040319.
[36] Saucedo L, Pfister IB, Schild C, Garweg JG. Association of inflammation-related markers and diabetic retinopathy severity in the aqueous humor, but not serum of type 2 diabetic patients. PLoS One 2023;18(10):e0293139. https://doi.org/10.1371/journal.pone.0293139.
[37] Sepah YJ, Do DV, Mesquida M, et al. READ-3 investigators. Aqueous humour interleukin-6 and vision outcomes with anti-vascular endothelial growth factor therapy. Eye (Lond) 2024;38:1–7. https://doi.org/10.1038/s.
[38] Thykjær AS, Andersen N, Bek T, et al. Attendance in a national screening program for diabetic retinopathy: a population-based study of 205,970 patients. Acta Diabetol 2022;59(11):1493–503. https://doi.org/10.1007/s00592-022-01946-4.