Bacterial infections as novel risk factors of severe diabetic retinopathy in individuals with type 1 diabetes

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ABSTRACT

Background/Aims Diabetic retinopathy (DR) is associated and shares many risk factors with other diabetic complications, including inflammation. Bacterial infections, potent inducers of inflammation have been associated with the development of diabetic complications apart from DR. Our aim was to investigate the association between bacterial infections and DR.

Methods Adult individuals with type 1 diabetes (n=1043) were recruited from the Finnish Diabetic Nephropathy Study (FinnDiane), a prospective follow-up study. DR was defined as incident severe diabetic retinopathy (SDR), identified as first laser treatment. Data on DR were obtained through fundus photographs and medical records, data on bacterial infections from comprehensive national registries (1 January 1995 to 31 December 2015). Risk factors for DR and serum bacterial lipopolysaccharide (LPS) activity were determined at baseline.

Results Individuals with incident SDR (n=413) had a higher mean number of antibiotic purchases/follow-up year compared with individuals without incident SDR (n=630) (0.92 [95% CI 0.82 to 1.02] vs 0.67 [0.62–0.73], p=0.02), as well as higher levels of LPS activity (0.61 [0.58–0.65] vs 0.56 [0.54–0.59] EU/mL, p=0.03). Individuals with on average ≥1 purchase per follow-up year (n=269) had 1.5 times higher cumulative incidence of SDR, compared with individuals with <1 purchase (n=774) per follow-up year (52% vs 35%, p<0.001). In multivariable Cox survival models, the mean number of antibiotic purchases per follow-up year as well as LPS activity were risk factors for SDR after adjusting for static confounders (HR 1.16 [1.05–1.27], p=0.002 and HR 2.77 [1.92–3.99], p<0.001, respectively).

Conclusion Bacterial infections are associated with an increased risk of incident SDR in type 1 diabetes.

INTRODUCTION

Diabetic retinopathy (DR) is the most common diabetic complication as well as the leading cause of vision loss in working age individuals.[1] DR shares many risk factors with other complications of diabetes, including poor glycaemic control, long duration of diabetes, hypertension and dyslipidemia.[2][3] Consequently, DR strongly associates with other late chronic diabetic complications. DR has been associated with diabetic kidney disease (DKD), through its association with reduced estimated glomerular filtration rate (eGFR) and macroalbuminuria,[4][5] and has been shown to serve as a predictor of DKD.[6] DR, especially proliferative DR, is further associated with macrovascular complications, and has been demonstrated to greatly increase the risk of cardiovascular morbidity and mortality.[7–9] Of note, the increased risk of cardiovascular disease attributed to proliferative DR has previously been observed to be independent of common cardiovascular risk factors, suggesting other potentially unknown, common pathogenic mechanisms.[10]

During the last two decades, an increasing amount of data have demonstrated how inflammatory processes participate in the development and progression of DR.[10–12] Experimental studies have shown that leucocytes accumulate and adhere to cells within the retina, a key process in the development of DR, already within 1 week of the onset of diabetes.[13][14] Inhibition of leucocyte adhesion further prevents retinal endothelial cell injury and death, both clinical hallmarks of DR.[15] Furthermore, individuals with DR exhibit higher levels of inflammatory markers in ocular tissues, compared with non-diabetic individuals, and the concentration of these cytokines increases as DR progresses.[16]

Bacterial infections are potent inducers of inflammation and are thought to play a role in the pathogenesis of macrovascular disease, in part through their acceleration of atherosclerosis by induction of inflammation.[17–19] Additionally, infections are associated with microvascular disease and are a common cause of acute kidney injury.[20] In line with these data, we have previously found that the number of bacterial infections correlate with both the development and progression of diabetic nephropathy.[21] Moreover, increased serum levels of bacterial lipopolysaccharides (LPS, endotoxemia), membrane components of gram-negative bacteria, have previously been associated with both the development and progression of DKD, as well as incident cardiovascular disease in individuals with type 2 diabetes.[22][23]

DR associates and shares many risk factors with other late complications of diabetes. As bacterial infections and endotoxemia have been associated with both DKD and cardiovascular disease in individuals with diabetes, and as inflammation plays a substantial role in the pathogenesis of DR, we hypothesised that bacterial infections and increased levels of LPS-activity may also be associated with the development of DR.

MATERIALS AND METHODS
Phenotype definition

The present study is part of the FinnDiane Study (the Finnish Diabetic Nephropathy Study), an ongoing nationwide multicenter study, started in 1997 to uncover risk factors for the chronic complications of type 1 diabetes. FinnDiane collects medical data on individuals with type 1 diabetes throughout Finland and the protocol is in accordance with the Declaration of Helsinki, further approved by the local ethics committees at each study centre as well as the Helsinki and Uusimaa Health District. Study subjects sign an informed consent prior to participation. At the baseline visit, subjects undergo a physical examination and the attending physician completes a standardised questionnaire, on which data on the presence of diabetic complications, comorbidities and medication are collected. Urinary and blood samples are collected for the detection of diabetic nephropathy and the measurement of relevant clinical laboratory parameters. After the baseline visit, subjects are prospectively followed with consecutive visits.

Type 1 diabetes was defined as an age at onset of diabetes <40 years and permanent insulin treatment started within 1 year after the diagnosis of diabetes. In a subset of FinnDiane-subjects (n=1983), data on the presence and severity of DR both before and after the baseline visit, were acquired by evaluation of retinal fundus photographs and medical records (1 January 1986 to 9 October 2010) by ophthalmologists and scored according to the Early Treatment Diabetic Retinopathy (ETDRS) 12 step severity scale. In the present study, DR was defined as severe diabetic retinopathy (SDR), identified as incident retinal laser treatment. Individuals without SDR were identified as individuals with no laser treatment during follow-up and with an ETDRS score of <30 at or after the baseline visit (figure 1). Individuals with a history of laser treatment before the baseline were excluded from the study. Data on laser treatment were retrieved from two sources: the standardised questionnaire completed at baseline and registered laser treatments within the national Finnish Hospital Discharge Register (HILMO, Finnish Care Register for Health Care, data available from 1 January 1986 to 31 December 2015). Laser treatments were identified from the hospital discharge register by using procedure codes based on the Nordic MedicoStatistical Committee [NOMESCO]: CKC10, CKC12, CKC15, CKD40, CKC50, CKD92, CKD93, 3721 and 3724.

Bacterial infections treated in outpatient care were identified using the Finnish National Drug Prescription Register (1 January 1995 to 31 December 2015) as medications with an Anatomical Therapeutic Chemical Classification System-code beginning with J01. This register includes all prescription purchases from pharmacies in Finland. As systemic antibiotics cannot be purchased without a prescription in Finland, the antibiotic purchases seen in this register reflect diagnoses of bacterial infections made by health professionals. Individual antibiotic data were merged with each FinnDiane subject using the personal identity code unique to each resident of Finland. To provide an estimate of the average exposure of antibiotic purchases through-out the follow-up for each individual, the mean number of antibiotic purchases per follow-up year was calculated as the total number of purchases divided by the follow-up time in years, and used as a constant infection risk score in the analyses. Serum LPS activity was measured using the Limulus amoebocyte lysate (LAL) chromogenic end-point assay (Hycult) from the baseline visit serum sample. As the levels of LPS activity can be affected by the time the serum sample has been frozen, the freezing time of each serum sample was used in the adjustment of the LPS activity levels in the analyses where necessary. Data on LPS activity were available for 879 individuals.
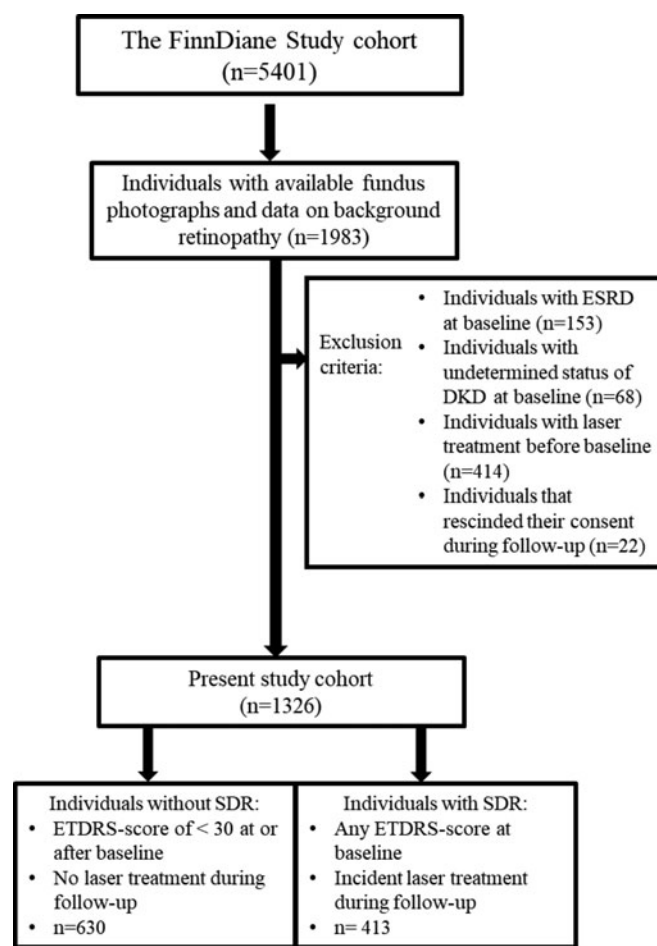

Figure 1 Flow chart of the selection of the cohort phenotypes. DKD, diabetic kidney disease; ESRD, end-stage renal disease.

The following risk factors for DR were included in the analysis: age, sex, duration of diabetes, systolic blood pressure, glycated haemoglobin (HbA_{1c}), body mass index (BMI), history of smoking, eGFR and dyslipidaemia. Data on these risk factors were obtained from measurements taken during the baseline visits. Smoking was used dichotomously, positive if the patient had a history of smoking or was smoking actively. Concentrations of low-density lipoprotein (LDL) were chosen to reflect dyslipidemia as LDL has been robustly associated with DR.[24] eGFR was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. The severity of DKD was assessed by the albumin excretion rate (AER) in at least two out of three overnight or 24 hour urine collections: normal AER (<20 µg/min or <30 mg/24 hour), microalbuminuria (≥20 <200 µg/min or ≥30 <300 mg/24 hour), macroalbuminuria (≥200 µg/min or ≥300 µg/24 hour), and end-stage renal disease (ESRD, defined as dialysis treatment or kidney transplantation). Individuals with ESRD at baseline were excluded due to the substantial effect of ESRD on mortality and susceptibility to infection.[22 25] For individuals who developed ESRD during the follow-up, follow-up years and data collected after the onset of ESRD were censored. After meeting exclusion criteria, 1326 subjects were available for analysis. Follow-up started at the baseline visit and ended when the individual received laser therapy, developed ESRD, died or 31 December 2015. The median length of follow-up was 14.2 years.

Statistical methods

Clinical characteristics are presented as mean ± SD, median (IQR) or percentages. Differences between groups were estimated using the Mann–Whitney U-test, Pearson's χ^2-test or Kruskal–Wallis' test, when appropriate. Cumulative incidence of SDR was estimated by Kaplan–Meier survival analyses, between-group differences were estimated using Pearson's χ^2-test. Further estimation of the impact of the infections and LPS activity on the risk of SDR was accomplished through multivariable Cox proportional hazards models. Results from the Cox models are presented as HRs with 95% CIs. All covariates were tested for univariate association and included in the model if the statistical significance was below 0.05. The potential effect of death and onset of ESRD as competing risks in the survival analysis were assessed using the Fine–Gray method. In the main survival analysis, the stage of DKD was not used as a covariate in order to avoid multicollinearity, however, in a sub-analysis the stage of DKD at baseline was also included.

Three additional sensitivity Cox regression analyses were performed. In the first analysis, individuals with an ETDRS score before baseline were included and longitudinal prospective ETDRS scores available during the follow-up were used as a time-dependent covariate, to adjust the models for existing background DR. Time-weighted average HRs were calculated for the prospective ETDRS scores for specific time windows, which were used to adjust the regression models. The ETDRS score closest to but before the baseline visit was used as the baseline score. The second and third sensitivity analyses were performed to more specifically evaluate the antibiotic purchases as risk factors for SDR. The antibiotic purchases were introduced as dichotomic categorical covariates in two separate regression models: The first stratified the individuals into two groups by the median of the mean number of antibiotic purchases per follow-up year in the entire cohort, the second analysis similarly compared the individuals with purchase frequencies in the lowest tertile versus individuals with purchase frequencies in the highest tertile. All analyses were conducted using the R open source software version 3.5.2 (URL: http://www.r-project.org).

RESULTS

Clinical characteristics of individuals with and without incident SDR

In this cohort of FinnDiane-subjects with available data on DR (n=1326), 413 individuals had incident SDR, while 630 individuals did not receive laser treatment during follow-up and had an ETDRS score <30 at or after the baseline visit (table 1). Individuals with incident SDR had a greater mean number of antibiotic purchases per follow-up year compared with individuals without SDR (0.92 [SD±1.04] vs 0.67 [SD±0.68], p=0.02). The types of antibiotics purchased according to their ATC codes can be found in online supplemental table I. Individuals with incident SDR also had higher mean serum LPS activity (0.62 [95% CI 0.58 to 0.65] vs 0.56 [0.54–0.59] EU/mL, p=0.03). Significant differences for all relevant risk factors for DR were observed between the two groups. Particularly, the distribution of DKD was heavily skewed, as 91% of the individuals without SDR had a normal AER at baseline and only 2% had macroalbuminuria, while only 49% of the individuals with SDR had a normal AER and up to 26% had macroalbuminuria (p<0.001). A significant difference in the distribution between the sexes was also seen as 59.3% of individuals with SDR were males, compared with 49.0% in individuals without SDR (p<0.001).

Effect of antibiotic purchases on the risk of incident SDR

The association between antibiotic purchases and SDR was first assessed by stratifying the individuals into two groups, based on if they had frequent antibiotic purchases (in average at least one purchase per follow-up year, n=269) or infrequent purchases

Table 1 Baseline clinical characteristics of the individuals with type 1 diabetes with (SDR+) and without (SDR−) incident severe diabetic retinopathy

Variables and baseline covariates	SDR−	SDR+	P value
N	630	413	NA
Sex, n (female %)	321 (51.0)	168 (40.7)	0.001*
Age (years)	30.6 (23.3–40.4)	33.7 (26.2–45.7)	<0.001†
Age at onset of diabetes (years)	17.2 (11.0–25.8)	12.1 (7.3–18.4)	<0.001†
Diabetes duration (years)	11.7 (7.4–18.7)	20.4 (14.5–28.2)	<0.001†
Normal AER, n (%)	576 (91.4)	203 (49.2)	
Microalbuminuria, n (%)	41 (6.5)	102 (24.7)	<0.001*
Macroalbuminuria, n (%)	13 (2.1)	108 (26.1)	
Onset of ESRD, n (%)	8 (1.3%)	23 (5.6%)	<0.001*
Systolic blood pressure (mm Hg)	127±14	136±19	<0.001†
Diastolic blood pressure (mm Hg)	77±9	81±10	<0.001†
Body mass index (kg/m²)	24.7±3.4	25.4±3.7	<0.001†
eGFR (mL/min/1.73 m²)	109 (96–120)	102 (82–116)	<0.001†
History of smoking, n (%)	254 (40.3)	208 (50.4)	0.001*
LDL-cholesterol (mmol/L)	3.08±0.85	3.53±0.94	<0.001†
Follow-up (years)	16.6 (14.2–17.5)	6.7 (2.6–11.1)	<0.001†
Deaths (n)	9	0	NA
LPS activity (EU/mL)	0.50 (0.36–0.69)	0.54 (0.37–0.81)	0.03†
Mean number of antibiotic purchases per follow-up year	0.67±0.68	0.92±1.04	0.02†

*Pearson's χ^2-test.
†Mann-Whitney U-test.
AER, albumin excretion rate; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HbA$_{1c}$, glycated haemoglobin; LPS activity, bacterial lipopolysaccharide activity; NA, not applicable; SDR+, individuals with incident severe diabetic retinopathy during follow-up; SDR−, individuals without severe diabetic retinopathy during follow-up and with absent/mild retinopathy at baseline. Data are presented as mean±SD, median (IQR) or percentages where appropriate.

(less than one purchase per year, n=774). Individuals with frequent antibiotic purchases were observed to have a greater risk of incident SDR (figure 2A) and a 1.5 times greater cumulative incidence of incident SDR compared with individuals with infrequent purchases (52% vs 35%, p<0.001). Neither death nor the onset of ESRD were significant competing risks using the Fine–Gray method (Gray's test: p=0.1).

The impact of the antibiotic purchases on the risk of incident SDR was further assessed using three separate multivariable Cox regression models with increasing numbers of covariates (figure 2B). The first model was the unadjusted HR of the infection covariate. The second model further included age, sex and diabetes duration. The third and fully adjusted model further included in addition to the covariates in the second model: history of smoking, systolic blood pressure, eGFR, BMI, LDL concentrations and HbA_{1c}. The mean

number of antibiotic purchases per follow-up year was found to be a significant risk factor for incident SDR, after adjusting for age, sex and diabetes duration with a HR of 1.16 (1.05–1.27, p=0.002). This corresponds clinically to a 16% higher risk of incident SDR for each annual antibiotic purchase. In the fully adjusted model, however, including all risk factors for DR, this HR was non-significant, although in the same direction (HR 1.09 [0.98–1.21], p=0.11).

Effect of bacterial LPS activity on the risk of incident SDR

The association between LPS activity and incident SDR was assessed by stratifying the individuals into quartiles, based on the IQR of the LPS activity (figure 3A): low LPS activity (LPS <0.36, n=203), moderately low LPS activity (LPS ≥0.36– <0.51, n=233), moderately high LPS (LPS ≥0.51–<0.73, n=216) and finally high LPS activity (LPS ≥0.73 EU/mL,
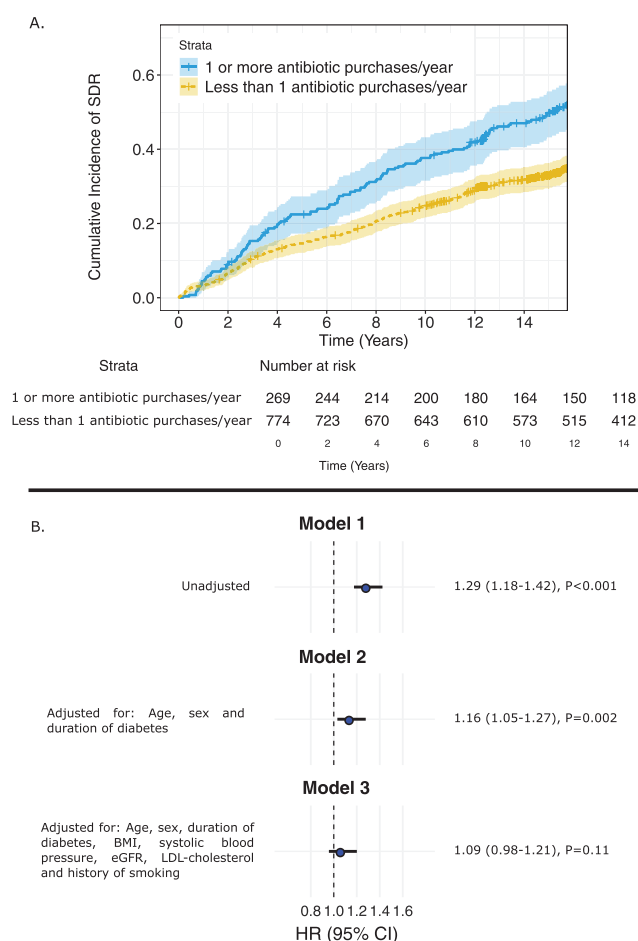
Figure 2 The impact of antibiotic purchases on the risk of incident severe diabetic retinopathy. (A) Kaplan–Meier cumulative incidence curves for incident severe diabetic retinopathy over a follow-up of 15 years in individuals with type 1 diabetes and frequent antibiotic purchases (≥1 antibiotic purchase in average/year, n=269) and infrequent purchases (<1 antibiotic purchase in average/year, n=774). (B) Forest plot portraying results from multivariable Cox proportional hazards regression models with the mean number of antibiotic purchases per follow-up year as main covariate, adjusted for relevant risk factors of diabetic retinopathy. BMI, body mass index; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein cholesterol; HbA_{1c}, glycated haemoglobin; SDR, severe diabetic retinopathy.
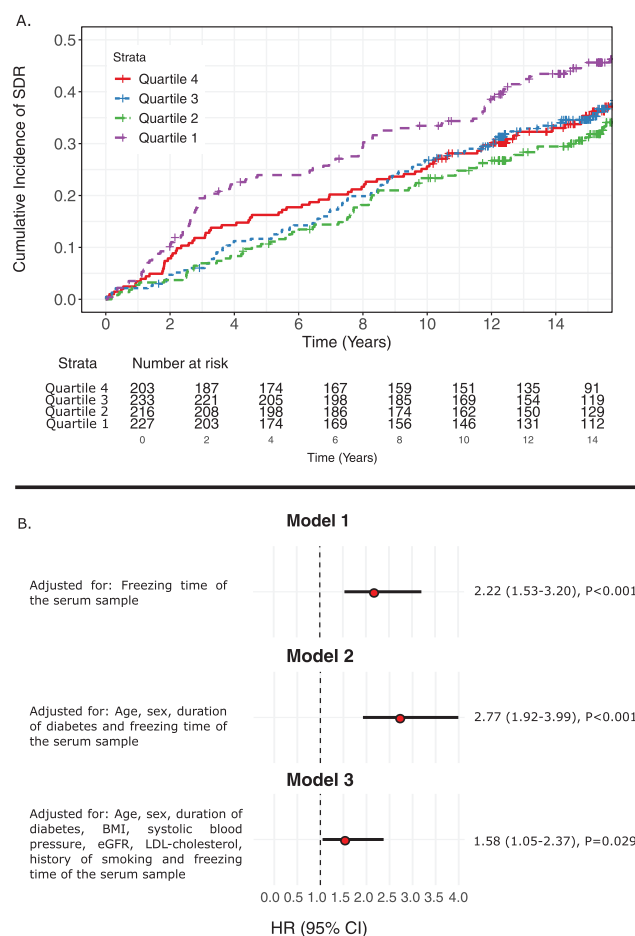
Figure 3 The impact of bacterial lipopolysaccharide activity on the risk of incident severe diabetic retinopathy. (A) Kaplan–Meier cumulative incidence curves for incident severe diabetic retinopathy over a follow-up of 15 years in individuals with type 1 diabetes stratified into quartiles based on their bacterial lipopolysaccharide activity: high LPS activity (Q1, LPS ≥0.73), moderately high LPS (Q2, LPS ≥0.51–<0.73), moderately low LPS activity (Q3, LPS ≥0.36–<0.51), and finally low LPS activity (Q4, LPS<0.36 EU/mL). (B) Forest plot portraying results from multivariable Cox proportional hazards regression models with the mean number of antibiotic purchases per follow-up year as main covariate, adjusted for relevant risk factors of diabetic retinopathy. BMI, body mass index; eGFR, estimated glomerular filtration rate; LPS, bacterial lipopolysaccharide activity; LDL, low-density lipoprotein cholesterol; HbA_{1c}, glycated haemoglobin; SDR, severe diabetic retinopathy.

n=227). Individuals in the highest quartile with the highest LPS activity had the greatest risk and cumulative incidence of incident SDR (46.7% [95% CI 40.1 to 53.2]) compared with all other groups: that is, the group with low LPS activity (36.5% [29.8–43.1], p=0.04), moderately low LPS activity (36.9% [30.7–43.2], p=0.04) and moderately high LPS (35.2% [28.8–41.6], p=0.02). No significant differences in cumulative incidence of SDR were observed between the other groups. No significant competing risks were either found in these analyses (Gray's test: p=0.44).

In multivariable Cox regression analyses, LPS activity was a significant risk factor for incident SDR (figure 3B). The HR for LPS activity was 2.77 (95% CI 1.92 to 3.99, p<0.001) when adjusting for sex, age and duration of diabetes at baseline. Further adjusting the LPS activity for HbA_{1c}, systolic blood pressure, eGFR, history of smoking, BMI and LDL-cholesterol reduced the HR although it remained significant (HR 1.58 [1.05–2.37], p=0.029). Importantly, this risk was still significant also after adjusting for the stage of DKD at baseline (online supplemental figure I).

Sensitivity analyses

In a subset of subjects with confirmed ETDRS scores before the baseline visit (n=680) prospective, longitudinal ETDRS scores were used as time-dependent covariates in two separate Cox regression models, containing the mean number of antibiotic purchases per follow-up year and LPS activity, respectively (table 2). Due to the substantial effect of this adjustment for background DR, the number of covariates were decreased in these models to static covariates (age, sex and duration of diabetes) as well as the time-weighted average HRs of the prospective ETDRS scores. In these analyses, the mean number of antibiotic purchases per follow-up year was not a significant risk factor (HR 1.05 [0.93–1.18], p=0.43), although LPS activity remained as a significant risk factor for incident SDR (HR 1.63 [1.02–2.60], p=0.04).

In further sensitivity analyses, with stratification according to the median as well as the intertertile range of the mean number of antibiotic purchases per follow-up year, Cox regression analyses

Table 2 Cox regression model results demonstrating (A) the mean number of antibiotic purchases per follow-up year, and (B) LPS activity, as risk factors for incident severe diabetic retinopathy with prospective, longitudinal ETDRS scores as a time-dependent covariate included in the models to adjust for background retinopathy

Covariate	HR (95% CI)	P value
A		
Mean number of antibiotic purchases per follow-up year	1.05 (0.93–1.18)	0.43
Age (years)	1.00 (0.98–1.01)	0.46
Duration of diabetes (years)	0.96(0.94–0.98)	**<0.001**
Sex	0.84 (0.65–1.0.8)	0.17
Longitudinal ETDRS scores*	1.07 (1.06–1.07)	**<0.001**
B		
LPS activity (EU/mL)	1.63 (1.02–2.60)	**0.04**
Freeze time (years)	0.97 (0.94–1.00)	0.06
Age (years)	1.00 (0.98–1.01)	0.26
Duration of diabetes (years)	0.96 (0.95–0.98)	**<0.001**
Sex	0.88 (0.68–1.15)	0.36
Longitudinal ETDRS scores*	1.06 (1.05–1.07)	**<0.001**

*Longitudinal scores used as a time-dependent covariate in the model. Bold values indicates P<0.05.
LPS activity, bacterial lipopolysaccharide activity; HR, hazard ratio; ETDRS, Early Treatment Diabetic Retinopathy Study.

demonstrated that individuals with an antibiotic purchase frequency above the median (n=521, mean number of annual antibiotic purchases >0.52) had a 1.2 times higher risk of incident SDR (HR 1.24 [95% CI 1.01 to 1.51], p=0.03) compared to individuals with purchase frequencies below the median (n=522, mean number of annual antibiotic purchases <0.52), after adjusting for sex, age and duration of diabetes. However, in corresponding regression models, when comparing individuals with antibiotic purchase frequencies in the highest tertile (mean number of antibiotic purchases/follow-up year >0.79, n=347) versus individuals in the lowest tertile (mean number of antibiotic purchases/follow-up year <0.32, n=348), no significant associations were seen (HR 1.23 [0.97 to 1.56], p=0.09).

DISCUSSION

As DR has been associated with inflammation and as bacterial infections and endotoxemia have been associated with other chronic complications of diabetes,[4–6 16 17 20 26] we hypothesised that bacterial infections and endotoxemia may associate with and even serve as risk factors for DR. We found that individuals with incident SDR had a higher mean number of antibiotic purchases per follow-up year compared with those who never developed SDR. We further found that individuals with on average at least one antibiotic purchase per follow-up year had a 1.5 times greater cumulative incidence of SDR compared with individuals with less frequent purchases, and that each annual antibiotic purchase increased the risk of incident SDR by 16%. Bacterial LPS activity proved to be an independent and significant risk factor for incident SDR, even after rigorous adjustment for traditional risk factors of SDR. Stratification according to the IQR of LPS activity in all study subjects revealed that this increased risk was mainly attributed to individuals with LPS activity in the highest quartile. Sensitivity analysis further demonstrated that individuals with antibiotic purchase frequencies above the median had a 1.2 times higher risk of incident SDR compared with individuals with purchase frequencies below the median. However, in stratifications according to the highest and lowest intertertile ranges, no significant associations between antibiotic purchase frequencies and incident SDR were observed. Although, of note, the latter analysis was potentially prone to insufficient statistical power as the size of the sample size was reduced by a third compared to the more robust main analysis or the former sensitivity analysis, where the association was more clearly seen.

The use of the drug prescription purchase register and the hospital discharge register offered an efficient method to assess infections treated in outpatient care as well as laser therapy for DR nationwide. The registries do however contain some limitations. The Finnish National Drug Prescription Register only specifies the compound that was prescribed and purchased, not the indication nor the infection for which it was prescribed. However, as previous research has shown that regardless of infection site, infections may accelerate chronic inflammatory processes, for example, atherosclerosis, through the induction and secretion of systemically circulating pro-inflammatory cytokines,[17] this limitation may be less relevant in this study if peripheral infections could in a similar manner contribute to the local inflammatory process in DR. Thus, our findings possibly demonstrate how peripheral infections increase the risk of diabetic complications where increased chronic inflammatory processes play a role in the aetiology of the disease.

In the present study, we found that increased levels of LPS-activity were a significant and independent risk factor for incident SDR. Previous research have found that LPS is to a large extent

dependent on cytokine interleukin (IL)-18 receptor signalling pathways and associated with NLRP3 inflammasome activation.[27 28] Of note, both IL-18 and NLRP3 have previously been associated with DR, and IL-18 has been shown to play a role in angiogenesis in retinal degenerative diseases.[29 30] The source of endotoxemia is however still uncertain. Studies have shown that translocation of LPS into the bloodstream can occur in the gut due to microbial dysbiosis and defects in the intestinal barrier, leading to endotoxemia,[31] or in the oral cavity where gram-negative flora is abundant and where superficial blood vessels may easily bleed during gingivitis, offering a path for LPS into the circulation.[22] But whether peripheral infections with gram-negative pathogens, for example, urinary tract infections can cause endotoxemia is unclear. Although interestingly, studies have found that dysbiosis increases endotoxemia,[32] and as recurrent antibiotic treatments may cause dysbiosis in the gut, an interesting possibility is that antibiotics could increase the risk of DR through increased LPS activity, and not solely through the direct effect of either the infection or the inflammatory response of the infection. However, as our study is observational, we can only speculate through which mechanisms bacterial infections and endotoxemia contribute to the pathogenesis of DR.

To conclude, we were able to study the association of bacterial infections and endotoxemia with DR and to demonstrate how both infections and high levels of LPS activity serve as risk factors for severe diabetic retinopathy in individuals with type 1 diabetes. To our knowledge, this association has not been shown previously and in line with previous research demonstrates how bacterial infections associate with the development of late diabetic complications.

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