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Angiographic evidence of proliferative retinopathy predicts neuropsychiatric morbidity in diabetic patients

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ABSTRACT

Introduction: Diabetic retinopathy (DR) is a common vasculopathy categorized as either non-proliferative (NPDR) or proliferative (PDR),characterized by dysfunctional blood-retinal barrier (BRB) and diagnosed using fluorescein angiography (FA). Since the BRB is similar in structure and function to the blood-brain barrier (BBB) and BBB dysfunction plays a key role in the pathogenesis of brain disorders, we hypothesized that PDR, the severe form of DR, is likely to mirror BBB damage and to predict a worse neuropsychiatric outcome.

Methods: A retrospective cohort study was conducted among subjects with diabetes ($N = 2982$) with FA-confirmed NPDR ($N = 2606$) or PDR ($N = 376$). Incidence and probability to develop brain pathologies and mortality were investigated in a 10-year follow-up study. We used Kaplan–Meier, Cox and logistic regression analyses to examine association between DR severity and neuropsychiatric morbidity adjusting for confounders.

Results: Patients with PDR had significantly higher rates of all-cause brain pathologies ($P < 0.001$), specifically stroke ($P = 0.005$), epilepsy ($P = 0.006$) and psychosis ($P = 0.024$), and a shorter time to develop any neuropsychiatric event ($P < 0.001$) or death ($P = 0.014$) compared to NPDR. Cox adjusted hazard ratio for developing all-cause brain impairments was higher for PDR (HR = 1.37, 95% CI 1.16–1.61, $P < 0.001$) which was an independent predictor for all-cause brain impairments (OR 1.30, 95% CI 1.04–1.64, $P = 0.022$), epilepsy (OR 2.16, 95% CI 1.05–4.41, $P = 0.035$) and mortality (HR = 1.35, 95% CI 1.06–1.70, $P = 0.014$).

Conclusions: This is the first study to confirm that angiography-proven microvasculopathy identifies patients at high risk for neuropsychiatric morbidity and mortality.

1. Introduction

Diabetes mellitus (DM) is an emergent, common and costly public health problem. In the United States, 9.3% of the population are estimated to have diabetes, including 21 million people diagnosed and 8.1 million undiagnosed (Centers for Disease Control and Prevention, 2014). Vascular complications including endothelial cells dysfunction and proliferation, diminished perfusion, and increased permeability are central to the pathogenesis and clinical manifestations of DM and commonly progress to end organ damage such as retinopathy, nephropathy and neuropathy (Stumvoll et al., 2005). Diabetic retinopathy (DR), manifested by microvascular leakage and occlusion, is an early consequence in the course of DM and eventually develops in the majority of the patients. DR is standardized and graded according to the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale ("Classification of diabetic retinopathy from fluorescein angiograms. ETDRS report number 11. Early Treatment Diabetic Retinopathy Study Research Group.," 1991). Fluorescein angiography (FA) is the most reliable

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and sensitive imaging method for detecting retinal microangiopathy and vascular leak, and FA imaging enables clinical decision support for treatment considerations and follow-up (Bandello et al., 1999). DR is categorized into two clinical types: non-proliferative and proliferative (NPDR and PDR, respectively). NPDR, the early and less severe stage of DR, is characterized by endothelial injury with dysfunctional blood-retinal brain (BRB) leading to microaneurysms, capillary occlusion, macular edema and result in retinal nonperfusion. PDR, the advanced form of the disease, exhibits abnormal vessel permeability and subsequent severe retinal ischemia with neovascularisation, hemorrhage, fibrosis and/or tractional detachment of the retina. As an extension of the central nervous system (CNS), the retina and the brain share common embryological, anatomical and physiological properties. Accordingly, similar features exist between the BRB and the blood-brain barrier (BBB) (Steuer et al., 2005) and clinical evidence suggest a correlated breakdown of the BRB and BBB under some clinical conditions (Greiner et al., 2015; Ozkan et al., 2014). Accumulating evidence indicate a key role for BBB dysfunction in the pathogenesis of a number of common CNS disorders, including ischemic, hemorrhagic and traumatic brain injuries, vascular dementia, epilepsy and mood disorders (Abbott et al., 2006). The common properties and pathological alterations of small vessels in the brain and retina signify the retina as a 'window' into the brain and suggest that retinal vessel pathology reflects a similar pathology in cerebral vasculature. Retinal pathology has been shown to co-exist in several major CNS disorders including dementia, cognitive dysfunction and brain imaging abnormalities (Cheung et al., 2010; Heringa et al., 2013; Lesage et al., 2009; London et al., 2013). Studies have related damage to the brain vasculature in diabetes as potentially the most significant contributor to psychiatric and cognitive comorbidities (Biessels et al., 2006; Qiu et al., 2014). However, the underlying etiology remains controversial and other mechanisms, including metabolic alterations and inflammatory response have also been suggested (McIntyre et al., 2007). While few studies have reported neurocognitive dysfunction in patients with DR detected by fundus photography alone (Cohen et al., 1997; Crosby-Nwaobi et al., 2013; Ding et al., 2010) there is lack of large scale studies evaluating the association between angiography-proven retinal vasculopathy and CNS morbidity. The purpose of the present study is to investigate whether the severity of retinal vascular pathology, in a well-defined sample of patients with FA-confirmed retinopathy, is associated with increased incidence of CNS complications and mortality and to determine the odds-ratio to develop brain pathology with respect to DR peri-diagnostic period.

2. Methods

2.1. Study subjects and design

We conducted a retrospective cohort study using data collected from the centralized electronic medical records (EMR) database of Clalit Health Services (CHS), the largest health fund in Israel. We analyzed records of 3085 diabetic patients who underwent retinal angiography at a single central eye facility of the southern district of CHS. Of these, 2982 patients were diagnosed with diabetic retinopathy. Eighty-eight cases were diagnosed with type 1 (mean age 46 ± 16 years) and 2894 with type 2 diabetes (mean age 63 ± 11 years). FA images were obtained between January 2005 to December 2012; clinical data (with censoring on death) was documented from a one-year pre-imaging period until August 1, 2014, the end of the follow-up period, in order to track outcomes documented over a contiguous time prior (and close) to the diagnosis of DR. Baseline measures of risk variables were used to study associations of observations taken at one time to subsequent end points. Inclusion criteria for the study were recorded diagnosis of DR and diagnosis of either NPDR or PDR based upon FA imaging. Patients were excluded from the study if they did not fulfill the inclusion criteria. The protocol for this study was approved by the Ethics Committees of Soroka Medical Center and CHS.

2.2. Measures

Data for all subjects were retrieved from CHS EMR central database, integrating administrative and clinical electronic health records, including data from community clinics, hospitals, laboratories, imaging facilities, and pharmacies. Socio-demographic data collected were sex, birth and death dates, country of birth, year of immigration to Israel and marital status. Clinical data included blood pressure monitoring, diagnoses of retinopathy, hypertension, chronic ischemic heart disease (CIHD), congestive heart failure (CHF), atrial fibrillation (AF), peripheral arterial disease (PAD), diabetic nephropathy and neuropsychiatric outcomes including cerebrovascular disease, epilepsy, all-cause dementia, affective and anxiety disorders, parkinsonism, schizophrenia and psychosis. All clinical diagnoses were defined by specified ICD-9 and 10 codes recorded for the first time in the year preceding the FA or until the end of the follow up period. Laboratory data reported from the year prior the FA included HbA_{1c} measurements, lipid profile, albumin/creatinine ratio and the estimated GFR (eGFR), calculated using the Modification of Diet in Renal Disease equation (MDRD). Retinal images were acquired according to a standard clinical protocol as described elsewhere (Bennett, 2001) using a Topcon 50EX camera (Topcon Corporation, Tokyo, Japan; excitation wavelength between 465–490 nm, emission of 520–530 nm). The grades of retinopathy were dichotomized for statistical analysis purposes. All cases were assigned to one of the two DR categories (NPDR vs. PDR) based on FA examinations and interpretation by two fellowship-trained retinal specialists (MS, JL) using OIS WinStation 5000 TM software (Ophthalmic Imaging Systems, Sacramento, CA). Retinopathy severity was determined by the pathological status of the worst affected eye.

2.3. Statistical analysis

Data analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL). Continuous variables are expressed as means $\pm$ SD and were tested by independent samples t-tests. Categorical variables are reported as frequencies and percentages and were tested by $\chi 2$ test. The impact of DR status on the 10-year follow-up probability to develop any neuropsychiatric event and on mortality rates was examined by time-to-event variables using Kaplan–Meier survival analysis and evaluated by using Breslow test. Patients who were diagnosed with any event in the year preceding the FA results were considered at time '0' for the purpose of survival calculation. Cox proportional hazards regression models were built to compare the adjusted risk to develop any neuropsychiatric disorder and mortality in PDR versus NPDR patients. Bivariate correlation and univariate logistic regression used to identify association between the outcome and each of the explanatory variables and to detect potential confounders. Clinically relevant factors previously identified in the literature to be associated with DR and brain diseases including cardiovascular comorbidities, renal function, age and gender were tested for adjustment purpose. For each outcome, all significant factors in the univariate regression models were included in the subsequent multivariable model. Adjusted multivariable binary logistic regression analysis was used to examine the odds ratios (OR) and 95% CIs of PDR for developing all-cause neuropsychiatric disorder (grouped) and specific CNS outcomes. The backward-LR variable selection method was used to eliminate the non-significant factors in the final model. For determination

of the robustness of our conclusions, a modified sensitivity analysis was performed by testing two models of logistic regression. Model I included all significant potential predicting variables but resulted in a dataset containing 1317 patients, a reduction of 55.8% of the original cohort. Model II is a complete case analysis ($N = 2974$) which discards predicting variables with >0.3% missing information, thus not adjusted for abnormal albuminuria, eGFR and total cholesterol. Eight patients did not have a recorded date of birth and were consequently excluded automatically from the regression analysis.

3. Results

The study cohort consisted of 2982 DM patients with a diagnosis of DR following FA, of whom 376 were diagnosed with PDR and 2606 with NPDR. There were no differences between the two groups in terms of sex, age, follow-up duration, and glycemic control (HbA_{1c}). The majority of patients with a valid HbA_{1c} measurement in the year preceding the FA examination had poor glycemic status with a mean HbA_{1c} of 8.6%. Patients with PDR had a higher incidence of all-cause hospitalization, micro- and macrovascular diabetes complications and increased total cholesterol and LDL levels (Table 1).

3.1. Retinopathy status and neuropsychiatric complications

The incidence of neurological and psychiatric outcomes was compared between DR sub-groups (Table 2). In comparison to patients with NPDR, participants with PDR had higher rates of all-cause neuropsychiatric impairments (42.6% vs. 33.3%, $P < 0.001$) and subsequent hospitalization attributed to these complications (23.7% vs. 15.2%, $P < 0.001$). With regard to new diagnoses of specific neuropsychiatric morbidity, significantly higher rates of cerebrovascular accident (CVA) ($P = 0.005$), epilepsy, recurrent seizures or convulsions ($P = 0.006$) and unspecified psychotic disorder ($P = 0.024$) were found in PDR patients compared with the NPDR group. No differences were demonstrated in the rates of affective or anxiety disorders, schizophrenia, brain malignancy or neurodegenerative diseases. The results suggest that diabetic small-vessel disease may underlie the pathophysiology of specific neuropathologies. We thus examined the rates of all-cause neuropsychiatric complications in the sub-group of patients with PDR with respect to other microvasculopathies. A significantly higher incidence of diabetes-associated endothelial pathology was found among PDR patients with any neuropsychiatric diagnosis ($N = 160$), compared to those without ($N = 216$); specifically PAD (43.1% vs. 32.9%, $P = 0.042$), nephropathy (60.6% vs. 49.1%, $P = 0.026$) and abnormal albumin/creatinine ratio (72.0% vs. 64.2%, $P = 0.043$). Table 3 summarizes patients' baseline characteristics and coexisting medical conditions by neuropsychiatric outcomes. Among the patients included, 1029 (34.5%) had at least one CNS complication diagnosed in the year preceding the established diagnosis of DR ($N = 249$), or during the subsequent follow up ($N = 780$). Patients who developed neuropsychiatric comorbidity were older (63.9 ± 11.4 vs. 61.3 ± 11.8 years, $P < 0.001$), had higher prevalence of cardiovascular impairments, higher total cholesterol and poorer renal function. No differences were found in sex, other serum lipids (LDL, HDL, triglycerides) and HbA_{1c} values. The Kaplan–Meier estimated mean time in years to develop any neuropsychiatric event was significantly shorter for patients with PDR compared with NPDR (5.7 vs. 6.3, respectively, Breslow $P < 0.001$). Cox proportional hazard ratio (HR) for developing any neurological or psychiatric events during the study period, adjusted by age, sex and hypertension, was also significantly higher for the PDR group (HR = 1.37, 95% CI 1.16–1.61, $P < 0.001$; Fig. 1A). Univariate regression analysis was used to shortlist possible controlling factors associated with CNS outcomes for adjusted logistic regression models. Interestingly, HBA_{1c} was not a significant predictor for any of the study outcomes. Although dementia and depression outcomes did not vary between DR severities, and diagnosis of PDR was not found to predict these diagnoses in a univariate analysis, a multivariate analysis was carried out given their extensively investigated association with diabetes. In the adjusted multivariable regression models no clear association was demonstrated between PDR and dementia, depression, CVA and psychosis. Participants with PDR were more likely to suffer from at least a single neuropsychiatric event compared to those with NPDR: in the unadjusted univariate logistic regression model, PDR appeared to be a significantly associated risk factor for all-cause neuropsychiatric outcomes (OR 1.48, 95% CI 1.18–1.84, $P < 0.001$). This association was maintained in a multivariable modeling. Two adjusted models of multivariable logistic regression were applied to examine the dependence of all-cause neuropsychiatric outcome on PDR, controlled to other recorded predicting variables. In model I, adjusted for ten independent variables (angiography-proven DR status, age, CIHD, PAD, CHF, AF, hypertension, abnormal albuminuria, eGFR score and high total cholesterol), PDR independently contributed to the prediction of all-cause neuropsychiatric event (OR 1.41, 95% CI 1.02–1.97, $P = 0.042$). In model II which excluded variables with missing observations, PDR remained associated with higher odds of events (OR 1.30, 95% CI 1.04–1.64, $P = 0.022$). PDR was also significantly associated with the diagnosis of epilepsy in both unadjusted logistic regression (OR 2.58, 95% CI 1.28–5.20, $P = 0.008$) and adjusted multivariable regression model (OR 2.16, 95% CI 1.05–4.41, $P = 0.035$).

3.2. Retinopathy status and mortality

A total of 512 participants (17%) died during the 10-year follow-up period. A significantly higher mortality rate was observed in patients with PDR compared with NPDR (22.1% and 16.5%, respectively, $P = 0.007$). Cumulative survival was calculated using Kaplan-Meier method for all-cause mortality. The survival time was significantly different among DR sub-groups, with estimated mean survival of 7.8 years for PDR vs. 7.9 years for NPDR ($P = 0.014$). Multivariate Cox regression was performed to examine the potential relationship between severe small-vessel disease, manifested as PDR, and survival throughout the study. Adjusted by age, sex and hypertension, the analysis showed increased risk of death for patients with PDR (HR = 1.35, 95% CI 1.06–1.70, $P = 0.014$; Fig. 1B).

4. Discussion

Angiography remains the criterion standard and the most reliable diagnostic technique for assessment of retinal circulation and is superior to ophthalmoscopy and fundus photography in the early detection of functional vascular abnormalities such as permeability and blood flow dynamics (Sim et al., 2014). FA provides detailed information on vessel structure and function and allows comprehensive assessment of retinopathy extent and progression. Despite the widespread use of FA, to the best of our knowledge, this is the first study to examine the significance and predictive value of FA-based diagnosis of vascular pathology severity on CNS morbidity. Our results indicate that patients with severe vasculopathy (PDR) are at higher risk for the development of neuropsychiatric morbidity than those with a less severe vasculopathy and suggest that microangiopathy underlies specific brain disorders. Patients with PDR suffered a significantly higher rate of neuropsychiatric burden, including CVA, epilepsy and psychotic disorders, with subsequent related hospitalizations. Interestingly, other pathologies, including dementia, affective and anxiety disorders did not show increased

Table 1
Baseline characteristics of study participants by retinopathy severity.

Baseline characteristics			NPDR (*N* = 2606)	PDR (*N* = 376)	*P* value
Age, years (at FA) (mean ± SD)			62.3 ± 11.7	61.3 ± 11.4	0.112
Gender N (%) (male)			1378 (53.0)	194 (51.6)	0.600
Follow up duration subsequent to the FA, years (median, inter-quartile range)			3.5, 0–5.1	2.4, 0–5.2	0.085
Any hospitalization during follow up N (%)			1801 (69.1)	313 (83.2)	<0.001
Co-morbid conditions N (%)	Hypertension	Recorded diagnosis	1541 (59.1)	227 (73.7)	<0.001
		Systolic >140 mmHg[a]	1249 (47.9)	186 (49.5)	0.576
		Anti-hypertensive drug purchase[a,b]	1956 (75.1)	300 (79.8)	0.046
		Total (combined)	2303 (88.4)	354 (94.1)	0.001
	PAD		514 (19.7)	140 (37.2)	<0.001
	CHF		410 (15.7)	87 (23.1)	<0.001
	Diabetic nephropathy		787 (30.2)	203 (54.0)	<0.001
	CIHD		891 (34.2)	163 (43.4)	0.001
	AF		331 (12.7)	51 (13.60)	0.640
Labs (mean)[a]	Albumin/creatinine ratio (*N* = 1328)	<30	578 (50.0)	62 (35.8)	<0.001
		30–300	400 (34.6)	56 (32.4)	
		>300	177 (15.3)	55 (31.8)	
	Estimated GFR (MDRD) (Mean ± SD) (*N* = 2003)		84.6 ± 31	71.7 ± 33	<0.001
	Estimated GFR (MDRD) N (%) (*N* = 2003)	<15	19 (1.1)	16 (5.3)	<0.001
		15–29	60 (3.5)	20 (6.6)	
		30–59	281 (16.5)	72 (23.8)	
		60–89	606 (35.6)	104 (34.4)	
		≥90	735 (43.2)	90 (29.8)	
	Serum lipid profile (mg/dL) N (%) (*N* = 2109)	Total cholesterol ≥240	868 (48.3)	176 (56.2)	0.010
		LDL ≥190	242 (13.5)	61 (19.5)	0.005
		LDL <100	1716 (95.5)	302 (96.5)	0.450
		HDL <40 for men or <50 for women	1589 (88.5)	273 (87.2)	0.524
		Triglycerides ≥200	1287 (71.7)	220 (70.3)	0.620
	HbA_{1c} N (%) (*N* = 1962)	≤6.5%	177 (10.6)	24 (8.1)	0.202
		6.5–8%	555 (33.3)	91 (30.7)	
		>8%	934 (56.1)	181 (61.1)	

AF: atrial fibrillation; CHF: congestive heart failure; CIHD: chronic ischemic heart disease; FA: fluorescein angiography; GFR: glomerular filtration rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MDRD: modification of diet in renal disease; NPDR: non-proliferative diabetic retinopathy; PAD: peripheral arterial disease; PDR: proliferative diabetic retinopathy; SD: standard deviation. Defined as ACE-I/ARB purchase without CHF background diagnosis or beta-blocker purchase without CIHD/AF/CHF background diagnosis or calcium channel blocker (dihydropyridines) or thiazides, during the year preceding the FA test.

[a] During the year prior the established FA diagnosis.

[b] Defined as ACE-I/ARB purchase without CHF background diagnosis or beta-blocker purchase without CIHD/AF/CHF background diagnosis or calcium channel blocker (dihydropyridines) or thiazides, during the year preceding the FA test.

Table 2
Incidence of neuropsychiatric outcomes and mortality rates by retinopathy.

Variables		NPDR (*N* = 2606)	PDR (*N* = 376)	*P* value
All-cause neuropsychiatric events N (%)		869 (33.3)	160 (42.6)	<0.001
Estimated mean time to develop any neuropsychiatric event, years (SE) Kaplan–Meier survival analysis		6.3 (0.08)	5.7 (0.22)	<0.001
Any hospitalization attributed to newly diagnosed neuropsychiatric disease N (%)		395 (15.2)	89 (23.7)	<0.001
Deaths, all-cause mortality N (%)		429 (16.5)	83 (22.1)	0.007
Estimated mean time to all-cause mortality, years (SE) Kaplan–Meier survival analysis		7.95 (0.06)	7. 86 (0.16)	0.014
Neurologic disease N (%)	Cerebrovascular disease	223 (8.6)	49 (13.0)	0.005
	Epilepsy diagnoses	30 (1.2)	11 (2.9)	0.006
	Peripheral Neuropathy	408 (15.7)	72 (19.1)	0.085
	All-cause dementia	158 (6.1)	17 (4.5)	0.234
	Parkinsonism	53 (2.0)	11 (2.9)	0.265
	Migraine	2 (1.0)	1 (0.3)	0.279
	Brain malignancy	31 (1.2)	6 (1.6)	0.506
	Intra-cranial hemorrhage	24 (0.9)	4 (1.1)	0.788
	Tremor	37 (1.4)	6 (1.6)	0.789
Psychiatric disease N (%)	Psychosis/Unspecified psychotic disorder	50 (1.9)	14 (3.7)	0.024
	Schizophrenia	13 (0.5)	3 (0.8)	0.458
	Anxiety disorders diagnoses[c]	50 (1.9)	9 (2.4)	0.536
	Affective disorder diagnoses[d]	128 (4.9)	19 (5.1)	0.906

NPDR: non-proliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy; SE: standard error.

[c] Include diagnoses of any anxiety disorder, generalized anxiety disorder, PTSD, OCD, panic disorder, agoraphobia, specific phobia.

[d] Include diagnoses of depression, major depressive disorder or any mood disorder.

incidence with more severe microangiopathy. To test for a temporal relationship between retinal vasculopathy and neuropsychiatric disorders, we sought to identify whether CNS events occurrence was increased in the peri-diagnosis period of DR and in the post-diagnostic follow up. We included in the analysis only cases with de novo diagnoses and determined a lag period of one year prior the FA as the uncertainty range. The burden of all-cause CNS illness was significantly higher for PDR, with shorter median time to develop CNS impairments and a significantly lower event-free survival. The greater burden of comorbidities in patients with PDR is further emphasized by the high rate of all-cause hospitalizations among this group.

Our results, showing a higher incidence of epilepsy among PDR patients are consistent with the high degree of comorbidity among vascular disease and epilepsy (often known as 'vascular epilepsy') (Stanimirovic and Friedman, 2012). Recent animal studies on

Table 3
Baseline characteristics of study participants by neuropsychiatric outcomes.

Baseline characteristics			No neuropsychiatric events (*N* = 1953)	Neuropsychiatric events (*N* = 1029)	*P* value
Age, Years (at FA) (mean ± SD)			61.3 ± 11.8	63.9 ± 11.4	<0.001
Gender N (%) (male)			1034 (53.1)	538 (52.3)	0.678
Co-morbid conditions N (%)	Hypertension	Recorded diagnosis	1072 (54.9)	746 (72.5)	<0.001
		Systolic >140 mmHg[a]	879 (45.0)	556 (54.0)	<0.001
		Anti-hypertensive drug purchase[a,b]	1469 (75.2)	787 (76.5)	0.444
		Total (combined)	1695 (86.8)	962 (93.5)	<0.001
	PAD		345 (17.7)	309 (30.0)	<0.001
	CHF		254 (13.0)	243 (23.6)	<0.001
	Diabetic nephropathy		573 (29.3)	417 (40.5)	<0.001
	CIHD		567 (29.5)	478 (46.5)	<0.001
	AF		198 (10.1)	184 (17.9)	<0.001
Labs (mean)[a]	Albumin/creatinine ratio (*N* = 1328)	<30	429 (51.9)	211 (42.1)	0.003
		3–300	264 (31.9)	192 (38.3)	
		>300	134 (16.2)	98 (19.6)	
	Estimated GFR (MDRD) (Mean ± SD) (*N* = 2003)		84.2 ± 32.4	80.4 ± 30.6	0.010
	Estimated GFR (MDRD) N (%) (*N* = 2003)	<15	22 (1.8)	13 (1.6)	0.072
		15-29	42 (3.5)	38 (4.7)	
		30-59	204 (17.0)	149 (18.5)	
		60-89	408 (34.0)	302 (37.6)	
		≥90	523 (43.6)	302 (37.6)	
	Serum lipid profile (mg/dL) N (%) (*N* = 2109)	Total cholesterol ≥240	593 (47.0)	451 (53.2)	0.005
		LDL ≥190	167 (13.2)	136 (16.1)	0.070
		LDL <100	1204 (95.4)	814 (96,1)	0.438
		HDL <40 for men or <50 for women	1109 (87,9)	753 (88.9)	0.473
		Triglycerides ≥200	895 (70.9)	612 (72.3)	0.505
	HbA1c N (%) (*N* = 1962)	≤6.5%	115 (9.8)	86 (110)	0.318
		6.5–8%	402 (34.2)	244 (31.1)	
		>8%	660 (56.1)	455 (58.0)	

AF: atrial fibrillation; CHF: congestive heart failure; CIHD: chronic ischemic heart disease; FA: fluorescein angiography; GFR: glomerular filtration rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MDRD: modification of diet in renal disease; NPDR: non-proliferative diabetic retinopathy; PAD: peripheral arterial disease; PDR: proliferative diabetic retinopathy; SD: standard deviation. Defined as ACE-I/ARB purchase without CHF background diagnosis or beta-blocker purchase without CIHD/AF/CHF background diagnosis or calcium channel blocker (dihydropyridines) or thiazides, during the year preceding the FA test.

[a] During the year prior the established FA diagnosis.

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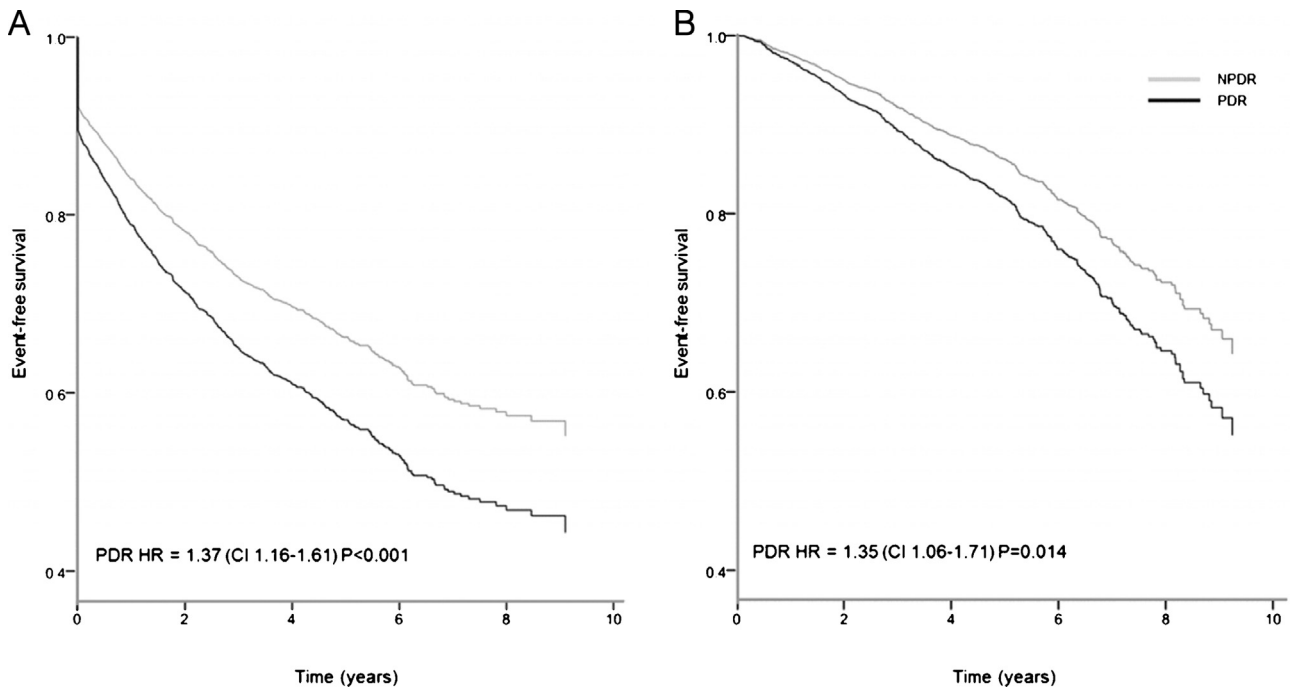

Fig. 1. Adjusted survival curves and hazard ratio derived from Cox proportional hazards regression for (A) all-cause neuropsychiatric events and (B) all-cause mortality.

the mechanism underlying BBB dysfunction in epileptogenesis, highlighted the role of transforming growth factor beta (TGF-β) signaling, mediated by ALK5 activation via Smad2/3 phosphorylation (Bar-Klein et al., 2014; Shlosberg et al., 2010). Interestingly, TGF-β and Smad signaling were shown to play role in the pathogenesis of diabetic nephropathy (Maezawa et al., 2015) and the

complete loop for TGF-β1 signaling via ALK-5 activation and phosphorylation of Smad2/3 was shown in a rat model of diabetic retinopathy (Gerhardinger et al., 2009). Moreover, losartan, an approved angiotensin II type 1 receptor antagonist, was shown to block brain TGF-β signaling, prevent epilepsy (Bar-Klein et al., 2014) and to ameliorate diabetic retinal neurodegeneration (Silva et al., 2009) in rodents. These shared pathologic features suggest that primary vascular pathology, manifested as severe DR, could underlie neuroinflammatory brain response clinically manifested by seizures and thus may be a target for preventive treatment.

Type 1 and 2 diabetes mellitus have been associated with increased risk of dementia and cognitive decline in large-scale studies (Biessels et al., 2006; Smolina et al., 2015). A consistent relation between retinal microvascular changes, dementia and cognitive impairment has been reported (reviewed by: (Heringa et al., 2013)). The rate of dementia in our cohort was 5.8%, slightly lower than 7.1% prevalence of all-cause dementia in a cohort of 438 participants with fundus photography graded retinopathy (Schrijvers et al., 2012). In this study, although a significant association was found between retinopathy and dementia, similarly to our study, no association was found between severity of retinopathy and dementia. The mechanism(s) underlying dementia in diabetes are unclear, and may not be entirely mediated through vascular pathology (Lovestone, 1999). Other explanations for the lack of association between DR severity and dementia include the non-quantitative, dichotomized nature of DR diagnosis, or the slower pathophysiological process, requiring a longer follow-up period. The relatively young age group of our cohort (62.2 ± 11.7 years) may also contribute to the insignificant difference among DR groups as the onset age of neurodegenerative pathologies typically begins at older ages.

Current molecular, genetic and neuroimaging advancements in neuroscience highlight the fundamental congruence between neurological and psychiatric disorders (Baker et al., 2002). An integrative approach merging these fields is evolving in lieu of the historical dichotomization of clinical conditions into strict "neurological" or "psychiatric" types (Arciniegas and Kaufer, 2006). This is the first study that examined the overall burden and time-to development of all-cause CNS pathology in patients with DR. In agreement with previous studies (Jacobson et al., 1985; Karlson and Agardh, 1997) no significant difference in the rate of affective comorbidity was demonstrated between DR subgroups and PDR was not found to predict affective disorders (Table 2). We did observe a higher incidence of diagnosed psychosis, yet again, without independent predictive value for PDR. A recent systemic review highlights the difficulties to provide a clear unidirectional relationship between affective disorders and diabetes (Roy and Lloyd, 2012), and it seems that complex diabetes-depression bidirectional interactions may be involved, consisting of various metabolic stresses, inflammatory response, glucocorticoid signaling, vascular and cellular-level alterations (McIntyre et al., 2007).

Dysglycemia is the only risk factor to be associated with progression of small-vessel disease in comparison to other risk factors such as smoking, inflammation and dyslipidemia which lead to large vessel disease (Aboyans et al., 2006). Diabetic vasculopathy, specifically PAD, has been demonstrated to be independently associated with cognitive impairment and dementia (Bruce et al., 2008). In our cohort, diagnosis of PAD was significantly more frequent in patients with PDR (in both inter- and intra-group analyses) as well as in patients with all-cause CNS morbidity. PAD itself, analyzed as a possible confounder, was shown to be a strong independent risk factor for all-cause neuropsychiatric disorders and CVA, but not for epilepsy, dementia, depression and psychosis (data not shown). Epidemiological data demonstrating high degree of comorbidity among patients with vascular disease and specific neuropathologies implicate the need for further investigation and suggest a primary etiological role for vascular pathology in CNS dysfunction. Dysglycemia is frequently recognized as an important adjustment covariant for vascular and neuropsychiatric complications in DM. HbA_{1c} levels, however, were not different between the subgroups and did not have an effect on the regression models tested. Glycemic status did not contribute to the association between retinopathy and mortality, as previously reported in prospective population-based cohorts (Van Hecke et al., 2005). Nevertheless, landmark clinical trials in type 1 and 2 diabetes patients have demonstrated the impact of intensive HbA_{1c} control on reducing the risk of diabetic retinopathy ("Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group.," 2000; Stratton et al., 2000). However, intensive glycemic control compared with standard glycemic lowering strategies had no impact on cognitive outcomes (Launer et al., 2011). While some reported that elevated HbA_{1c} was associated with depression (Richardson et al., 2008) and lower cognitive function (Avadhani et al., 2015), others reported that patients with elevated HbA_{1c} values performed better on cognitive testing than those with lower HbA_{1c} (Crosby-Nwaobi et al., 2013). It thus appears that the direct and indirect contribution of HbA_{1c} control on cognitive functions in DM is far from being elucidated and a variety of metabolic and vascular interactions may play a role (Kodl and Seaquist, 2008).

This study was limited by its retrospective nature. Patient data were retrieved from medical records, and therefore clinical assessments may not be consistent between patients. Additionally, while renal function indicators, namely albuminuria and eGFR were proven in a univariate regression analysis to have a potential predictive value for the all-cause neuropsychiatric end point, 32.8% of the patients had no valid eGFR measurement and 55.5% missed evaluation for albumin/creatinine ratio. An incomplete record of these variables could introduce a potential missing bias in the final adjusted regression models. Optimization approaches to reduce this imbalance, i.e., multiple imputation techniques to impute missing covariate values for patients with incomplete data, were unsuitable in the context of extensive missing information which was demonstrated to be a 'missing not at random' (MNAR) data. To overcome this constraint and to prove the validity of our result in the fully-adjusted model I (in a dataset of 44.2% of the original cohort), a complete case multivariate logistic regression, omitting variables with many missing values, was tested (model II). In both models, PDR was a strong predictor for all-cause neuropsychiatric outcomes and maintained significant odds ratios.

Overall, we found a significantly higher death rate in patients with PDR; PDR was also found to be associated with increased risk of all-cause mortality, especially during the early part of the study interval. Breslow test, emphasizing differences in early survival (Parmar and Machin, 1995), was thus a more suitable statistical test for data analysis. It is plausible that mortality rates for patients with PDR are actually higher than observed, since progression of NPDR to PDR is the natural course of retinopathy, but not vice versa. The presented results are in line with studies associating severe retinopathy in type 1 and 2 diabetes with greater all-cause or cardiovascular mortality (Kramer et al., 2011). Our study included a substantially larger sample size than previously studied with a considerably greater number of patients with documented PDR, allowing inter- and intra-patient comparisons. Our results emphasize the importance of accurate identification of individuals at risk and support the potential role of FA in predicting the risk for neurological complications, and highlight the need of novel preventive strategies. Imaging methods for quantitative analysis of FA studies (Serlin et al., 2013), and new modalities such as optical coherence tomography (OCT) angiography may prove to be superior with

regards to objectivity, sensitivity and accuracy in detecting changes in blood flow and permeability.

In conclusion, this is the largest ever studied cohort of diabetic patients with FA-confirmed retinal vasculopathy demonstrating a clear association with the total burden of CNS pathology. Our results highlight the potential of BRB evaluation to reflect brain small vessel pathology and support the key role of compromised blood-neural barriers in the pathophysiology of specific brain disorders. Diagnosis of retinal vasculopathy seems to identify individuals at high risk for neuropsychiatric adverse events and emphasize the need to develop sensitive, quantitative and more accurate evaluation methods to allow for early identification of retinal vasculopathy. Future pre-clinical and clinical studies are required to investigate endothelial dysfunction as a novel target for the prevention of brain impairments in the presence of diabetic retinopathy.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

YS contributed to literature search, study design, data analysis and interpretation and wrote the manuscript. TS contributed to data acquisition, analysis and interpretation. JL, MS, AW, HS and EU contributed to data interpretation and discussion. YP contributed to the statistical analysis and data interpretation. AF contributed to study design, data analysis and interpretation and is responsible for the integrity of the work as a whole. All authors contributed to the critical revision of the manuscript and approved the final version of the manuscript before submission.

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