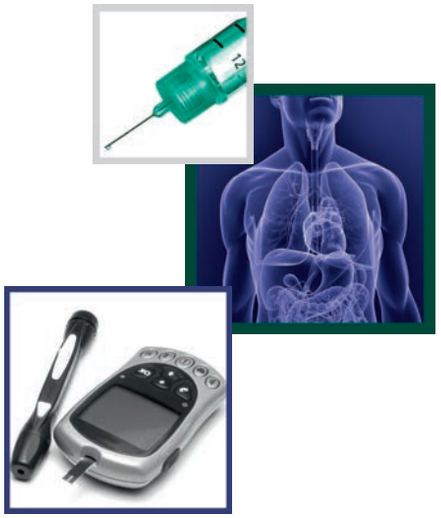
REVIEW

Circulating biomarkers of diabetic retinopathy: a systematic review and meta-analysis



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Practice Points

- Blindness from diabetic retinopathy can be prevented with timely intervention.
- Screening all at-risk patients represents a considerable clinical burden.
- Validated circulating biomarkers of diabetic retinopathy may be used to prioritize the clinical screening of patients at high risk of developing sight-threatening complications.
- Systematic review and meta-analysis identified circulating ADMA, AM, ACE, AGEs, E-selectin, ICAM-1, VCAM-1 and vWF as being significantly associated with any form of diabetic retinopathy compared with people with no retinopathy.
- The most significant group of interacting biomarkers for diabetic retinopathy identified to-date, include the adhesion molecules: VCAM-1, ICAM-1 and E-selectin, which are found to cluster in the receptor cascade of the VEGF pathway.
- The diabetic retinopathy biomarker literature is limited by the small number of replication studies and lack of consistent reporting of results.

SUMMARY Approximately a third of adults with diabetes have diabetic retinopathy (DR), a potentially blinding eye disease. This study aimed to summarize known biomarkers associated with DR and investigate their potential roles in retinopathy phenotype. All available data from studies analyzing human peripheral blood samples were extracted for meta-analysis. Pathway analysis was subsequently performed on the biomarkers found to be associated with DR. We identified a significant difference in circulating levels of: soluble ICAM-1 ($p < 0.0001$), asymmetric dimethylarginine ($p < 0.001$), adrenomedullin ($p = 0.04$), soluble E-selectin ($p = 0.0017$), soluble VCAM-1 ($p < 0.001$) and von Willebrand factor ($p = 0.0004$), between patients with various stages of DR and patients with diabetes but no retinopathy. Several distinct biomarkers were consistently found to be associated with DR. Many of these biomarkers are known to function in pathways that promote leukocyte adhesion and transmigration into cells, presumably contributing to the characteristic vascular dysfunction observed in DR.

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Background

Diabetes mellitus is emerging as an epidemic, with diabetic retinopathy (DR) being the leading cause of blindness in working-age individuals [1]. Approximately a third of adults in the USA with diabetes have DR, and this number is expected to triple by 2050 [2]. Pivotal studies have confirmed the importance of optimal diabetes control in the prevention of development and progression of DR [3,4]. Furthermore, in people who progress clinically, the results from the Early Treatment of Diabetic Retinopathy Study (ETDRS) demonstrated the effectiveness of laser photocoagulation in reducing severe visual loss in sight-threatening disease, maculopathy and proliferative DR [5]. Pharmaceutical intervention using anti-VEGF agents (such as ranibizumab, bevacizumab or possibly aflibercept) have shown successful outcomes in preventing diabetes-related vision loss [6,7]. It can be difficult, however, to predict the future clinical course of some patients in clinical practice. Duration of diabetes, glycemic and blood pressure control only explain approximately 15% of the variance in DR development and progression [3,8,9]. For instance, patients with good glycemic control may manifest with early or rapidly progressive DR, while other people with relatively poor glycemic control may take several years to manifest signs of DR [10]. This discrepancy highlights the importance for better understanding of the pathogenesis of DR. An increased understanding of the underlying molecular pathologies leading to DR development and the identification of novel biomarkers for DR progression may aid in early diagnosis, clinical monitoring and risk stratification among patients with diabetes.

An understanding of gene expression profiles and their *in vivo* regulators, both systemically and locally (within the retina) is crucial to elucidate the complex pathogenesis of DR. Recently, Abhary and colleagues published a systematic meta-analysis of genetic associations for DR and reported a strong association with variants in the *ARK1B1* gene [11]. Polymorphisms of *NOS3*, *VEGF*, *ITGA2* and *ICAM1* were also nominally associated with DR. Nonetheless, the pathogenesis of DR is multifactorial, with genetic, epigenetic and environmental contributions likely to be important. DR development is thought to largely be the result of diabetic-induced retinal microvascular dysfunction [12–14]. Although several molecular mechanisms have been proposed, proinflammation has recently been attributed as a major factor in the underlying pathogenesis of DR.

Inflammation is a multifaceted response of vascular tissue to a pathogen, which initiates the immune system [15]. While proinflammation is generally homeostatically protective, overall it can be deleterious if chronic [16]. Inflammation involves a variety of molecular mediators, such as the recruitment and activation of leukocytes [15]. Proinflammatory molecules in the microvasculature have been implicated in vascular ischemia, as well as vasopermeability, and thus are potentially intimately linked with DR progression [17].

Ischemia creates progressive vascular compromise in the diabetic microcirculation. Widespread autoregulatory disturbance ensues, with progressive ischemia and the liberation of substances compensating for such dysregulation, clinically recognizable as the progression from background DR (BDR) to proliferative diabetic retinopathy (PDR). At the proliferative stage of retinopathy there is neovascularization, a consequence of nonperfusion and tissue hypoxia [12].

The process of vasopermeability derives from an increased elaboration of vasopermeable factors in the microvasculature that contributes to widespread breakdown of the blood–retinal barrier, resulting in protein exudation and leukocyte migration leading to the clinical spectrum of maculopathy. *In vitro* work has revealed dysregulation of immune and angiogenesis factors in the diabetic microenvironment [12].

To date there is no molecular biomarker used clinically for the detection of DR, apart from those used to diagnose diabetes and monitor glycemic control. Furthermore, in the early stages of retinopathy there is no current means to identify patients specifically at risk of DR progression. Hence, assessing the circulating nongenetic biomarkers of DR would be useful for risk prediction and understanding disease pathogenesis. Early detection would allow for timely treatment intervention, thus preventing blindness from diabetes. Herein, we report the results of a systematic review and meta-analysis of molecular biomarkers for DR. The aim of this study was to identify circulating markers that are consistently associated with the severity of DR; define their molecular interactions; and re-assess potentially relevant pathological pathways.

Research design & methods

■ Data sources

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses

(PRISMA) guidelines [18]. Published articles were extracted from the following databases: PubMed [101]; science citation index at web of science (ISI) [102]; web of science (ISI) [103]; MEDLINES [104] and Johns Hopkins University [105]. The following search terms: "biomarker"; "antibody"; "protein"; "histone"; "epigenetic", were searched independently with the terms "diabetic retinopathy" and "human". Search dates were left open (1963 until October 2010) to include all available research. All articles were retrieved and then searched for duplicates, first using Endnote's Endnote X3® (Thomson Reuters, Carlsbad, CA, USA). Subsequent manual checking of title, year of publication and author was conducted.

■ Study selection

Articles were grouped based on title and abstract screening. Inclusion criteria were defined as; research presenting results from nongenetic, human, peripheral blood samples **(Figure 1)**. Exclusion criteria included; *in vitro*/*in vivo* studies, nonsystematic reviews, diabetic studies with no reference to retinopathy and interventional clinical trials with pharmacological agents. Cohorts were also excluded if patients were known to have gestational diabetes or nondiabetic retinopathy. Patient cohorts with known diabetic nephropathy were excluded owing to individual's variation in the inadequate clearance of circulating molecules and the likely confounding effect of this on results. Studies lacking an abstract were grouped separately using the full text article, and if they met the eligibility criteria they were included for the meta-analysis. Studies specifically investigating the role of HbA1C were excluded.

■ Data extraction

Data from all eligible studies were entered into a database and grouped according to the severity of DR and type of diabetes, if applicable. Following data entry, one in five papers were randomly selected and checked independently for extraction errors prior to analysis. Recorded characteristics included study demographic features; sample size, age range, diabetes duration, diabetes subtype and HbA1c levels. For each investigated molecular biomarker, the mean, standard deviation (SD), range, confidence interval (CI) and reported significant level p-values were recorded for each study group reported. Given the change in molecular nomenclature with time, and different abbreviations of the same biomarker across articles, all biomarkers studies were assessed for consistency using the UniProt [106] and NCBI [107] databases. Markers with two or more datasets per subgroup were included in the meta-analysis.

■ Statistical analysis

Data were subgrouped into biomarkers based on their unique UniProt or NCBI number. Within each biomarker subgroup the units of measure were standardized. Where possible, unit conversions were carried out to ensure homogeneity of units between studies for each biomarker. When units could not be converted, a standardized mean difference was calculated, allowing the data to be analyzed on a uniform scale. For each biomarker, data were categorized by type of diabetes (Type 1 and 2) and severity of DR (none; nonproliferative DR [NPDR]; PDR; any DR). Comparisons were performed for studies stratified by diabetes subtype (Type 1 and 2) and by control groups used; the latter were classified as being either diabetic patients without retinopathy or healthy nondiabetic participants. A mixed control type was defined to include studies that used both normal subjects and diabetes patients without DR as controls.

The Der Simonian and Laird random-effects model was used [19]. This model utilizes weights that incorporate both within-study and between-study variance. Forest and funnel plots were constructed in the statistical software R version 2.7.1 [108] using the 'meta' plugin. A p-value of <0.05 was considered to be statistically significant in all analyses, except for testing publication bias (Egger's test), where a p-value of <0.1 [20] was considered statistically significant. Mean fold change (MFC) between case and control groups was calculated from the weighted means and SD. Heterogeneity between studies was calculated as the inverse variance estimate. For datasets with significant heterogeneity; outlying studies were removed in a stepwise fashion until homogeneity was achieved.

■ Molecular pathway analysis

With the aim of identifying additional biomarkers for future study and to identify interacting molecules important in the pathogenesis of DR, a pathway analysis was performed. Using a trial license, the biomarkers found to be consistently associated with DR were entered into the MetaCore search function of GeneGO [109], a

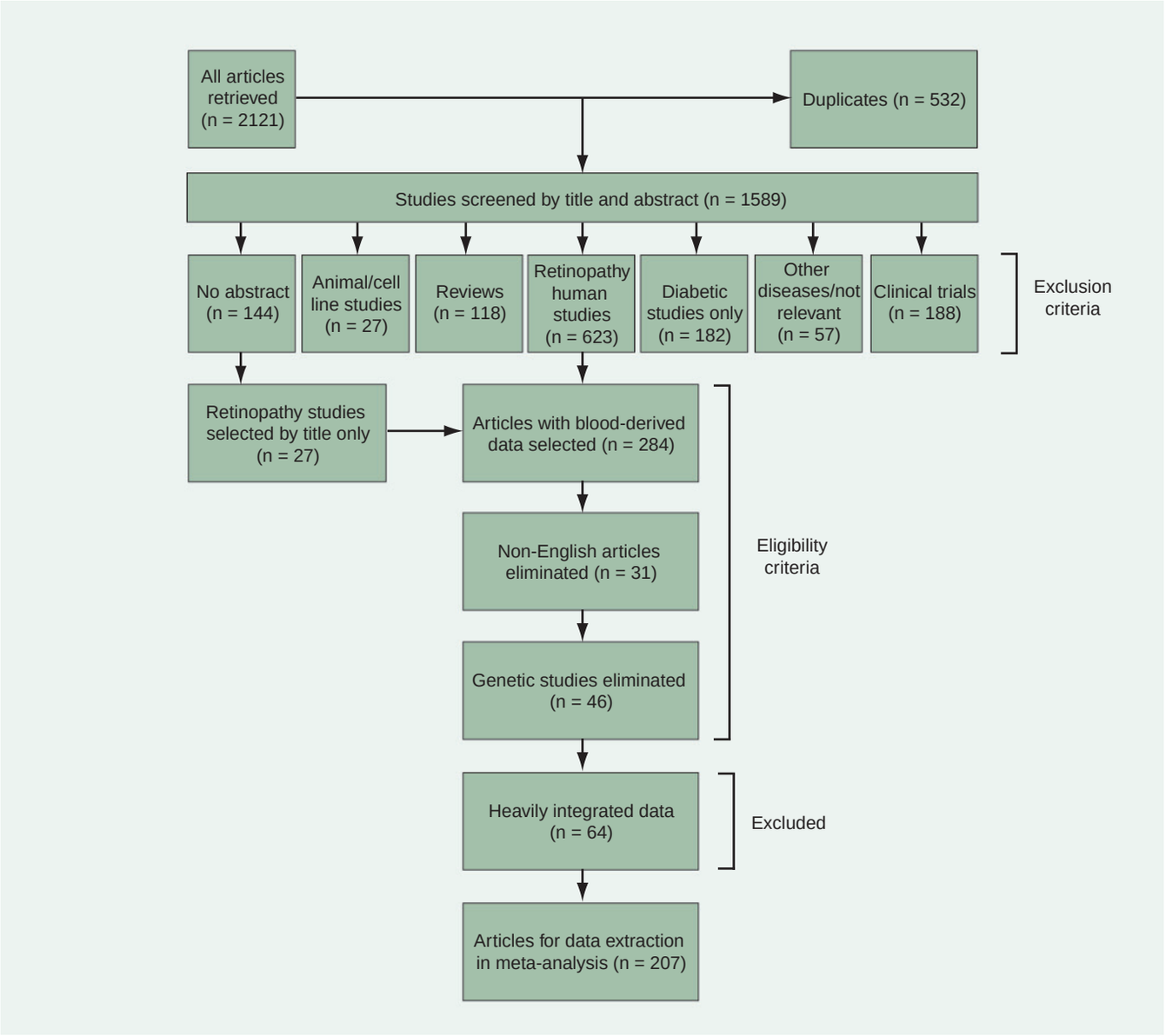

Figure 1. Study selection for systematic meta-analysis.

curated database of gene and protein expression pathways. Expression pathways for each biomarker were manually reviewed from MetaCores pathway analysis to explore potential interactions between pathways.

Results

Meta-analysis results and MFC relative to controls for 12 biomarkers assessable in the 'any DR' category are presented in **Table 1**. For Type 1 and 2 diabetes combined with any DR compared with diabetics without retinopathy, a significant increase in biomarkers levels was observed for soluble ICAM-1 ($p < 0.0001$), asymmetric dimethylarginine (ADMA; $p < 0.001$), adrenomedullin (AM; $p = 0.04$), advanced glycation end products (AGEs; $p = 0.02$), angiotensin-converting enzyme (ACE; $p = 0.001$) and von Willebrand factor (vWF; $p = 0.0004$) and no significant variation in fructosamine ($p = 0.42$). With any DR specifically associated with Type 1 diabetes a significant increase in soluble E-selectin ($p = 0.0017$) and soluble VCAM-1 ($p < 0.001$) was identified compared with diabetic patients without retinopathy **(Figures 2 & 3)**. Among Type 2 diabetic patients, we found no significant differences in C-reactive protein (CRP; $p = 0.61$) or VEGF ($p = 0.624$) between

Table 1. Biomarkers for any diabetic retinopathy.

Biomarker	DM type	Control type	Number of studies	Number of cases	Number of controls	Mean fold change	Mean difference[†]	Units	p-value
ADMA	T1 and T2	Diabetic, no DR	2	196	348	1.317	0.220	mm/l	<0.001
AM	T1 and T2	Diabetic, no DR	3	75	175	1.256	15.83	pg/ml	0.04
ACE	T1 and T2	Mixed[‡]	5	143	180	0.701	2.23[§]	N/A	0.001
AGEs	T1 and T2	Diabetic, no DR	3	66	148	0.219	1.82[§]	N/A	0.020
CRP	T2	Diabetic, no DR	3	168	409	1.115	0.050	mg/l	0.62
Soluble E-selectin	T1	Diabetic, no DR	3	96	131	1.562	28.93	ng/ml	0.0017
ET-1	T2	Non-Diabetics	2	24	22	1.024	1.090	pg/ml	0.33
Fructosamine	T1 and T2	Diabetic, no DR	4	132	103	0.008	0.110	mmol/l	0.42
sICAM-1	T1 and T2	Diabetic, no DR	2	92	66	1.226	62.630	ng/ml	<0.0001
sVCAM-1	T1	Diabetic, no DR	3	96	131	1.225	168.82	ng/ml	<0.001
vWF	T1 and T2	Mixed[‡]	2	38	74	1.221	0.75	%	0.0004
VEGF	T2	Diabetic, no DR	2	37	54	0.590	-1.35	pg/ml	0.624

[†]Based on random effects model, whereby negative values imply lower expression levels.
[‡]Mixed control type defined as studies that included both normal subjects and diabetes patients without DR.
[§]Standardized mean difference, calculated for studies with differing units of measure.
ACE: Angiotensin-converting enzyme; ADMA: Asymmetric dimethylarginine; AGE: Advanced glycation end product; AM: Adrenomedullin; CRP: C-reactive protein; DM: Diabetes mellitus; DR: Diabetic retinopathy; ET-1: Endothelin 1; N/A: Not applicable; sICAM-1: Soluble inter-cellular adhesion molecule 1; sVCAM-1: Soluble vascular cell adhesion molecule-1; T1: Type 1 diabetes; T2: Type 2 diabetes; vWF: von Willebrand factor.

patients with and those without retinopathy. There was no significant difference in endothelin (ET-1; p = 0.33) levels when Type 2 diabetic patients with DR were compared with healthy nondiabetic controls.

A total of ten biomarkers were similarly included for meta-analysis of studies specifically investigating patients with NPDR (Table 2). Including both diabetes types, soluble E-selectin, ICAM-1 and soluble VCAM-1 were all increased in those with NPDR compared with controls, but none reached statistical significance. When compared with diabetic patients without retinopathy, significant increases in vWF (p = 0.006) and lipoprotein A (p = 0.0001) were found in NPDR patients of either Type 1 and 2 diabetes. Specifically for Type 1 diabetes, the association between levels of apolipoprotein A-I (APOA; p = 0.38) or apolipoprotein B (APOB; p = 0.27) and NPDR compared with diabetic patients without retinopathy did not vary statistically significantly.

Table 3 presents the results of a meta-analysis of 11 biomarkers assessable for PDR. For Type 1 and 2 diabetes combined, an increase in ACE (p = 0.024) was identified in patients with PDR compared with diabetic patients without retinopathy. For Type 1 diabetic patients there was no significant difference in the circulating levels of APOA (p = 0.55) or APOB (p = 0.78) in PDR compared with controls mixed with diabetic patients without DR and nondiabetic subjects. There was a significant increase in circulating levels of soluble E-selectin (p < 0.0001) and soluble VCAM-1 (p < 0.0001) in PDR cases compared with healthy normal controls. For patients with Type 2 diabetes, a statistically significant difference was found for soluble VCAM-1 (p < 0.0001) only in PDR cases compared with diabetic patients without retinopathy. There were six biomarkers meeting study inclusion criteria, which had been studied in the comparison of PDR and NPDR (Table 4). None were found to be significant in a random effects model.

The interaction pathways of all biomarkers from the meta-analysis were analyzed using MetaCore. The major pathways identified were involved in blood coagulation, cell adhesion,

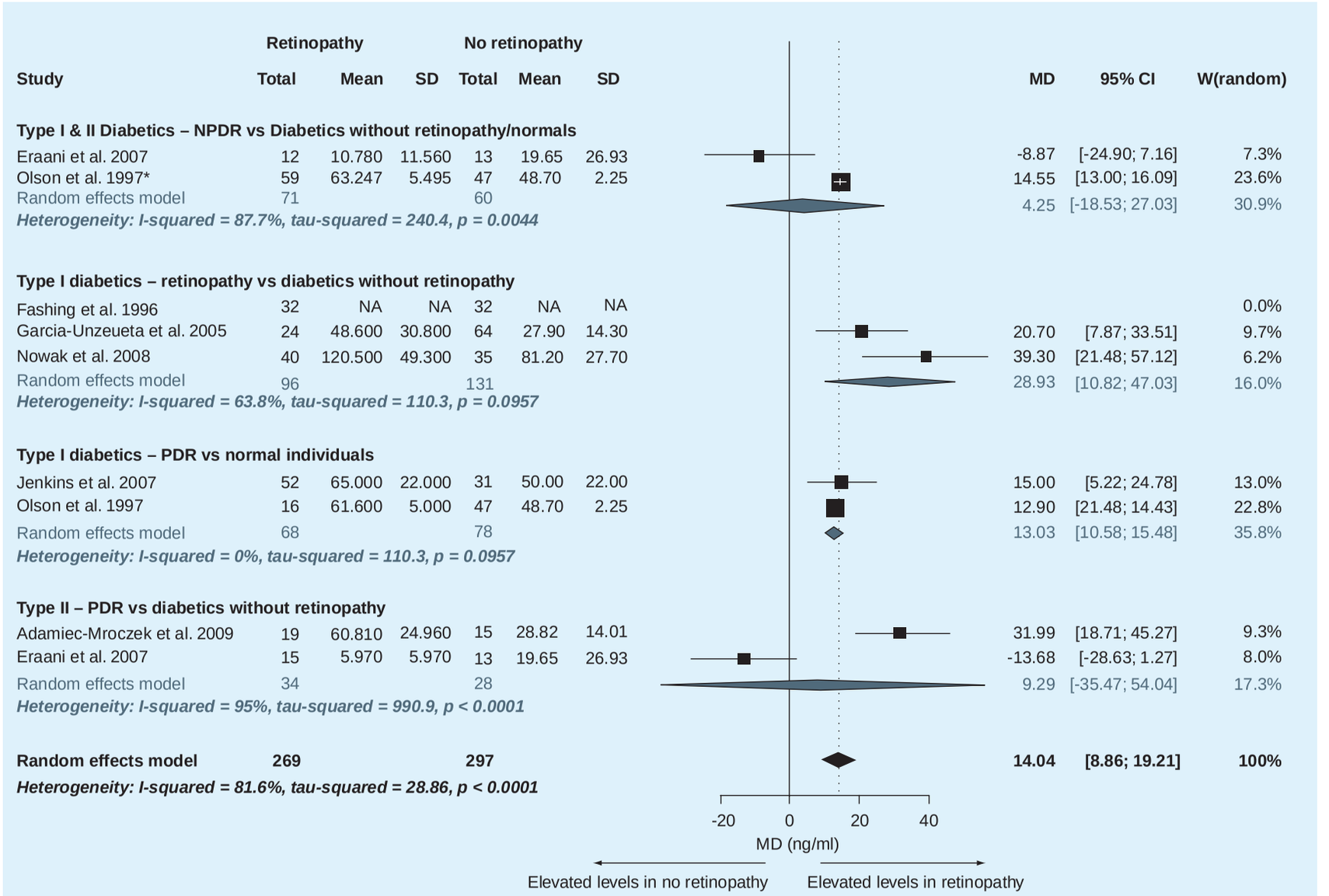

Figure 2. Forest plot of studies investigating soluble E-selectin in Type I and II diabetes for all diabetic retinopathy, nonproliferative diabetic retinopathy and proliferative diabetic retinopathy compared with diabetic patients without retinopathy.

MD: Mean difference; PDR: Proliferative diabetic retinopathy; SD: Standard deviation; W(random): Weight under a random effects model.

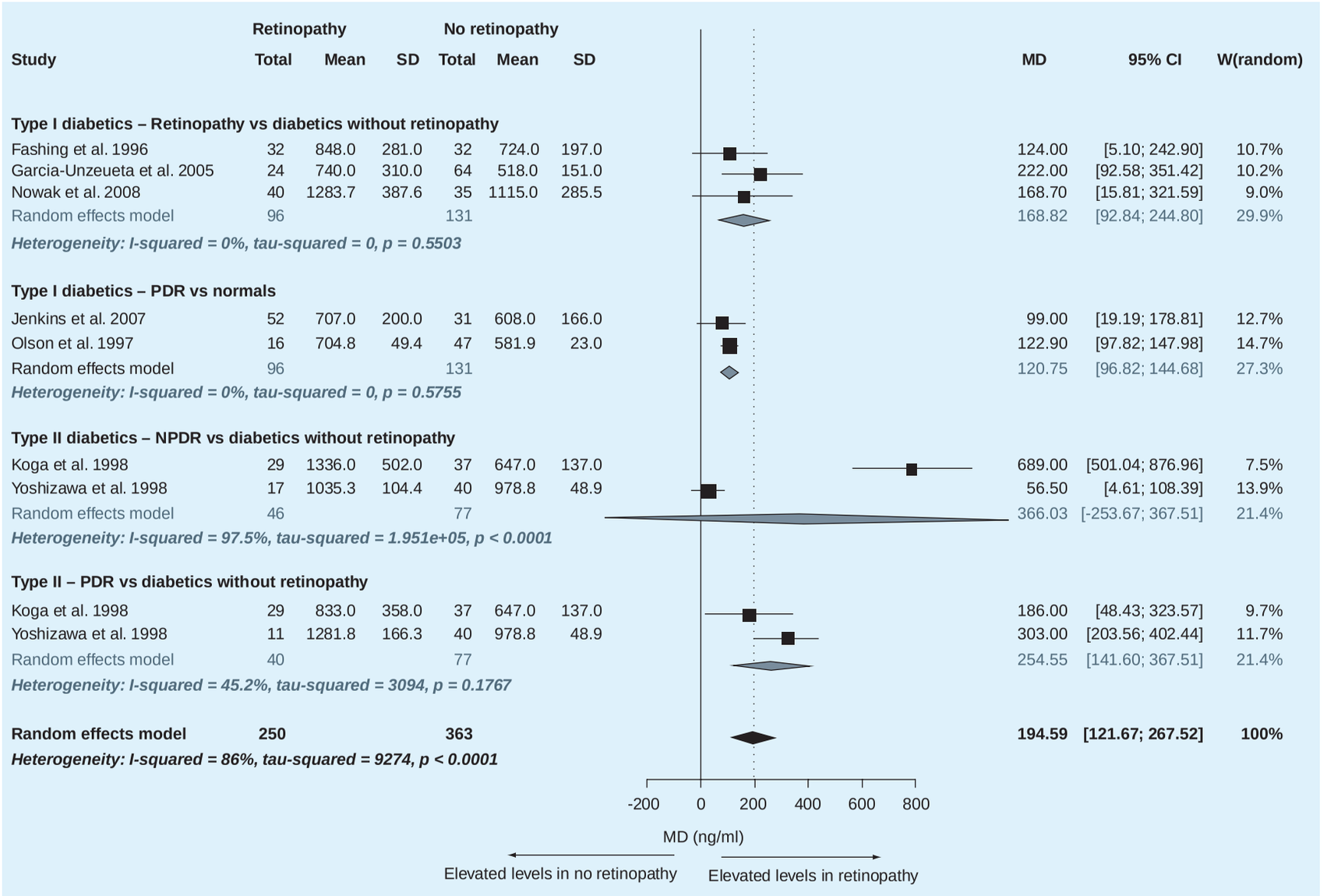

Figure 3. Forest plot of studies investigating soluble VCAM-1 in Type I and II diabetes for all diabetic retinopathy, nonproliferative diabetic retinopathy and proliferative diabetic retinopathy compared with diabetic patients without retinopathy.

MD: Mean difference; PDR: Proliferative diabetic retinopathy; SD: Standard deviation; W(random): Weight under a random effects model.

Table 2. Biomarkers for nonproliferative diabetic retinopathy.

Biomarker	DM type	Control type	Number of studies	Number of cases	Number of controls	Mean fold change	Mean difference†	Units	p-value
APOA	T1	Mixed‡	2	213	161	1.025	-1.150	mg/dl	0.38
APOB	T1	Mixed‡	2	213	161	0.962	-1.596	mg/dl	0.27
ACE	T1 and T2	Diabetic, no DR	4	78	180	0.074	1.28§	N/A	0.456
Soluble E-selectin	T1 and T2	Nondiabetics	4	71	154	1.118	4.254	ng/ml	0.71
ET-1	T1 and T2	Diabetic, no DR	2	33	34	1.330	27.930	pg/ml	0.20
Fibrinogen	T1 and T2	Mixed‡	2	33	90	1.071	0.400	mg/dl	0.06
Lipoprotein A	T1 and T2	Diabetic, no DR	2	151	239	1.326	6.93	mg/dl	0.0001
sICAM-1	T2	Diabetic, no DR	2	73	40	1.446	135.640	ng/ml	0.35
TM	T1 and T2	Diabetic, no DR	3	52	169	1.880	0.770	ng/ml	0.07
sVCAM-1	T2	Diabetic, no DR	2	46	77	1.448	366.030	ng/ml	0.25
vWF	T1 and T2	Mixed‡	4	66	148	0.483	1.70§	N/A	0.006

†Based on random effects model, whereby negative values imply lower expression levels.

‡Mixed control type defined as studies that included both normal subjects and diabetes patients without DR.

§Standardized mean difference, calculated for studies with differing units of measure.

ACE: Angiotensin-converting enzyme; APOA: Apolipoprotein A-I; APOB: Apolipoprotein B; CRP: C-reactive protein; DM: Diabetes mellitus; DR: Diabetic retinopathy; ET-1: Endothelin 1; N/A: Not applicable; sICAM-1: Soluble inter-cellular adhesion molecule 1; sVCAM-1: Soluble vascular cell adhesion molecule-1; T1: Type 1 diabetes; T2: Type 2 diabetes; TM: Thrombomodulin; vWF: von Willebrand factor.

chemotaxis, development, immune response and muscle contraction. The major interactions between multiple biomarkers were observed within the 'any DR' category when compared with diabetic patients without retinopathy. The analysis revealed that VEGF, ET-1 and CRP appear in the development of the leptin signaling pathway. In the leptin signaling pathway, VEGF activates angiogenesis, with ET-1 also being intimately involved in vasoproliferation. Interestingly, CRP provides a negative feedback on this pathway, correlating with the lower levels of CRP observed in the meta-analysis of DR. However, the most significant cluster of biomarkers in the 'any DR' category appeared in a separate pathway, where the adhesion molecules VCAM-1, ICAM-1 and E-selectin were found to cluster in the receptor cascade of the VEGF pathway (**Supplementary material,** see online www.futuremedicine.com/doi/suppl/10.2217/DMT.12.4).

Discussion

This study confirmed the strong association of specific biomarkers with DR, implicating pathways involved in ischemia, vasopermeability and

Table 3. Biomarkers for proliferative diabetic retinopathy.

Biomarker	DM type	Control type	Number of studies	Number of cases	Number of controls	Mean fold change	Mean difference†	Units	p-value
APOA	T1	Mixed‡	2	55	161	1.037	-0.700	mg/dl	0.55
APOB	T1	Mixed‡	2	55	161	1.048	0.781	mg/dl	0.19
ACE	T1 and T2	Diabetic, no DR	3	39	147	0.329	0.29§	N/A	0.024
Soluble E-selectin	T1	Nondiabetics	2	68	78	1.267	13.030	ng/ml	<0.0001
Soluble E-selectin	T2	Diabetic, no DR	2	34	28	1.181	9.290	ng/ml	0.68
ET-1	T1 and T2	Diabetic, no DR	2	33	34	1.367	13.850	pg/ml	0.09
Fibrinogen	T1 and T2	Mixed‡	2	28	90	1.281	1.290	mg/dl	0.0001
Lipoprotein A	T2	Diabetic, no DR	2	108	239	0.320	12.60	mg/dl	0.250
TM	T1 and T2	Diabetic, no DR	3	32	169	1.368	-1.270	ng/ml	0.37
sVCAM-1	T1	Nondiabetics	2	68	78	1.207	120.750	ng/ml	<0.0001
sVCAM-1	T2	Diabetic, no DR	2	40	77	1.303	254.550	ng/ml	<0.0001
vWF	T1 and T2	Mixed‡	5	59	163	0.608	1.46§	N/A	0.051

†Based on random effects model, whereby negative values imply lower expression levels.

‡Mixed control type defined as studies that including both normal subjects and diabetes patients without DR.

§Standardized mean difference, calculated for studies with differing units of measure.

ACE: Angiotensin-converting enzyme; APOA: Apolipoprotein A-I; APOB: Apolipoprotein B; CRP: C-reactive protein; DM: Diabetes mellitus; DR: Diabetic retinopathy; ET-1: Endothelin 1; N/A: Not applicable; sICAM-1: Soluble inter-cellular adhesion molecule 1; sVCAM-1: Soluble vascular cell adhesion molecule-1; T1: Type 1 diabetes; T2: Type 2 diabetes; TM: Thrombomodulin; vWF: von Willebrand factor.

Table 4. Biomarkers for proliferative diabetic retinopathy compared with nonproliferative diabetic retinopathy.

Biomarker	DM Type	Control type	Number of studies	Number of cases	Number of controls	Mean difference†	Units	p-value
APOA	T1	NPDR	2	55	213	0.590	mg/dl	0.28
ET-1	T1 and T2	NPDR	2	33	33	10.710	pg/ml	0.34
Fibrinogen	T1 and T2	NPDR	2	28	33	1.460	mg/dl	0.43
sVCAM-1	T2	NPDR	2	40	46	-121.610	ng/ml	0.75
TM	T1 and T2	NPDR	3	32	52	-0.160	ng/ml	0.83

†Based on random effects model, whereby negative values imply lower expression levels.
APOA: Apolipoprotein A-I; DM: Diabetes mellitus; DR: Diabetic retinopathy; ET-1: Endothelin; NPDR: Nonproliferative diabetic retinopathy; sVCAM-1: Soluble vascular cell adhesion molecule-1; T1: Type 1 diabetes; T2: Type 2 diabetes; TM: Thrombomodulin.

inflammation. Interestingly, different markers for DR were found in Type 1 and 2 diabetes. In Type 1 alone, soluble E-selectin and soluble VCAM-1, and in Types 1 and 2 combined, ADMA, AM, ACE, AGEs, soluble ICAM-1 and vWF were significantly elevated in patients with DR compared with diabetic patients without retinopathy, suggesting that these biomarkers are specific for DR as opposed to markers of diabetes itself. In particular, vWF and lipoprotein A were indicated to be markers for NPDR in Type 1 and 2 diabetes. Significant markers found to be increased in PDR included soluble E-selectin for Type 1 diabetes **(Figure 2)** and soluble VCAM-1 for Type 1 and 2 diabetes **(Figure 3)**. The remaining biomarkers have been found to be present at differing levels in patients with NPDR and PDR, and while the MFC was not statistically significant, it is noteworthy that differential biomarker expression was associated with DR severity. Indeed, subtle alteration in biomarker level may prove to have a profound effect on the clinical phenotype.

The upregulated markers in DR and PDR patients with Type 1 diabetes include soluble E-selectin and soluble VCAM-1. E-selectin is a cell adhesion molecule exclusively expressed in endothelial cells. Its actions allow the endothelial wall to become unselectively permeable, and because of this E-selectin has been suggested to be a marker of endothelial dysfunction [21]. VCAM-1 is also a cellular adhesion molecule that is extracellular and located on the endothelial cell membrane. VCAM-1 is suggested to mediate leukocyte adhesion and signal transduction, thus playing a role in leukocyte emigration to sites of inflammation [22,23]. Both soluble forms of E-selectin and VCAM-1 are suggested to have similar functions to their nonsoluble forms; however, given that these soluble molecules are not membrane bound they are likely to have a greater function across the endothelial wall [24,25]. Furthermore, previous studies have demonstrated that soluble E-selectin and VCAM-1 are strong chemoattractants for monocytes, which play a pivotal role in the inflammatory cascade [26–28]. Generally, E-selectin, VCAM-1 and ICAM-1 differ in their ligand binding and expression duration [26–28]. Results from this study suggest that DR pathogenesis in Type 1 diabetes involves endothelial dysfunction and increased permeability, allowing the increase in leukocytes, monocytes and other inflammatory cells to transmigrate across the vessel wall. This indicates that vasopermeability and inflammation have a significant role in retinopathy in Type 1 diabetics.

For Type 1 and 2 diabetes combined, our findings suggest a large systemic increase in adhesion molecule, soluble VCAM-1 and soluble ICAM-1, cellular adhesion molecules found in endothelial cells, macrophages and lymphocytes in persons with DR. When elevated, ICAM-1 binds leukocytes to endothelial cells and facilitates their transmigration into the tissue, producing tissue infiltration [22,23,29]. Soluble versions of cellular adhesion molecules are readily detectable in peripheral fluids [30]. Soluble VCAM-1 and soluble ICAM-1 are extracellular leukocyte mediators in the VEGF pathway involved in the pathogenesis of neovascularization [17]. Interestingly, significantly upregulated VEGF levels were not consistently detectable, suggesting that adhesion molecules, in particular soluble VCAM-1, may be superior systemic markers of leukostasis leading to neovascularization and macular edema, clinical signs of DR. Similarly, markers of leukostasis, AM and ADMA, were found by the meta-analysis to be elevated for retinopathy in Type 1 and 2 diabetes combined. AM is a peptide that is secreted by monocyte and macrophage lymphocytes, and functions to modulate

macrophages [31,32]. ADMA is a naturally occurring compound, the concentration of which is largely influenced by LDL cholesterol. When ADMA is elevated it inhibits nitric oxide (NO), which is important for vascular homeostasis, as NO inhibits leukocyte adhesion to the endothelium [33,34] as well as having direct effects on vascular autoregulation in the retina [35]. These findings suggest that the homeostatic effects of NO are inhibited and that leukocyte adhesion is promoted and caused increased expression in the endothelial cells of patients with DR.

When grouping patients with either Type 1 or 2 diabetes in the meta-analysis, the AGE proinflammatory mediators were found to be elevated in patients who had retinopathy. AGEs were found to accumulate after prolonged periods of hyperglycemia, collecting in the membranes of vessels and macrophages [36,37]. AGEs have been found to be precursors to VEGF and ICAM-1 production and inhibitors of NO [38,39]. Thus, AGEs have a major role in regulating leukostasis and vascular homeostasis, thereby outlining an inflammatory response in retinopathy.

This meta-analysis identified two markers for NPDR development in Type 1 and 2 diabetes combined: lipoprotein A and vWF. Interestingly, lipoprotein A is involved in inducing the adhesion of molecules, ICAM-1 and VCAM-1, on endothelial cells [40,41]. vWF is involved in the adhesion of leukocytes, and has been implicated in cell homeostasis and in the promotion of the inflammatory process by functioning as an adhesive surface for leukocytes, allowing them to stick to the endothelial wall, possibly acting synergistically with the adhesion molecules above [42,43]. Our findings suggest that vWF remains elevated in PDR. Thus, lipoprotein A and vWF detection can be viewed systematically as early indicators, prior to the elevation of adhesion molecules from the VEGF pathway that are involved in leukostasis [41].

Vascular ischemia and inflammation have clearly been implicated in the pathogenesis of DR in Type 1 and 2 diabetes when combined. Each of the above biomarkers may have a major role in the immune response system in the endothelium of individuals with DR. Specifically, they are all involved in promoting leukocyte adhesion to the endothelium and leukocyte transmigration into tissue cells. However, it would appear that vasopermeability is selective to patients with Type 1 diabetes.

The precise underlying pathogenesis of DR is likely to vary between individuals. Therefore, the NPDR and PDR groups assessed most likely contain ischemic, as well as vasopermeable, factors important for disease development and progression. Consequently, this potential heterogeneity may have diluted significant associations between the studied biomarkers and disease severity observed in this study. Given that PDR is an advanced disease process that may in many ways be distinct from the diabetic *in vivo* environment alone, it was interesting that none of the circulating biomarkers differed significantly between patients with the nonproliferative and proliferative forms of the disease. It is important to note, however, that the findings are generally only based on a small number of studies (generally two to three). Few replication studies have been performed, and additionally many studies were excluded because they reported only summary statistics (see **Supplementary material**). This resulted in many biomarkers needing to be excluded from the meta-analysis owing to a lack of consistency in the presentation of rseults across studies. In general, there is a lack of uniform reporting of results and we feel that future biomarker reporting would benefit from established criteria, which could act as a minimum requirement for data presentation. Although reduced by using a random-effects model, the major limitation of this analysis was the relatively high degree of heterogeneity between studies. Owing to the predominance of cross-sectional data available for analysis, the assessment of factors important or specific for DR progression was not possible. It would be beneficial for future studies to assess and validate biomarkers longitudinally prior to clinical implementation or monitoring for DR disease progression. Future work could also be directed towards investigating any molecular overlap in the pathogenesis between DR and diabetic nephropathy.

Conclusion

This study has confirmed that distinct biomarkers of DR can be detected in the peripheral circulatory system. Different levels of specific circulating biomarkers, such as soluble VCAM-1, soluble E-selectin and vWF, correlate with DR severity. The major limitation of this review is the relatively small number of studies available for individual analysis. Nonetheless, these results suggest that robust tests of peripheral blood

could be developed, aiding early DR diagnosis and allowing for the rational use of finite clinical resources. Furthermore, results from this work have corroborated specific biomarkers and pathological pathways that could be potential targets for drug intervention to prevent DR-related blindness.

Future perspective

An escalating effort is being directed towards disease-specific biomarker discovery. It is very likely that, over the upcoming decade, dramatic technological advances in the fields of proteomics and next-generation transcriptomic sequencing will enable the high-throughput analysis of many biological specimens [44]. The ability to detect markers of a small volume (as would be predicted if an ocular specific marker for DR susceptibility exists) is also improving [45]. Taken together, we foresee that a more precise marker (as compared with and replacing HbA1c) for risk of DR development will be identified. Rapid clinical translation is certainly plausible should such a biomarker be found to have a robust degree of sensitivity, as well as specificity, for DR progression.

Given rising healthcare costs, a simple laboratory test, which could aid and improve current clinical screening protocols, would allow for the rational use of finite resources. To date, studies investigating the circulating profiles of candidate proteins in DR have generally been underpowered, thereby leading to inconsistent results. Just as the genetics field has shifted from small candidate association studies, to the adoption of dramatically larger *a priori*, genome-wide association study design [46], a transcriptomic, proteomic or system-wide method for investigation for circulating biomarkers will begin to prosper. The stage is certainly set for the application of 'big science' in circulating biomarker discovery [47]. By the end of this decade our understanding of the molecular pathways involved in the pathogenesis of DR will be definitively dissected.

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