• Clinical Research •

Risk factors for an atherothrombotic event in patients with diabetic macular edema treated with intravitreal injections of bevacizumab

Alon Tiosano, Aviel Hadad, Noam Yanculovic

Department of Ophthalmology, Soroka University Medical Center, Ben-Gurion University of the Negev, Beer Sheva 84101, Israel

Correspondence to: Alon Tiosano. Department of Ophthalmology, Soroka University Medical Center, Ben-Gurion University of the Negev, Beer Sheva 84101, Israel. alon.tiosano@gmail.com

Received: 2019-09-20 Accepted: 2020-06-22

Abstract

● **AIM:** To identify risk factors for an atherothrombotic event (ATE) among patients who were treated for diabetic macular edema (DME) with intravitreal bevacizumab injections.

● **METHODS:** This retrospective study enrolled all consecutive patients with DME who were treated by intravitreal bevacizumab from 2009 through 2016 in a single center. They were divided into one group treated by bevacizumab and subsequently had an ATE and a second group also treated by bevacizumab and did not have an ATE.

● **RESULTS:** A total of 455 patients with DME were enrolled. Seventy-two of the patients had an ATE. A multivariate model adjusted for age, gender, smoking, body mass index, hemoglobin A1c (HbA1c), duration of diabetes, creatinine, and blood pressure revealed an increased risk for ATE in the patients with diabetic duration of more than 13y, a systolic blood pressure over 153.5 mm Hg at first treatment, or having been treated by more than 4 intravitreal bevacizumab injections. Additionally, patients that had an ATE within 3mo from the last intravitreal treatment underwent more bevacizumab injections (5.2±3.4 *vs* 3.07±1.86; *P*<0.001).

● **CONCLUSION:** The risk factors for an ATE identified in this study are systolic blood pressure >153.5 mm Hg, a history of diabetic mellitus for more than 13y, and treatment with more than 4 intravitreal bevacizumab injections. These factors need to be borne in mind when bevacizumab is being considered in the management of patients with DME.

● **KEYWORDS:** atherothrombotic event; bevacizumab; diabetic macular edema

DOI:10.18240/ijo.2020.09.12

Citation: Tiosano A, Hadad A, Yanculovic N. Risk factors for an atherothrombotic event in patients with diabetic macular edema treated with intravitreal injections of bevacizumabs. *Int J Ophthalmol* 2020;13(9):1411-1416

INTRODUCTION

It is estimated that diabetes mellitus (DM) affects 415 million people worldwide, and it is predicted to escalate to 642 million by 2040[1]. Possible complications of DM include heart attack, stroke, kidney failure, leg amputation, loss of vision, and neurological damage[2]. Diabetic macular edema (DME) is the leading cause of loss of vision in the working-age population[3]. Initial focal or grid laser photocoagulation was established as the treatment of choice by the Early Treatment Diabetic Retinopathy Study group[4]. Anti-vascular endothelial growth factor (VEGF) treatment became an invaluable tool in the management of a number of retinal diseases, based on the understanding that VEGF has a central role in DME pathology[5-6].

VEGF is a group of growth factor consisting of 6 members of which VEGF-A is most prominent. These growth factors are important signaling proteins in the formation, proliferation, and survival of new blood vessels[7]. The 3 anti-VEGF agents currently in clinical use for intravitreal treatment are bevacizumab, ranibizumab (Lucentis, Genentech, Inc., San Francisco, USA), and aflibercept (Eylea, Regeneron Pharmaceuticals, Inc. New York, USA). Anti-VEGF from intravitreal injections passes to the systemic circulation and suppresses VEGF levels[8]. Among the various anti-VEGF agents, bevacizumab was found to be highly suppressive of systemic VEGF even after 1mo of treatment[9]. Importantly, Avery *et al*[10] demonstrated a systemic accumulation effect of bevacizumab after 3mo of intravitreal treatment.

A major concern with the use of anti-VEGF agents is the potential increased risk of major serious adverse events, from ophthalmic ones, such as retinal pigment epithelial tear which may cause significant visual impairment, to systemic ones, such as cardiovascular disease (CVD) secondary to long-term systemic suppression of VEGF[11-13]. There are several reports on adverse systemic effects after intravitreal anti-

VEGF treatment, but most of them involved patients with age-related macular degeneration patients and not those with DME[14-15]. In a recent Meta-analysis of DME patients treated with anti-VEGF, an increased risk for death, hypertension, and cerebrovascular accident was demonstrated in association with aflibercept and ranibizumab treatment compered to sham treatment[15]. Another recent large study aimed to assess the risk for a systemic adverse event among the DME patients treated with anti-VEGF compared to other treatments reported that there was no difference in risk, however, bevacizumab treatment was not singled out nor was there any identification of risk factors for atherothrombotic event (ATE) among the DME patients who were treated by bevacizumab[16].

The main objective of this study was to identify risk factors for the occurrence of an ATE among DME patients who were treated with intravitreal injections of bevacizumab.

SUBJECTS AND METHODS

Ethical Approval This retrospective study was approved by the Soroka University Medical Center's Helsinki Committee which waived informed consent.

Study Overview The patients' medical records were obtained from the Ophthalmology Department in the Soroka University Medical Center. All consecutive patients who were treated for DME by intravitreal injections of bevacizumab from January 1, 2009 to December 31, 2016 were enrolled into the study. Their records were searched for the occurrence of an ATE, including a myocardial infarction, stroke, pulmonary embolism, and deep vein thrombosis, that occurred after the initial treatment. Patients with an ATE that occurred before the first intravitreal bevacizumab injection were excluded, as were patients that were treated with other anti-VEGF medications.

Study Groups The cohort was divided into 2 groups (Figure 1). The patients in the ATE group were treated with bevacizumab and subsequently had an ATE, and the patients who were treated with bevacizumab and did not have an ATE were included in the second group. The dosage of intravitreal bevacizumab was 1.25 mg.

Follow-Up Follow-up began at the time of the first intravitreal injection of bevacizumab. The end of the follow-up was determined by the date of an ATE or study closure on December 31, 2016.

Statistical Analysis The statistical analysis was performed with SPSS version 23 (SPSS Inc., Chicago, IL, USA), Prism version 7 (GraphPad Software, San Diego, USA) and R version 3.4.2 (R Development Core Team 2017). Descriptive statistics were used to compare baseline and endpoint characteristics. Analyses of covariance statistics were used to assess observed differences between the groups. The independent *t* test and paired samples *t* test were applied for continuous variables, and the Chi-square test was applied for nominal variables. A receiver operating characteristic (ROC) analysis was used in order to determine the optimal value of specific characteristic for the prediction of an ATE. The Gehan-Breslow-Wilcoxon test was used to assess the time to ATE. A logistic multivariate analysis was used to identify factors relevant to the prediction of an ATE among the bevacizumab-treated patients. Estimations are given as odds ratios (OR) with 95% confidence intervals (CI). The level of significance was set to a P value <0.05. Post-hoc analysis was by G*Power software version 3.1.9.2 (Heinrich-Heine-Universität, Düsseldorf, Germany) in order to validate a sufficient power of 80%.

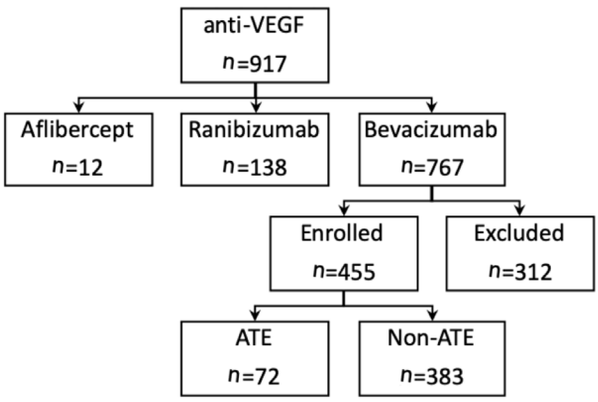

Figure 1 Enrollment and distribution of the patients with DME treated with intravitreal injections of bevacizumab.

RESULTS

All consecutive patients who were treated for DME between January 1, 2009 to December 31, 2016 (n=917) were enrolled into the study. Among them were 455 patients who were treated with intravitreal bevacizumab injections, and their medical records were searched for the occurrence of an ATE, including myocardial infarction, stroke, pulmonary embolism, and deep vein thrombosis, that occurred after the initial treatment.

Study Groups A total of 455 patients were treated with intravitreal bevacizumab injections, and 72 of them subsequently had an ATE and they comprised the ATE group. The intravitreal bevacizumab dose given in each treatment was 1.25 mg. The 383 patients who were treated with bevacizumab and did not have an ATE were included in the non-ATE group.

Baseline Characteristics The baseline characteristics of the 2 study groups are listed in Table 1. The ATE patients' mean±standard deviation age at study entry (first intervention) was 66.2±11.7 *vs* 63.3±11.5y for the non-ATE patients (P=0.054). The percentage of females and the percentage of smokers did not differ between the groups (P=0.394 and P=0.906, respectively). There were no differences in the baseline hemoglobin A1c (HbA1c) levels, body mass index (BMI), or diastolic blood pressure (dBP). Significant differences were, however, found in the systolic BP (sBP) and creatinine levels. The mean sBP and creatinine levels

Table 1 Characteristics of the study groups

Characteristics	ATE (*n*=72)	Non-ATE (*n*=383)	*P*
Gender, *n* (%)			0.394
F	41 (57)	194 (51)	
M	31 (43)	189 (49)	
Smoking history, *n* (%)	13 (18)	48 (12)	0.906
Characteristics at first treatment			
Age at treatment initiation	66.2±11.7	63.3±11.5	0.054
HbA1c, %	8.56±2.1	8.65±2	0.729
Creatinine, mg/dL	1.38±1.6	1.01±0.6	0.001
BMI	28.61±5.8	28.9±5.6	0.716
Systolic BP, mm Hg	154.4±25.9	145.5±26.2	0.011
Diastolic BP, mm Hg	72.2±13.9	74.2±12.9	0.236
Diabetes duration, y	15.48±17.9	10.55±5.1	<0.001
Characteristics at adverse event or end of follow-up			
HbA1c, %	8.36±2.3	8.47±1.9	0.655
Creatinine, mg/dL	1.57±1.9	1.36±1.4	0.277
BMI	28.23±5.9	28.77±5.5	0.482
Systolic BP, mm Hg	159.53±32.5	138.91±23.5	<0.001
Diastolic BP, mm Hg	71.69±14.0	74.24±13.1	0.154
Follow-up, y	1.61±1.06	3.16±1.27	<0.001
No. of injections	3.78±2.8	6.46±5.6	<0.001

ATE: Atherothrombotic event; BMI: Body mass index; BP: Blood pressure; No. of injections: From first intervention to adverse event or study closure.

were higher at the beginning of the study in the ATE group compared to the non-ATE group (154.4±25.9 *vs* 145.5±26.2 mm Hg, respectively, *P*=0.011 and 1.38±1.6 *vs* 1.01±0.6 mg/dL, respectively *P*=0.001).

DM Characteristics The patient's diabetic profile was determined by the time since DM diagnosis until first therapeutic intervention and the HbA1c level upon study admission. The patients in the ATE group had the longest duration since diagnosis (15.48±17.9y) compared to the patients in the non-ATE group (10.55±5.1y, *P*<0.001). HbA1c levels at the beginning of the study were similar for the 2 groups (*P*=0.729; Table 1). A ROC curve was applied to identify the duration of DM until first intravitreal injection and the critical sBP that characterized patients at high risk to develop an ATE at the first intervention. The DM duration was 13.2y (AUC =0.577, *P*=0.025) and the sBP was 153.5 mm Hg (AUC=0.625, *P*<0.001) according to the Youden index (Figure 2).

Change in Characteristics During the Study Period There was a rise of 0.2 mg/dL in creatinine levels in the ATE group (*P*=0.004) and an elevation of 0.35 mg/dL in the non-ATE group (*P*<0.001). The sBP demonstrated a significant decrease in the non-ATE group (*P*<0.001; Table 2).

Analysis of Time to Adverse Event A subgroup analysis of the ATE patients revealed that 34% of them had an adverse event within 100d (early event) from the last injection. The mean number of days to the event was 47.87±31.09 for the early event subgroup *vs* 561.77±325.71 for late events subgroup, *P*<0.001). An additional analysis was made according to the median amount of 4 injections as a cutoff (*P*<0.001, Gehan-Breslow-Wilcoxon test; Figure 3). The amount of bevacizumab injection was higher (5.2±3.4) in the early ATE group (<100d) compared to the late ATE group (>100d, 3.07±1.86, *P*<0.001).

Table 2 Characteristics of each study group over time

Characteristics	Start	End	*P*
ATE group, *n*=72			
HbA1c, %	8.56±2.1	8.36±2.3	0.410
Creatinine, mg/dL	1.38±1.6	1.57±1.9	0.004
BMI	28.61±5.8	28.23±5.9	0.276
Systolic BP, mm Hg	154.4±25.9	159.53±32.5	0.32
Diastolic BP, mm Hg	72.2±13.9	71.69±14.0	0.662
Non-ATE group, *n*=383			
HbA1c, %	8.65±2	8.47±1.9	0.056
Creatinine, mg/dL	1.01±0.6	1.36±1.4	<0.001
BMI	28.9±5.6	28.77±5.5	0.54
Systolic BP, mm Hg	145.5±26.2	138.91±23.5	<0.001
Diastolic BP, mm Hg	74.2±12.9	74.24±13.1	0.981

ATE: Atherothrombotic event; Start: Start of treatment; End: ATE or end of follow-up; BMI: Body mass index; BP: Blood pressure.

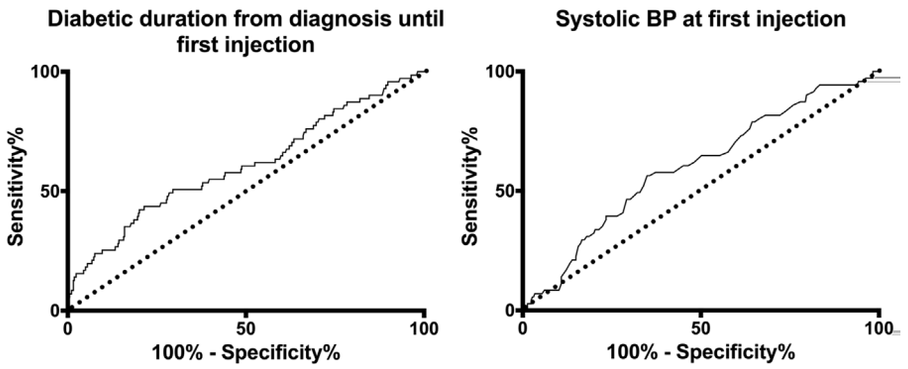

Figure 2 ROC curves of systolic blood pressure of patients with DME at first intervention and time from diabetes diagnosis to first therapeutic intervention.

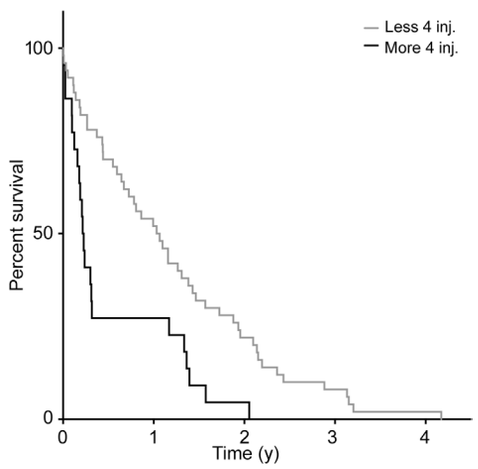

Figure 3 Duration from the first intervention to an ATE among patients with DME who were treated by bevacizumab inj: Bevacizumab injections.

Table 3 Multivariate analysis at first treatment

Characteristics	*P*	OR	95%CI
Gender	0.466	0.784	0.408-1.507
Age	0.048	1.032	1.00-1.064
Smoking history	0.466	0.784	0.306-1.619
Diabetes duration over 13.2y	0.004	2.659	1.372-5.153
BMI	0.771	1.008	0.955-1.064
HbA1c	0.317	1.008	0.926-1.269
Creatinine	0.228	1.206	0.890-1.624
Systolic BP over 153.5 mm Hg	0.002	3.049	1.497-6.212
Diastolic BP	0.228	0.984	0.955-1.010
>4 injections	0.003	2.652	1.389-5.063

OR: Odds ratio; CI: Confidence interval; BMI: Body mass index; HbA1c: Hemoglobin A1c; BP: Blood pressure.

Multivariate Analysis A multivariate analysis revealed 3 main covariates that carried a high risk for the occurrence of an ATE: an sBP over 153.5 mm Hg (OR=3.049; *P*=0.002; 95%CI 1.497-6.212), the presence of DM over 13.2y (OR=2.659; *P*=0.004; 95%CI 1.372-5.153), and an accumulation of more than 4 injections prior to the event (OR=2.652; *P*=0.003; 95%CI 1.389-5.063). None of the other examined factors, such as age, gender, smoking, BMI, HbA1c, creatinine, or dBP, carried a significant risk (Table 3).

DISCUSSION

The aim of this study was to identify characteristics of DME patients that can alert to an impending or future ATE. In total, 8% of the patients with DME had an ATE. A similar high rates of ATE in diabetic patients had been detected by others[17]. A recent Meta-analysis showed that 15.8% of 7604 patients with type 2 DM had a cardiovascular event after a mean follow-up of 6y. Furthermore, patients with DME were reportedly more likely to have an ATE compared with those without DME[11]. Although anti-VEGF therapy is a well-established treatment for DME, there are still concerns about its long-time safety. By entering the systemic circulation, intravitreally administered anti-VEGF decreases systemic VEGF levels, eventually leading to a decline in nitric oxide and an increase in thrombotic activity[18-19]. The various meta-analyses on the use of anti-VEGF have not yet established definitive safety guidelines. Additionally, the follow-up period of those studies did not extend beyond 2y[20].

This study focused on bevacizumab treatment among the anti-VEGF agents currently in use due to its low price and high availability. The results revealed 2 main factors that can identify patients who are at risk to develop ATE before they initiate treatment with intravitreal injections of bevacizumab: the first is the duration from the time from diagnosis of DM to the first intravitreal injection, and the second is the sBP level at the time of the first proposed intervention.

DM induces changes in the microvasculature, causing extracellular matrix protein synthesis and capillary basement membrane thickening[21]. These changes and the advanced glycation end-products, *i.e.*, oxidative stress, low-grade inflammation, and neovascularization of the vasa vasorum, can lead to macrovascular complications[22]. In the current study, patients that were known to be diabetic for more than 13.2y

before bevacizumab intervention had a nearly a threefold risk for an ATE. A patient's diabetic profile consists of a number of factors, among them the HbA1c and the creatinine levels. HbA1c is a highly important parameter in the DM follow-up due its ability to describe the last 3mo of glycemic control[23]. In the setting of the current study, the HbA1c level was similar in the ATE and non-ATE study groups.

The second risk for an ATE that emerged was the sBP level at the time of the first intravitreal injection. A vast majority (70%-80%) of patients with type 2 DM have hypertension, which increases their risk of a myocardial infarction, stroke, and all-cause mortality[24]. One of the adverse effects of anti-VEGF treatment is an increase in sBP that can lead to CVD events[18]. The results of the current study demonstrated that a sBP greater than 153.5 mm Hg almost tripled the risk for an ATE.

More injections were delivered to the patients in the non-ATE group compared to those in the ATE group (6.46±5.6 *vs* 3.78±2.8, respectively; $P<0.001$) and the follow-up time was longer (3.1±1.27 *vs* 1.61±1.06y; $P<0.001$). A subsequent subgroup analysis of the of early versus late ATE events revealed a cutoff of 4 intravitreal injections. Furthermore, in the multivariate analysis, the accumulated effect of bevacizumab after 4 injections emerged as additional risk for ATE. There are several treatment regimens of intravitreal injections in current use and, regardless of the chosen course of treatment, most will exceed 4 intravitreal injections altogether, thus calling for discretion[25].

Study Limitations There are several limitations to this study. First, its retrospective nature may have led to incomplete data that could affect the results. Second, this single-center study included a relatively small cohort, and findings from a larger cohort are needed to draw firm conclusions. Third, the retrospective design precluded the possibility to establish cause and effect.

In conclusion, anti-VEGF is the first-line of treatment for patients with DME, but patient assessment for selected parameters is mandatory before initiating intravitreal bevacizumab injections. Patients in the current study population with sBP readings over 153.5 mm Hg as well as those who had DM for more than 13y carried a high risk for undergoing an ATE during the first 18mo from first intervention. During treatment, it will be prudent to monitor the accumulated amount of bevacizumab injection due to its increase in risk for ATE. Tight control of BP may be recommended for patients with those risk factors who are receiving intravitreal bevacizumab injections, as well as close follow-up of those with multiple risk factors.

ACKNOWLEDGEMENTS

Esther Eshkol is thanked for editorial assistance.

Authors' contributions: Tiosano A, Hadad A, and Yanculovic N contributed to the conception and design of this study. Tiosano A provided the statistical analysis. All authors contributed to the analysis and interpretation of data and have read and approved the final manuscript.

Foundation: Supported by Department of Ophthalmology at Soroka University Medical Center Research Fund.

Conflicts of Interest: Tiosano A, None; **Hadad A,** None; **Yanculovic N,** None.

REFERENCES

1 Al-Lawati JA. Diabetes mellitus: a local and global public health emergency. *Oman Med J* 2017;32(3):177-179.

2 Nathan DM. Long-term complications of diabetes mellitus. *N Engl J Med* 1993;328(23):1676-1685.

3 Lee R, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. *Eye Vis (Lond)* 2015;2:17.

4 Photocoagulation for diabetic macular edema. Early Treatment Diabetic Retinopathy Study report number 1. Early Treatment Diabetic Retinopathy Study research group. *Arch Ophthalmol Chic Ill* 1985;103(12):1796-1806.

5 Scott IU, Edwards AR, Beck RW, *et al*. A phase II randomized clinical trial of intravitreal bevacizumab for diabetic macular edema. *Ophthalmology* 2007;114(10):1860-1867.

6 Arevalo JF, Fromow-Guerra J, Quiroz-Mercado H, Sanchez JG, Wu L, Maia M, Berrocal MH, Solis-Vivanco A, Farah ME. Primary intravitreal bevacizumab (avastin) for diabetic macular edema: results from the pan-American collaborative retina study group at 6-month follow-up. *Ophthalmology* 2007;114(4):743-750.

7 Shibuya M. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and pro-angiogenic therapies. *Genes Cancer* 2011;2(12):1097-1105.

8 Matsuyama K, Ogata N, Matsuoka M, Wada M, Takahashi K, Nishimura T. Plasma levels of vascular endothelial growth factor and pigment epithelium-derived factor before and after intravitreal injection of bevacizumab. *Br J Ophthalmol* 2010;94(9):1215-1218.

9 Zehetner C, Kirchmair R, Huber S, Kralinger MT, Kieselbach GF. Plasma levels of vascular endothelial growth factor before and after intravitreal injection of bevacizumab, ranibizumab and pegaptanib in patients with age-related macular degeneration, and in patients with diabetic macular oedema. *Br J Ophthalmol* 2013; 97(4):454-459.

10 Avery RL, Castellarin AA, Steinle NC, Dhoot DS, Pieramici DJ, See R, Couvillion S, Nasir MA, Rabena MD, Le K, Maia M, Visich JE. Systemic pharmacokinetics following intravitreal injections of ranibizumab, bevacizumab or aflibercept in patients with neovascular AMD. *Br J Ophthalmol* 2014;98(12):1636-1641.

11 Xie J, Ikram MK, Cotch MF, Klein B, Varma R, Shaw JE, Klein R, Mitchell P, Lamoureux EL, Wong TY. Association of diabetic macular edema and proliferative diabetic retinopathy with cardiovascular disease: a systematic review and meta-analysis. *JAMA Ophthalmol* 2017;135(6):586-593.

12 Leon PE, Saviano S, Zanei A, Pastore MR, Guaglione E, Mangogna A, Tognetto D. Spontaneous or secondary to intravitreal injections of anti-angiogenic agents retinal pigment epithelial tears in age-related macular degeneration. *Int J Ophthalmol* 2014;7(4):681-685.

13 Sarraf D, Joseph A, Rahimy E. Retinal pigment epithelial tears in the era of intravitreal pharmacotherapy: risk factors, pathogenesis, prognosis and treatment (an American Ophthalmological Society thesis). *Trans Am Ophthalmol Soc* 2014;112:142-159.

14 Nicholson BP, Schachat AP. A review of clinical trials of anti-VEGF agents for diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2010;248(7):915-930.

15 Avery RL, Gordon GM. Systemic safety of prolonged monthly anti-vascular endothelial growth factor therapy for diabetic macular edema: a systematic review and meta-analysis. *JAMA Ophthalmol* 2016;134(1):21-29.

16 Maloney MH, Schilz SR, Herrin J, Sangaralingham LR, Shah ND, Barkmeier AJ. Risk of systemic adverse events associated with intravitreal anti-VEGF therapy for diabetic macular edema in routine clinical practice. *Ophthalmology* 2019;126(7):1007-1015.

17 Carnethon MR, Biggs ML, Barzilay J, Kuller LH, Mozaffarian D, Mukamal K, Smith NL, Siscovick D. Diabetes and coronary heart disease as risk factors for mortality in older adults. *Am J Med* 2010;123(6):556.e1-556.e9.

18 Shord SS, Bressler LR, Tierney LA, Cuellar S, George A. Understanding and managing the possible adverse effects associated with bevacizumab. *Am J Health Syst Pharm* 2009;66(11):999-1013.

19 Matsuyama K, Ogata N, Matsuoka M, Wada M, Nishimura T, Takahashi K. Effects of intravitreally injected bevacizumab on vascular endothelial growth factor in fellow eyes. *J Ocul Pharmacol Ther* 2011;27(4):379-383.

20 Virgili G, Parravano M, Menchini F, Evans JR. Anti-vascular endothelial growth factor for diabetic macular oedema. *Cochrane Database Syst Rev* 2014(10):CD007419.

21 Ashton N. Vascular basement membrane changes in diabetic retinopathy. Montgomery lecture, 1973. *Br J Ophthalmol* 1974;58(4):344-366.

22 Goldfine AB, Shoelson SE. Therapeutic approaches targeting inflammation for diabetes and associated cardiovascular risk. *J Clin Investig* 2017;127(1):83-93.

23 Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A. Correlation of glucose regulation and hemoglobin AIc in diabetes mellitus. *N Engl J Med* 1976;295(8):417-420.

24 Deedwania PC. Diabetes and hypertension, the deadly duet: importance, therapeutic strategy, and selection of drug therapy. *Cardiol Clin* 2005;23(2):139-152.

25 Freund KB, Korobelnik JF, Devenyi R, Framme C, Galic J, Herbert E, Hoerauf H, Lanzetta P, Michels S, Mitchell P, Monés J, Regillo C, Tadayoni R, Talks J, Wolf S. Treat-and-extend regimens with anti-VEGF agents in retinal diseases. *Retina* 2015;35(8):1489-1506.