

The Role of VEGF in the Diabetic Patients Undergoing Endovascular Therapy of Symptomatic Aortic Valve Stenosis

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Summary

The aim of this study was to explore changes in plasma vascular endothelial growth factor (VEGF) in aged patients who undergone transcatheter aortic valve implantation or balloon angioplasty for the treatment of aortic stenosis. Plasma VEGF was measured in subjects with diabetes mellitus type 2 (DM) (n=21, age 79.2±1.6 years) and in non-diabetic subjects (non-DM) (n=23, age 84.4±0.7 years), using an ELISA kit. Before the procedure plasma levels of VEGF were significantly lower in DM than in non-DM patients (P<0.05). Plasma VEGF significantly increased in both groups (DM and non-DM) 24 h (387±64 vs. 440±30 pg/ml, P<0.05) and 72 h (323±69 vs. 489±47 pg/ml, P<0.05) after the endovascular procedure. However, the VEGF in DM patients was significantly lower compared to non-DM subjects up to one month after the endovascular procedure (283±47 vs. 386±38 pg/ml, P<0.05). We conclude that increased plasma VEGF in aged patients associates with atherosclerotic aortic valve stenosis. In spite of that plasma VEGF in DM was constantly significantly lower than in non diabetic patients, both before and after the endovascular procedure, possibly reflecting a disturbance of angiogenic/anti-angiogenic balance in diabetes.

Key words

Type 2 diabetes mellitus • Aortic stenosis • Vascular endothelial growth factor

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Introduction

Aortic stenosis (AS) is the most frequent heart valve disease and the most common cause of heart valve replacement in the elderly (Otto *et al.* 1999). It is characterized by massive fibrous thickening of valve leaflets and extensive focal calcification (Ortlepp *et al.* 2006). The development and progression of AS is an active inflammatory process, including disruption of the basement membrane, subendothelial accumulation of intracellular lipids, lipoproteins and molecular mediators of calcification, together with infiltration of inflammatory cells and activation of local and systemic inflammation (Mohty *et al.* 2008, Yetkin and Waltenberger 2009). The concept of AS as an atherosclerosis-like process is supported by a number of studies showing that its development is associated with cardiovascular risk factors such as smoking, hypercholesterolemia, and arterial hypertension (Ngo *et al.* 2001, Pate 2002). However, several lines of evidence suggest differences

with regard to AS as an atherosclerosis-like process and the occurrence of diabetes mellitus (DM). Atorvastatin (Panahi *et al.* 2013) nor simvastatin with ezetimibe (Rossebø *et al.* 2008) in the randomized trials did not reduce the composite outcome of combined aortic valve events and ischemic events in non DM patients with AS. Such therapy reduced the incidence of ischemic cardiovascular events but not events related to aortic-valve stenosis (Rossebø *et al.* 2008). On the contrary, DM is associated with enhanced inflammation within AS valves, which may contribute to faster AS progression (Natorska *et al.* 2012). Diabetes mellitus has also been reported to represent a risk factor for AS, however few is known about this association despite the strong impact of DM on coronary atherosclerosis (Katz *et al.* 2006). Diabetes accompanied by inflammation is thought to play an important role in enhancing the pathophysiological mechanism of atherosclerosis (Jawień 2008).

Vascular endothelial growth factor (VEGF), a 45 kDa heparin-binding growth factor, is a determinant of the rate and extent of angiogenesis in embryonic vasculogenesis and adult vascular remodelling following physiological repair and regeneration (Battagay 1995, Dvorak *et al.* 1995). VEGF belongs to the growth factors believed to act predominantly on vascular endothelial cells. VEGF is mitogenic for endothelial cells, acting in early phases and at most of the steps in the angiogenic cascade (Ferrara 2001). Increasing evidence suggests a role for VEGF in the pathophysiology of cardiovascular disease (CVD). Elevated plasma VEGF has been shown in patients with hypertension (Felmeden *et al.* 2003) and diabetes (Hovind *et al.* 2000, Blann *et al.* 2002), with levels correlating with measures of endothelial damage/dysfunction and overall cardiovascular risk in hypertensive patients (Felmeden *et al.* 2003). Furthermore, VEGF has an independent prognostic significance in patients with acute coronary syndromes (Heeschen *et al.* 2003).

Normal angiogenesis depends on the intricate balance between angiogenic (e.g. VEGF, fibroblast growth factor – FGF2, transforming growth factor – TGF- β , and angiopoietin) and antiangiogenic factors (angiostatin, endostatin, thrombospondins) (Goodwin 2007). Although the impairment of collateral vessel development is well established in diabetic patients (Abaci *et al.* 1999), the factors responsible for this alteration are not well known. In a previous study on twenty patients that placed undergone coronary artery bypass surgery, increased VEGF expression in the

myocardium of diabetic patients in comparison with non-diabetic patients, and decreased expression levels of VEGF receptor 1 and 2, has revealed a relationship between diabetic cardiovascular complications and neovascularization dysfunction (Sasso *et al.* 2005).

For patients with severe symptomatic AS, endovascular intervention with transcatheter aortic valve implantation (TAVI) or ballon angioplasty (BA) is an effective treatment modality. Successful valve implantation or angioplasty results in marked symptom relief and age-corrected survival becomes nearly normal (Conrotto *et al.* 2014). However, there are no data on plasma VEGF and its relationship with endovascular interventions with TAVI or BA in patients with type 2 diabetes.

Thus, the aim of this work is to study the role of plasma VEGF as the strongest known angiogenic factor in the elderly diabetic patients with advanced atherosclerosis and evaluate the impact of the endovascular therapy of symptomatic severe aortic valve stenosis. We hypothesized that increased plasma VEGF would be associated with aortic stenosis. We tested our hypothesis in a cross-sectional study with elderly patients suffering from severe aortic stenosis. In addition we hypothesized that plasma VEGF levels would change after the endovascular treatment independently from diabetes mellitus. For that, we divided the study group in patients with and without diabetes.

Material and Methods

Subjects

We performed a cross-sectional study on 44 elderly patients (Table 1) aged 79.6 \pm 5.6 years who undergone transcatheter aortic valve implantation (TAVI) or ballon angioplasty (BA) for the treatment of severe aortic stenosis. The subjects were admitted at the Cardiology department of the Faculty Hospital of Hradec Králové, Czech Republic. Two of these patients had BA before TAVI (one 8 months before and the second patient 1 month before), one of them needed BA 3 months after TAVI due to perivalvular leak. We then divided the patients into diabetic and non-diabetic subjects. The study was approved by local ethical committee and all patients sign a written consent.

Study design

Fasting blood samples were obtained immediately before and then 24 h, 72 h and one month

after the cardiac procedure from every patient. Blood was drawn and centrifuged immediately. The serum was then aliquoted and stored at -80°C .

Table 1. Characteristics of subjects.

	Non DM	DM
<i>n</i>	23	21
Age (years)	84.4 ± 0.7	79.2 ± 1.6
Male	10	10
Female	13	11
BMI (kg/m^2)	26.7 ± 4.9	28.6 ± 0.9
Systolic BP (mm Hg)	133 ± 16	133 ± 19
Diastolic BP (mm Hg)	78 ± 9	71 ± 10
Total patients after TAVI	20	16
a) apical route	5	4
b) femoral route	15	12
Total patients after BA	3	5
Coronary artery disease	17 (74 %)	16 (76 %)
Peripheral artery disease	2 (9 %)	4 (19 %)
Carotid artery disease	6 (26 %)	1 (5 %)
Total cholesterol (mmol/l)	4.43 ± 1.10	3.88 ± 1.10
HDL-C (mmol/l)	1.46 ± 0.46	1.27 ± 0.34
LDL-C (mmol/l)	2.58 ± 0.96	2.25 ± 0.92
Triglycerides (mmol/l)	1.24 ± 0.60	1.13 ± 0.46

BMI – body mass index, BP – blood pressure, TAVI – transcatheter aortic valve implantation, DM – diabetes mellitus, HDL-C – high-density lipoprotein concentration, LDL-C – low-density lipoprotein concentration, BA – balloon angioplasty.

Analytical methods

The levels of plasma VEGF were obtained using an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine human VEGF, R&D Systems, USA). Intra-assay variations were <6.7 %, and interassay variations were <8.8 %. The detection range of the assay was 15.6-2000 pg/ml. We included a control group using 50 healthy blood donors without diabetes mellitus or atherosclerotic cardiovascular disease, giving us a population with 39.8±9.4 years of age, with a mean level of VEGF of 232.95±51.18 pg/ml.

Serum levels of total cholesterol (enzymatic colorimetric method), LDL, HDL cholesterol (homogenous enzymatic colorimetric test) and triglycerides (enzymatic colorimetric test) were analyzed with automatic analyzer Modular (Roche/Hitachi) using reagents from Roche diagnostics GmbH, Mannheim,

Germany. Levels of high sensitivity CRP (hsCRP) were measured by chemiluminescent immunometric assay (Immulate, DPC, USA). A value less than 0.05 denoted the presence of a statistically significant difference.

Statistical methods

The statistical evaluation was performed using SigmaStat (Systat Software, Chicago IL, USA). All data are expressed as mean ± SD, or as total number, or as median (lower quartile, upper quartile). The statistical significance was determined based on a probability level of less than 0.05. For comparison of the VEGF levels before and after the cardiac intervention, the paired t-test was used. The correlations between the levels of VEGF and other variables were calculated using Pearson's correlation coefficient. To calculate the differences between 2 groups (diabetic and nondiabetic, TAVI and BA), independent (unpaired) t-tests were used. The numerical data were tested according to normal distribution. Mann Whitney test was used to assess the differences between obese DM patients vs. non-DM patients and Wilcoxon test for the evaluation of differences between the individual phases of observation of DM and non-DM patients.

Results

Concerning in-hospital combined safety endpoints (all-cause mortality, major stroke, periprocedural myocardial infarction, life-threatening bleeding, major vascular complication, and acute kidney injury), one patient (3 %) died due to a cerebrovascular event after TAVI.

Besides the treatment of DM (Table 1), the patients were given statins (77 %), antiplatelet agents (91 %), B-blockers (93 %), diuretics (98 %), warfarin (7 %), LMWH (2 %), sartans (36 %), or ACE inhibitors (45 %). Among DM patients, 83 % were treated by statins and 97 % by antiplatelet drugs.

Plasma VEGF in DM ($P=0.032$) and non-DM ($P<0.001$) patients at the baseline was significantly higher in comparison to the healthy subjects before any intervention was done (Table 2).

The patients were then further divided into diabetic and nondiabetic groups to compare the levels of VEGF between them. In the diabetic group, all patients were included without differentiation, if they were on diet, peroral antidiabetics, or insulin therapy. Using the independent samples t-test, we concluded that the levels

VEGF are statistically significantly different between them. Plasma VEGF significantly increased after the endovascular procedure in both groups (with and without DM, $P < 0.05$) and returned to the baseline values after one month after treatment. However, the VEGF in DM patients was constantly significantly lower than in non diabetic patients ($P < 0.05$) (Fig. 1). Recently, it has been shown that statins used in patients with type 2 diabetes reduced the serum concentration of VEGF (Dworacka *et al.* 2014), however when we analyzed our DM patients based on the concomitant use of statins, we did not measure any significant differences in plasma VEGF.

Table 2. Plasma VEGF in the non-DM and DM patients.

Group	n	Age (years)	Plasma VEGF (pg/ml)	P
Non-DM	23	84.4 ± 0.7	418 (329-555)	-
DM	21	79.2 ± 1.6	331 (242-467)	0.017

Data are shown as medians (interquartile range). Plasma VEGF in healthy blood donors without diabetes mellitus or any atherosclerotic cardiovascular disease, aged 39.8±9.4 years was 219 (209-260) pg/ml. Statistical significance is indicated by P between non-DM and DM patients.

Plasma CRP concentration before treatment was non-significantly higher in the DM patients. Its level however increased with the endovascular intervention in both non-DM and DM patients 24 and 72 h after treatment. 1 month after treatment CRP returned to the baseline (Table 3).

There were no statistical differences between the diabetic and nondiabetic groups concerning levels of total cholesterol, HDL, LDL, and triglycerides as well as BMI, and systolic and diastolic blood pressures. There was not any significant correlation between VEGF and total

cholesterol, HDL, LDL, and triglycerides as well as BMI, and systolic and diastolic blood pressures.

Furthermore, using the independent samples t-test we noticed that the levels of VEGF before and after the procedure are not statistically significant when comparing it between the TAVI and BA groups ($P = 0.55$ and $P = 0.75$, respectively). This observation concluded that the plasma VEGF significantly increased in the same proportion after manipulation with the aortic valve, regardless of the interventional method.

Discussion

In this study, we have shown that in elderly patients with nonrheumatic stenotic aortic valves, 1) there is a profile of raised plasma VEGF levels; 2) endovascular intervention with transcatheter aortic valve implantation or balloon angioplasty is associated with increases in plasma levels of VEGF and returns to the baseline values 1 month after treatment; 3) changes in plasma levels of VEGF are not related to indexes of metabolic control, i.e. total cholesterol, HDL, LDL, and triglycerides as well as BMI, and systolic and diastolic blood pressures; 4) plasma VEGF in DM patients was constantly significantly lower than in non diabetic patients, suggesting an altered metabolism of proangiogenic VEGF and thus neovascularization dysfunction by overt disease.

Several lines of evidences suggest that the mechanism of aortic valve degeneration is similar to that of atherosclerosis, and accordingly, more patients are suffering from degenerative aortic valve disease. Although the molecular mechanisms underlying the onset of aortic valve degeneration are largely unknown, research in this area is advancing rapidly. Among other factors, investigations into angiogenesis in the aortic valve have been reported (Hakuno *et al.* 2009). Having

Table 3. Plasma CRP in the non-DM and DM patients.

CRP (mg/l)	Non-DM	DM	P
Before procedure	2.91 (1.91-4.37)	3.40 (2.54-7.46)	0.139
After 24 h	23.80 (15.32-43.97)	28.00 (12.72-45.07)	0.860
After 78 h	67.10 (26.40-127.00)	68.30 (22.30-97.80)	0.488
After 1 month	5.67 (3.24-8.54)	3.80 (2.00-6.23)	0.059

Plasma CRP (mg/l) in patients with advanced atherosclerosis and diabetes mellitus type 2 (DM) (n=21, age 79.2±1.6 years) and non-diabetic subjects (non-DM) (n=23, age 84.4±0.7 years) with symptomatic severe aortic stenosis undergone endovascular therapy by transcatheter aortic valve implantation. Data are shown as medians (interquartile range). CRP was measured before treatment, 24 h, 72 h and 1 month after treatment. P indicates statistical significance in non-DM patients in comparison to the DM patients.

noted avascularity of normal cardiac valves, disturbances between crucial angiogenic and anti-angiogenic factors in human atherosclerotic valves have recently been identified and the role of vascular endothelial growth factor is being recognized. The normal cardiac valves have specific protective mechanisms to inhibit pathologic angiogenesis, and once avascularity is lost and pathologic vascular invasion occurs in these tissues, severe diseases may ensue. In contrast, histopathologic analysis of tissues from human calcified aortic valves typically shows the expression of the potent angiogenic factor VEGF with numerous blood vessels growing into these valves (Hakuno *et al.* 2009). The potent proangiogenic factors, VEGF, and its receptors, VEGF receptor 1 (VEGFR-1) and VEGFR-2, are all locally expressed in aortic valves and upregulated in stenotic valves (Soini *et al.* 2003). Possible sources of VEGF in aortic valves include endothelial cells, myofibroblasts (Rajamannan *et al.* 2005) and mast cells, which have been shown to release VEGF when activated/stimulated (Grutzkau *et al.* 1998). Raised plasma VEGF levels in our group of non-DM patients with nonrheumatic aortic stenosis are in agreement with that concept.

The procedure of transcatheter aortic valve implantation or balloon angioplasty for the treatment of aortic stenosis lead to a dramatic increase in plasma VEGF concentration. While myocardial infarction following TAVI is relatively rare, with reported rates in the range of one percent (Rodés-Cabau *et al.* 2010), myocardial injury that does not meet the universal definition of myocardial infarction appears to be more common, with reported troponin elevations in as many as 95 to 100 percent of patients (Barbash *et al.* 2013). The mechanism of extremely elevated VEGF levels post TAVI in our patients should be a finding on a similar basis to the study of Heeschen *et al.* (2003), where the patients with acute coronary syndrome were stratified into quartiles according to their measured plasma VEGF levels. Their study population was dichotomized according to the level of VEGF 0.3 pg/l, and the patients with VEGF levels equal or above 0.3 pg/l had more frequently ST segment depression, transient ST-segment elevation, recurrent ischemia CK-MB elevation and troponin elevation. VEGF elevation thus correlated with the evidence of myocardial ischemia.

The patients with DM and nonrheumatic aortic stenosis showed a similar pattern of plasma VEGF as the non-DM patients in our study. Their plasma VEGF was significantly increased at the baseline, and further

increased after TAVI or BA. These findings were in agreement with the previous studies of March *et al.* (2000) and Valabhji *et al.* (2001) who observed that serum VEGF increased significantly in diabetic patients compared with healthy control subjects. Malamitsi *et al.* (1998) reported the same observation that serum VEGF increased significantly in diabetic patients compared with control subjects, and added that diabetes is characterized by increased angiogenic response in various organs.

Recent observations made in human aortic valves from patients with AS demonstrated increased expression of CRP and showed that the amount of pro-inflammatory proteins increased with the progression of AS (Toli *et al.* 2008). Plasma CRP concentration before treatment was non-significantly higher in our aged DM patients. In agreement with the above findings its level increased with the endovascular intervention in both non-DM and DM patients 24 and 72 h after treatment. One month after treatment CRP concentration returned to the baseline. It has been proposed that inflammation plays a crucial intermediate role in the pathogenesis of glucose disorders in the elderly (Barzilay *et al.* 2011), thereby linking diabetes with a number of commonly coexisting chronic inflammatory conditions thought to originate from inflammatory mediators, such as CRP. An independent association of CRP with AS progression has been also previously documented by Galante *et al.* (2001) giving a new understanding of the physiological role of CRP in stenotic valves, by promoting local proinflammatory effects.

We report that plasma VEGF in our elderly DM patients with nonrheumatic aortic stenosis was constantly significantly lower than in non diabetic patients, both before TAVI or BA and after the endovascular procedure. Our findings are in consistence with the general debate regarding the role of VEGF in diabetes and atherosclerotic cardiovascular disease and add information regarding diabetic patients (Inoue *et al.* 1998, Blann *et al.* 2001, Malamitsi-Puchner *et al.* 2001). From literature, serum VEGF is not raised in children with type I diabetes compared with age matched controls (Malamitsi-Puchner *et al.* 2001).

Moreover, VEGF is raised in the patients with diabetes plus atherosclerosis, but not in the group with diabetes alone (Blann *et al.* 2002); and our data (Fig. 1) suggest that this may be also the case in the elderly DM patients. Atherosclerotic tissue, within the artery wall, plaque or in microvessels, overexpress VEGF (Inoue *et al.* 1998), and a positive correlation between coronary

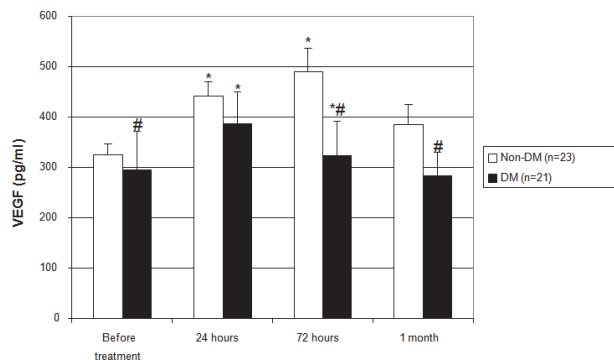


Fig. 1. Plasma VEGF before and after endovascular procedure in non-DM and DM patients. Vascular endothelial growth factor (VEGF) in patients with diabetes mellitus type 2 (DM) (n=21, age 79.2 ± 1.6 years) and non-diabetic subjects (non-DM) (n=23, age 84.4 ± 0.7 years) with symptomatic severe aortic stenosis undergone endovascular therapy by transcatheter aortic valve implantation (n=35) or balloon angioplasty (n=9). Measurements are expressed as mean \pm SD. VEGF was measured before treatment, 24 h, 72 h and 1 month after treatment. Statistical significance is indicated by * $P < 0.05$ during the time, # $P < 0.05$ DM vs. non-diabetic patients.

artery collaterals and intra-coronary artery levels of plasma VEGF has been reported (Fleisch *et al.* 1999). Although we found lowered plasma VEGF levels in the group of patients with nonrheumatic aortic stenosis and DM, but not in those without DM, it is not clear whether, overall, VEGF levels are reduced in diabetic atherosclerosis free cases. It is therefore curious that plasma VEGF levels were lower in patients within the diabetic group (Fig. 1). The reasons for lower plasma VEGF in our elderly DM with aortic stenosis are unclear. One of the possible explanation could be some metabolic or treatment aspects of diabetes and dyslipidaemia. When we analyzed our nonDM or DM patients based on their treatment modality (diet, per oral antidiabetic agents or insulin) or the concomitant use of statins or antiplatelet drug therapy, we did not measure any significant differences in plasma VEGF. Another explanation was highlighted in the observations of Valabhji *et al.* (2001) who reported a significant correlation between serum VEGF and glycaemic control detected by HbA1c. However, we do not have a complete set of data on HbA1c in our patients. Other metabolic parameters used in our study such as total cholesterol, HDL, LDL, and triglycerides as well as BMI did not correlate significantly with plasma VEGF. Other molecular mechanisms which were not measured in our study are the paradoxical changes in the expression of VEGF and its receptors in the myocardium and microvessels. The major cell types in cardiac tissue include cardiac

myocytes, fibroblasts, and endothelial cells, all of which have been shown to produce VEGF. In the study of Chou *et al.* (2002), VEGF and its receptors in myocardium were decreased in insulin-resistant and diabetic states. One potential mechanism for decreased VEGF expression in myocardium in diabetic and insulin-resistant states was reported to be a loss of insulin-induced VEGF expression (Miele *et al.* 2000). Whether VEGF receptors could be similarly altered, and which signaling pathways may be involved, has yet to be assessed. Nevertheless, these findings are suggestive that the ability of insulin to induce VEGF expression may be inhibited in myocardium exposed to metabolically abnormal states such as insulin resistance, which is often present in poorly controlled diabetic and insulin-resistant patients. The decreased plasma VEGF in our DM patients may reflect the impaired production of VEGF, but we did not address that issue in our study and thus it would be unwise to speculate further without additional power.

Several limitations of our study should be taken into account. The first and most important drawback is the small number of subjects included in these investigations. However, patients were investigated four times during the observation period of one month to obtain a detailed impression of the plasma VEGF levels before and after transcatheter aortic valve implantation or balloon angioplasty for the treatment of aortic stenosis. The plasma concentrations of VEGF were measured up to one month of the experimental protocol but no longer, which might have underestimated the association between the investigated factors and VEGF during the natural development of the disease. Nevertheless, both groups were well matched. Second, the severity, and the duration of DM may not be the same in patients from the DM group. Thirdly, the mean age of these subjects implies the coexistence of multiple comorbidities, some of which may influence the VEGF metabolism. Additionally, the present study is focused on the patients with severe symptomatic aortic stenosis. Once the valve is heavily stenotic and calcified, inflammation may play less of a role in progression of AS than in a valve with less severe disease and the influence of DM on AS may be masked to some extent. Therefore, our results cannot be evaluated using the current approach and extrapolated on subjects with mild AS. Fourth, patients from DM group had significantly higher BMI (28.3 kg/m^2 ; $26.6\text{-}31.4$) than the non-DM patients (26.2 kg/m^2 ; $23.2\text{-}28.2$) (medians; interquartile range) ($P=0.04$), and obesity mediated production of atheroinflammatory

markers is an important mechanism for its systemic elevation (Barzilay *et al.* 2011), which could partially influence the results. Finally, glycosylated hemoglobin, a retrospective DM indicator, has not been measured which could be another reason of the changes in plasma VEGF in DM patients (Valabhji *et al.* 2001).

In summary, the results of the present study bear out the involvement of increased plasma VEGF in advanced nonrheumatic aortic valve stenosis in elderly patients. Because plasma VEGF levels are selectively lower in patients with AS and diabetes we suggest that VEGF can act as a target for many new therapeutic modalities with the aim to induce angiogenesis and improve collateralization in the elderly diabetic patients with reparative endovascular therapy of aortic stenosis. Further studies are warranted to determine the role of

angiogenic/anti-angiogenic balance including the role of both systemic and locally acting VEGF in aortic valve disease and in the patients with diabetes mellitus. Also, further investigations are needed to define whether interventions that directly target VEGF balance in diabetes might retard or reverse the progression of nonrheumatic aortic stenosis.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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