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INTRA-VITREAL ANTI-VEGF AGENTS AND CARDIOVASCULAR RISK

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Abstract

Antagonists of Vascular Endothelial Growth Factor (Anti-VEGF) are widely administered by intravitreal injection for the treatment of ocular pathologies such as Age-related Macular Degeneration, Diabetic Macular Edema, Proliferative Diabetic Retinopathy and occlusion of retinal vessels. Anti-VEGF agents, in particular bevacizumab, were introduced in oncology to inhibit tumor-induced angiogenesis feeding neoplastic tissues. Subsequently, other specific agents were developed for intraocular administration. Whereas systemic administration of anti-VEGF agents in oncology is burdened by increased risk of arterial hypertension and embolism, agents administered for ophthalmic indications are delivered locally into the eye globe in much smaller quantities. Nevertheless, clinical observations have raised the possibility that, even in these conditions, anti-VEGF agents may increase cardiovascular risk in patients who, being elderly and/or diabetic, are intrinsically prone to such events. This paper aims at reviewing the current knowledge on VEGF and its pharmacologic antagonists from mechanistic and side-effect points of view, with specific reference to patients with sight-threatening conditions. Internists should be aware of the need to collaborate with ophthalmologists and pharmacovigilance operators to define as best as possible the risk/benefit balance of intravitreal agents in patients who might lose their sight if left untreated, or increase their risk of suffering a cardiovascular event if treated.

Key Words:

Vascular Endothelial Growth Factor, diabetes mellitus, diabetic retinopathy, anti-VEGF agents, cardiovascular risk.

Glossary:

AMD = Age-related Macular Degeneration

BRB = Blood Retinal Barrier

CSME = Clinically Significant Macular Edema

DME = Diabetic Macular Edema

DR = Diabetic Retinopathy

HIF-1 α = Hypoxia Inducible Factor 1 α

PDR = Proliferative Diabetic Retinopathy

RVO = Retinal Vessel Occlusion

VEGF = Vascular Endothelial Growth Factor

Introduction

Antagonists of Vascular Endothelial Growth Factor (Anti-VEGF) are widely administered by intravitreal injection for the treatment of ocular pathologies such as Age-related Macular Degeneration (AMD), Diabetic Macular Edema (DME), Proliferative Diabetic Retinopathy (PDR) and Retinal Vessel Occlusion (RVO). Apart from economic and regulatory issues which will not be discussed in this review, their administration presents a number of potential clinical problems because they may intrinsically increase cardio-embolic risk in patients who, being elderly and/or diabetic, are already prone to such events. Consequently, internists should be aware of the need to collaborate with ophthalmologists and pharmacologists or pharmacovigilance operators to define as best as possible the risk/benefit balance of intravitreal agents in patients who might lose their sight if left untreated, or increase their risk of suffering a heart attack or stroke if treated.

This paper aims at reviewing the current knowledge on VEGF and its pharmacologic antagonists from mechanistic and side effect points of view, with specific reference to patients with sight-threatening conditions.

VEGF and angiogenesis

VEGF plays a key role in the process of angiogenesis, both in physiological conditions, such as wound repair and endometrial reconstruction during the menstrual cycle, and in tumor growth or retinal neo-vascularization during PDR, retinopathy of prematurity, and sequelae of retinal vein thrombosis. Senger et al (1) described in 1983 a "Vascular Permeability Factor" (VPF) with the ability to increase vascular permeability. Ferrara and Henzel, in 1989, highlighted its mitotic effect on endothelial cells and proposed the name VEGF (2). Later, it was understood that there is a whole family of VEGFs: VEGF-A (the most important, usually referred to), VEGF-B, -C, and -D (implicated in embryonic and lymphatic angiogenesis), and placental PlGF, which may act to promote uterine angiogenesis and vascular permeability within the placental bed (3).

VEGF isoforms are the result of different splicing of the same 8-exon gene. The first product of transcription leads to forms that are structurally similar but have specific functions. Further molecules of the same family are: VEGF-E, of viral origin, and VEGF-F isolated from snake venom (4-5). VEGFs are produced, among others, by endothelial cells, pericytes, keratinocytes, fibroblasts, macrophages and placenta. They bind to trans-membrane tyrosine kinase receptors, inducing their dimerization and trans-phosphorylation. These receptors have an extracellular domain similar to immunoglobulins, a portion located in the thickness of the cell membrane and an intra-cytoplasmic one. The VEGFR-2 receptor (KDR/FLK1) mediates almost all the effects of VEGF-A, while VEGFR-1 (Flt-1) appears to modulate the activity of VEGFR-2 (6). A third receptor (VEGFR-3) binds VEGF-C and D. Finally, it was recently found that Neuropilin-1, a trans-membrane protein lacking tyrosine kinase activity, acts as a co-receptor for VEGF-A (7) (**Figure 1**)

There are two isoforms of VEGF-A, coded 165-a and 165-b. The former increases vascular permeability, promotes the development of new vessels and has a pro-inflammatory effect, whereas VEGF 165-b has opposite properties as it inhibits new vascularization and is an anti-inflammatory, cyto- and neuro-protective factor (8). VEGF 165-a is increased, relative to 165-b, in PDR (9) and appears to promote inflammation and retinal exudates. Indeed, the development of new vessels from pre-existing vessels through morphogenesis requires endothelial cell destabilization, increased vascular permeability, mesenchymal transition of endothelial cells, proliferation and differentiation of new endothelial cells (10). Such destabilization requires the intervention of angiopoietin 2, which is produced by the same cells and induces the transition to mesenchymal cells expressing markers such as calponin, snail, and transgelin FSP-1 (11). These cells detach from the others and lose their relationship with supporting pericytes, becoming at high risk of “anoikis”, a type of apoptosis due to the lack of adhesive consent with the other cells and the basal membrane. However, they are “saved” by VEGF, which activates the anti-apoptotic PI3K-AKT pathway and causes them to proliferate and migrate to form new immature vessels (angiogenic switch and sprouting) (12).

Role of VEGF in systemic retinopathies

Most retinal conditions associated with systemic diseases such as diabetes, hypertension and thrombosis are characterized by neovascularization consequent to vascular occlusion and ischemia.

Hypoxia stimulates the release of Hypoxia Inducible Factor 1 α (HIF-1 α), which is usually hydroxylated in its proline residues by prolyl-hydroxylases (a reaction that requires oxygen) and degraded by ubiquitin VHL in the proteasome within a “futile cycle” (13-14). In hypoxia, HIF-1 α is not adequately hydroxylated and, consequently, not degraded and enters the nucleus where it binds to HIF-1 β . This complex stimulates the transcription of the VEGF-A gene (15) by endothelium, pericytes and retinal pigment epithelium (13-14). The free radical nitric oxide (NO) produced by endothelial NO Synthase (eNOS) plays the role of mediator in the angiogenic response supported by VEGF either through the activation of PI3K or through the PLC-gamma pathway. Once activated, both pathways converge on AKT phosphorylation of eNOS, releasing NO. VEGF also regulates vascular permeability by promoting endocytosis of VE-Cadherin (also known as cadherin 5) and regulation of Rho GTPase at the cell surface (16).

Thus, NO is crucial for the regulation of cytoskeletal architecture, leading to reversible changes in vascular permeability *in vivo*. However, the molecular mechanisms through which endogenous NO modulates endothelial permeability are not completely clear (17). Hence, VEGF is necessary for the survival of retinal endothelial cells too. Experimental studies in animals show that local inhibition of VEGF causes thinning and atrophy of the choriocapillaris and degeneration of photoreceptors (18).

There are many other regulatory factors of retinal angiogenesis, acting in response to hypoxic stimuli (19-20). Some are inhibitors, such as thrombospondin for fibroblasts, which cause proliferating cells to release

Fas ligand in a soluble form. This factor has no action on endothelial cells of mature vessels but induces apoptosis of immature ones in new vessels, which express a specific receptor (21). Null mice that do not produce thrombospondin exhibit marked vascularization and tumor growth (22). Pigment Epithelium Derived Factor (PEDF) is important for the integrity of the Blood-Retinal Barrier (BRB) and its deficiency seems to favor proliferation of retinal vessels and the development of DME (23). Basic fibroblast growth factor (bFGF) can also induce angiogenesis. Systemic administration of nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, to rabbits bearing a corneal implant blocked VEGF but not bFGF induced angiogenesis: bFGF induce angiogenesis by different mechanistic pathway (24).

The imbalance between these factors promotes the transition from non-proliferative to proliferative retinopathy. Loss of autoregulation and capillary occlusions, especially in the peripheral retina, are the initial events providing the hypoxic stimulus that increases release of HIF-1 α (25). At the same time, factors closely related to hyperglycemia, such as AGE, PKC, RAS, IGF-1, FGF, and PDGF, contribute to stimulate the release of VEGF-A (26-27).

New vessels originate from small and medium caliber venules, though not capillaries. However, the first visible lesions, microaneurysms developing at the edge of non-perfused retinal areas, involve retinal capillaries. Microaneurysms are regarded as unsuccessful attempts to give rise to new-vessels, and their count in early diabetic retinopathy (DR) is an important prognostic indicator for its worsening (28).

New vessels represent an attempt to vascularize ischemic areas in the peripheral retina. Unfortunately, the consequences are harmful because new vessels bleed easily and are associated to fibrosis due to myofibroblasts partly derived from circulating fibrocytes and partly from the same endothelial cells undergoing the above-mentioned mesenchymal transition (**Figure 2**) (29).

In 1994, Aiello et al measured VEGF in intra-ocular fluids of 164 patients with retinal neovascularization associated with diabetic retinopathy, central retinal vein occlusion and *rubeosis iridis*, who had been subjected to vitrectomy or cataract surgery. They found increased levels of VEGF in those with proliferative compared to those with non-proliferative DR, quiescent PDR or who were not diabetic and were able to observe reduction of VEGF after laser treatment, confirming the efficacy of pan-retinal photocoagulation in reducing hypoxia by photodynamic ablation of the ischemic peripheral retina. In addition, VEGF stimulated the growth of retinal endothelial cells *in vitro*, as did vitreous fluid containing measurable VEGF, and this stimulation was inhibited by VEGF-neutralizing antibodies (30).

The other type of high-risk DR, DME, is today the leading cause of visual impairment in diabetes. Initially cytotoxic, from intracellular accumulation of sorbitol, lactate and phosphates, DME becomes vasogenic, due to the intervention of inflammatory factors that loosen the tight junctions of the BRB. Small cystic fluid collections form in the inner layer of the macular area and can extend over the retinal surface with detachment of the neuro-retina (31). Both DME and PDR are mediated by an increase in VEGF-A 165a (32).

Intravitreal administration of VEGF-A 165 b reduces retinal and choroidal vascularization (33-34) and increases the release of protective factors (35).

Anti-VEGF agents and cardiovascular risk

Anti-VEGF agents, in particular Bevacizumab, were initially introduced in oncology to inhibit tumor-induced angiogenesis feeding neoplastic tissues. Subsequently, Bevacizumab and other specific agents were developed for intraocular administration in the treatment of AMD, high-risk DR and other conditions characterized by retinal edema and/or neovascularization (36-37). Since VEGF protects blood vessel integrity, prolonged systemic administration of anti-VEGF agents in oncology is burdened by side effects (due to the lack of normal function of VEGF) as increased risk of arterial hypertension and embolism (**Figure 3**) (38-39). Hypertension is commonly observed during bevacizumab systemic treatment for cancer. All these patients are recommended to have blood pressure monitored every two to three weeks and in case of hypertension to initiate anti-hypertensive therapy. Hypotheses for the mechanisms of hypertension induced by systemically administered Bevacizumab include: increased vascular tone due to inhibition of VEGF-mediated vasodilatation with decreased NO and prostacyclin, increased endothelin-1, reactive oxygen species (ROS) and arterial stiffness, reduction of capillary density, activating of renin-angiotensin system with renal injury (40).

When used for ophthalmic indications, these agents are administered locally into the eye globe and in much smaller quantities but many studies have investigated the possibility that anti-VEGF agents may increase cardiovascular events in patients with diabetes, old age or both even if administered intraocularly. Some considerations should be made in this respect:

- Diabetes *per se* is associated with a risk of myocardial infarction equivalent to that of non-diabetic people who have already suffered a heart attack (41), so much so that the American Diabetes Association and American Heart Association recommend to consider diabetes a coronary artery heart disease equivalent rather than a mere risk factor (42).
- DR is a manifestation of generalized micro- and macrovascular degeneration and is associated with a further increase in cardiovascular risk (43-44).
- Type 2 diabetic patients with retinopathy are at greater risk of small vessel occlusions in the brain, indicating that the central nervous system may be as much a site of microangiopathy as the retina and kidneys (45).
- The hospitalization rate for stroke and myocardial infarction in individuals with DME is respectively 2 and 2.5 times higher than in diabetic patients without DME (46).
- Retinal lesions, even isolated small microhemorrhages and cotton wool spots, are found in up to 10% of the non-diabetic, non-hypertensive population and are associated with increased risk of ischemic heart disease, stroke and heart failure (47).

Anti-VEGF agents used in ophthalmology include Bevacizumab (Avastin), Pegaptanib (Macugen), Ranibizumab (Lucentis) and Aflibercept (Eylea) (**Fig 4**). Bevacizumab is a humanized function-blocking monoclonal murine antibody with a molecular weight of 149 kDa. It was the first anti-VEGF agent approved by the FDA for systemic use in oncology. Pegaptanib (no longer in use) is a pegylated aptamer, a small RNA fragment with a molecular weight of 5 kDa and is the only anti-VEGF designed to bind only the VEGF 165 isoform blocking its interaction with its receptors on endothelial cells. Ranibizumab is a recombinant humanized immunoglobulin G1k isotype antibody fragment with a molecular weight of 48 kDa. It can bind all VEGF-A isoforms. Ranibizumab is three times smaller than bevacizumab, and its intravitreal half-life is 75% shorter. Aflibercept, the latest approved in Europe and America, is a 115 kDa soluble decoy receptor is made up by the second domain of VEGF-R1 and the third domain of VEGF-R2, fused to the Fc region of a human IgG1. It acts as a competitive inhibitor of VEGF, also binding to PlGF.

Two more drugs will become available in the near future: Combercept and Abicipar Pegol. The former, currently approved in China, is a 141 kDa recombinant fusion protein of the VEGF receptor domains with the Fc fragment of a human immunoglobulin. The difference between Combercept and Aflibercept is the presence of a fourth VEGF-R2 binding domain in Combercept, which stabilizes the receptor-ligand complex (48). Abicipar Pegol, already used in oncology and asthma, binds with high affinity to all soluble isoforms of VEGF-A, similar to Ranibizumab, but has smaller molecular weight (34 kDa vs 48 kDa), longer ocular half-life and higher efficacy, as 6-8 injections are comparable to 13 Ranibizumab injections (49).

Bevacizumab does not have an ophthalmic indication and is administered off label in doses on average 150 times lower than those used in oncology (50), whereas the other three are registered specifically for ophthalmic use.

Bevacizumab and Aflibercept maintain plasma levels close to their IC50 (the concentration necessary to inhibit 50% of VEGF biological activity) as long as 30 days after intravitreal administration, which makes cardiovascular side-effects potentially possible (51). Ranibizumab has a lower half-life because, in contrast with the other two molecules, does not contain the Fc component (52). Matsuyama et al showed that the blood concentrations of both VEGF and PDGF are reduced within 24 hours of a single intravitreal administration of Bevacizumab and that the effect lasts for at least 1 month (53). Avery et al in 2017 demonstrated that Ranibizumab clears from the bloodstream more quickly than Bevacizumab or Aflibercept. Aflibercept results in the greatest reduction in plasma free VEGF, whereas Ranibizumab produces the smallest decrease in plasma free VEGF. Generally, the reduction in plasma free VEGF levels correlates with elevated levels of circulating anti-VEGF (54).

Lee et al in 2018 assessed VEGF plasma levels after treatment with intravitreal Aflibercept, Bevacizumab or Ranibizumab in 436 participants at baseline and 4, 52 and 104 weeks later. Their study suggests that

decreases in plasma VEGF levels are greater with Aflibercept or Bevacizumab compared to Ranibizumab confirming Avery's findings (55).

Usually, these agents are injected once a month for 3 months and can be further administered monthly or every 2 months, according to clinical judgement based upon anatomical and visual outcomes. In 2019 Busch et al, reported the 2-year follow-up outcomes of non responding DME after three monthly anti-VEGF injections vs early switch to dexamethasone (DEX) implant and showed a beneficial effect of an early switch to DEX implant in DME non responders, at month 12, maintained during the second year, suggesting a role for VEGF-independent inflammatory mechanisms. A later switch from anti-VEGF to steroids still provides a significant improvement of visual acuity (56) .

Kamba et al reported a decrease in both NO and prostacyclin after systemic anti-VEGF administration, accompanied by increased arterial pressure and endothelial cell detachment, potentially favoring the occurrence of thrombotic events (38). Zarbin, in 2018, recognizing that systemic administration of anti-VEGF agents is associated with these complications, stated that the reduction in free plasma VEGF levels following intravitreal injection may lead to similar consequences. Although the frequency of systemic serious adverse events after intravitreal administration is low, patients with recent stroke or other events may be at higher risk of systemic complications (57). More so, the possibility that repeated intraocular administration of anti-VEGF agents is associated with systemic disorders is real, although it remains difficult to discriminate how much of the risk is due to the drug and how much to the underlying systemic pathology itself, old age and lifestyle.

Clinical trials, reviews and meta-analyses

Several studies were carried out over the last 20 years, including systematic reviews and meta-analyses, to shed light on this problem but the results are still inconclusive. Potential biases include the facts that patients enrolled in clinical trials, and therefore in the meta-analyses that followed, are often not representative of people subjected to intravitreal treatments in real life.

The Pan American Collaborative Study group (PACORES), published in 2008, was a retrospective survey of 1173 patients with and without diabetes, followed monthly for one year after treatment with bevacizumab. Systemic adverse events were as follows: 7 (0.59%) cases of acute elevation of systemic blood pressure, 6 (0.5%) vascular accidents, 5 (0.4%) myocardial infarctions, 2 (0.17%) iliac artery aneurysms, 2 (0.17%) toe amputations and 5 (0.4%) deaths. Ocular complications included 7 (0.16%) cases of bacterial endophthalmitis, 7 (0.16%) tractional retinal detachments, 4 (0.09%) cases of uveitis and 1 each of retinal detachment and vitreous detachment (58). Rajendram et al, in 2012, reported the 2-year outcomes of the BOLT study, a prospective randomized controlled trial evaluating intravitreal Bevacizumab in 80 patients with persistent clinically significant macular edema (CSME). This study provided evidence supporting longer-term use of intravitreal Bevacizumab for CSME but found myocardial infarction in 4.8% of patients

and one fatality. However, no significant differences were noticed between the patients on Bevacizumab and those treated with laser, suggesting that off label Bevacizumab may be safe in patients with DME (59). On the other hand, one more recent retrospective case-control study, with a maximum follow-up of 73 months, enrolled over 5000 patients with AMD treated with Bevacizumab and showed an increased risk of death from all causes (OR 1.69; CI 95% 1.54-1.84, $p < 0.001$) (60).

RISE and RIDE, two phase-3, randomized, multicenter, double-masked trials enrolled 759 patients with DME. These were randomized equally to 0.3 or 0.5 mg monthly Ranibizumab or sham injections. The incidence of adverse events was higher in the patients who received 0.5 mg Ranibizumab compared to those receiving 0.3 mg Ranibizumab or sham (19.7, 16.8 and 13.1 respectively). The incidence of central nervous system hemorrhage, congestive heart failure, hypertension, gastrointestinal perforation, proteinuria and wound-healing complications appeared increased in patients on Ranibizumab (61). In 2014 the extension of RESTORE, a phase-3 randomized multicenter study, evaluated long-term efficacy and safety of Ranibizumab during 3 years of treatment in 240 patients with DME. No cases of endophthalmitis, retinal tear or retinal detachment were reported. The most frequently reported adverse effects over 3 years were cataract (16.3%) and nasopharyngitis (23.3%). Eight deaths were reported during the extension study, but none were suspected to be related to the drug (62).

Aflibercept was studied in the VIVID and VISTA trials, which compared efficacy and safety of intravitreal injection against laser photocoagulation for DME over 3 years (148 week) in 872 eyes, showing good safety (63). However, two years later (2018), Sobolewska et al, showed a potential risk of thromboembolic events for Aflibercept explained by increased activation of the fibrinogen receptor GPIIb/IIIa and the presence of a direct interaction between the drug and platelets (64).

However, most trials are sponsored by pharmaceutical companies for registration purposes, adding potential biases. For example, in VIVID and VISTA adverse effects were not related to Aflibercept if they occurred 30 or more days after the last injection even if we know that, as mentioned above, the drug is still present in the blood. Finally, the interactions between anti-VEGF and other therapies and the remark that angiogenesis is partially independent of VEGF (65-66) make it difficult to analyze the results of each study. Some reviews comparing Bevacizumab to Ranibizumab suggested that the former increases systemic events by 20-35% (67-68). In particular, two randomized controlled trials in patients with AMD: CATT, including 128 patients in the USA, and IVAN on 610 patients in the UK suggest a marginal superiority of Ranibizumab in terms of visual acuity gain. In IVAN there were more atherothrombotic events and heart failure with Ranibizumab. Other adverse events were more frequent with Bevacizumab, including infections, palpitations, and gastrointestinal disorders. However, CATT and IVAN were not powered to look at safety, as their sample sizes were too small (69).

Other authors have associated Ranibizumab with increased risk of extraocular bleeding (70). However, publication bias should be considered, and some conclusions were not confirmed in 2014 by Moja et al,

who also examined unpublished studies. Their systematic review of non-industry sponsored studies could not reveal differences between intravitreal Bevacizumab and Ranibizumab for death and all serious systemic adverse events in the first two years of treatment, with the exception of gastrointestinal disorders, for which there was a higher risk with Bevacizumab (71).

In a meta-analysis of 13 RCT, Cheng et al, in 2012, evaluated 4942 patients with AMD, DR and RVO treated with anti-VEGF vs controls without showing increased arterial thromboembolic events (72).

In 2014, Thulliez et al, in a meta-analysis of 21 trials that evaluated 9557 patients with DME, AMD and RVO subjected to anti-VEGF therapy with Bevacizumab or Ranibizumab compared to no anti-VEGF treatment, concluded for no increase in the risk of major cardiovascular events (OR 1.18; CI 95% 0.81-1.71), stroke (OR 1.61; CI 95% 0.85-3.05), overall mortality (OR 1.53; CI 95% 0.92-2.56), non ocular hemorrhagic events (OR 1.42; CI 95% 0.95-2.13), hypertension (OR 0.97; CI 95% 0.71-1.32), or myocardial infarction (OR 0.92; CI 95% 0.54-1.59). Furthermore, no significant difference was found between patients treated with lower or higher doses of Ranibizumab (0.3 vs 0.5 mg, only the latter being registered in Europe) with regard to the risk of major cardiovascular events (OR 0.86; CI 95% 0.62-1.21) and extraocular hemorrhages (OR 0.92; CI 95% 0.67-1.26). However, that paper showed a greater risk of venous thromboembolism associated with Bevacizumab compared to Ranibizumab (OR 3.45; CI 95% 1.25-9.54) and an increased risk of non-fatal extraocular hemorrhages associated with Ranibizumab compared to controls not on anti-VEGF agents in patients with AMD (OR 1.55; CI 95% 1.01-2.44) (73).

Braithwaite et al in 2014 issued a Cochrane review about macular edema secondary to RVO, comparing anti-VEGF to sham injections or no treatment and showed improved visual outcomes in the anti-VEGF group without safety concerns (74).

Avery and Gordon in 2016 reported increased risk of death from all causes (OR 2.98; CI 95% 1.44-6.14, $p=0.04$), cardiovascular accidents (OR 2.33; CI 95% 1.04-5.22, $p=0.04$) and vascular death (OR 2.51; CI 95% 1.08-5.82, $p=0.03$) in 1328 patients with DME treated with Aflibercept or Ranibizumab compared to laser or sham. It should be noted, though, that they considered patients treated monthly with high doses for 2 years while other studies (which did not produce similar results) had enrolled patients subjected to less intensive and shorter treatments. The main reason for increased risk of death in the study by Avery and Gordon was ischemic stroke (75).

Zarbin et al in 2017 evaluated cardio- and cerebrovascular risk in DME with 0.3 or 0.5 mg Ranibizumab vs sham or laser in a pooled-analysis that considered 6 phase-2 and -3 registrative trials for a total of 1867 patients (1186 on Ranibizumab). Unlike Avery and Gordon, they did not report increased risk of death from all causes or systemic vascular events associated with the active drug (76).

Thulliez et al in 2018 published another overview considering 21 meta-analyses about AMD, DME and RVO, paying particular attention to the selection criteria in order to eliminate possible confounding factors and suggested that anti-VEGF do not increase the risk of systemic adverse events, although caution is advisable

in AMD patients who may be at higher risk of hemorrhagic events when receiving Ranibizumab. They also stated that the increased risk of cardiovascular death reported by Avery and Gordon might have been due to patient old age and/or repeated higher doses of the drug (77).

Starr, Dalvin, et al authored a population-based, retrospective cohort study including 2541 patients with AMD, DME and RVO which suggested no increased risk of stroke (either ischemic or hemorrhagic), MI or death in patients receiving intravitreal anti-VEGF compared with control populations (78-79).

In a Cochrane analysis of 2018 on data relevant to diabetic patients with DME at the longest available follow-up (1 or 2 years), Virgili et al (80) found that Aflibercept, Ranibizumab and Bevacizumab do not differ in terms of serious systemic adverse events. They noted that more than 25 million injections have been delivered worldwide and that, by now, we should be able to draw some firm conclusion about this problem.

In 2019 Maloney et al, reported the risk of systemic adverse events associated with intravitreal Anti-VEGF therapy for DME in routine clinical practice compared with controls treated with macular laser photocoagulation or intravitreal corticosteroid in a cohort of 23348 patients. Anti-VEGF treatment was not associated with an increased hazard ratio of cerebrovascular disease (HR 0.96; CI 95% 0.65-1.41; p= 0.83), major bleeding (HR 1.23; CI 95% 0.76-1.99; p=0.41), or myocardial infarction (HR 1.03; CI 95% 0.73-1.44; p=0.88) when compared with macular laser for DME; however there was an increased risk of post treatment all-cause hospital admission (HR 1.17; CI 95% 1.05-1.30; p= 0.01) (81).

However, risk of bias still makes estimates not precise enough for low-frequency events like arterial thromboembolism or death. Most industry-sponsored studies became open-label after one year of follow up and one large independent study found no difference among the three drugs at 2 years.

A synopsis of published studies is shown in Table 1.

In conclusion, the risk of atherothrombotic events is low compared with their natural incidence in elderly patients, and acceptable if we consider the advantage on vision gain. However, caution is necessary in patients with recent thromboembolic events or myocardial infarction. It could be safer to use drugs with short systemic half-life that do not accumulate in the serum, like Ranibizumab or Pegaptamib or alternate drugs with longer plasma half-lives, which have the Fc fragment, with drugs with short plasma half-lives, without Fc fragment (82).

Is there a role for the internist?

Systemic use of anti-VEGF agents in oncology is associated with increased risk of arterial hypertension, cardiovascular events and arterial embolism. However, it is not established whether also their intravitreal administration increases such risks. Limits in the above reported studies make the interpretation of available data uncertain. Moreover, it would be important to increase pharmacosurveillance, in order to uncover complications arising from the administration of these drugs in real life. A decision to administer

anti-VEGF drugs should be made on the basis of a case by case risk/benefit analysis, taking into account the patient's comorbidities, age and current therapies. Of the three anti-VEGF agents available for intra-ocular administration, with different structures and half-lives, Bevacizumab might be associated with greater risks but is by far less expensive (very important in developing Countries). Ranibizumab has a shorter half-life and may involve fewer risks, while Aflibercept may be effective in the rescue of more advanced cases of DME. The decision to treat requires the collaboration not only between ophthalmologist, diabetologist and internist, but also pharmacologist and pharmacovigilance operators, weighing the need to save sight (DR and AMD remain major causes of blindness (83)) and the apparently minimal additional possibility of further increasing the already high cardiovascular risk related to diabetes and/or old age (82). Clinicians should minimize the dose and frequency of injections without compromising results case by case rather than adopting rigid monthly protocols. Abicipar Pegol which can be administered every 3 months after the loading phase, could be an interesting option in the next future (82). It should be underlined anyway that this review cannot provide indications about the best preferred use of anti-VEGF agents from a strictly ophthalmological point of view.

Finally, one should bear in mind that DR meets all the criteria for screening: DR is an important public healthcare problem and screening is simple, safe, and effective treatments are available. Internists should not overlook the importance of screening to detect patients at risk for sight-threatening DR at a stage when they can be effectively treated by less invasive options (laser) (84-85). However, once a need for anti-VEGF arises, they may be requested to assess the overall cardiovascular risk of individual candidates before intravitreal treatment, much like anesthesiologists assess overall risk before elective surgery. This may sound bizarre but the continuing evolution of medical knowledge and therapies carries the emergence of novel tasks and responsibilities, which include timely and correct patient information on the different options available, as established by legal and deontological considerations.

Compliance with Ethical Standards

This paper does not directly involve studies in humans or animals.

Conflicts of interest

MP has received speaker honoraria from Allergan, Novartis and SIFI.

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Figure legends

Fig. 1 – The VEGF family and receptors. The figure depicts the vascular endothelial growth factor (VEGF) isoforms VEGF (also known as VEGF-A), VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF), and their binding to the receptor tyrosine kinases VEGFR-1, VEGFR-2 and VEGFR-3 and the co-receptors neuropilin-1 (NRP-1) and NRP-2. Major effects on receptor-expressing cell types in the vasculature and CNS are indicated. sVEGFR-1 can trap VEGF-A, VEGF-B and PlGF and reduce their biological actions. sVEGFR-1, soluble VEGFR-1.

From: Lange C, Storkebaum E, Ruiz de Almodóvar C, Dewerchin M, Carmeliet P. Vascular endothelial growth factor: a neurovascular target in neurological diseases. *Nature Reviews Neurology* 12, 439–454, 2016. <https://www.nature.com/articles/nrneuro.2016.88/figures/1>.

Figure 2. Proliferative diabetic retinopathy

Top: Image of left eye with new vessels, pre-retinal haemorrhages and diabetic macular edema.

Bottom: Schematic representation of new vessels growing at the vitreo-retinal interface, retinal traction following vitreous detachment and, finally, retinal traction and detachment from newly formed fibro-vascular tissue.

Figure 3 – Effects of VEGF blockade.

From: Chen, H. X. & Cleck, J. N. *Nat. Rev. Clin. Oncol.* 6, 465–477 (2009)

Fig 4. Structure of anti-VEGF drugs used in the treatment of retinal diseases

From: Thakur S. *Health & Medicine* May 10, 2017)

<https://www.slideshare.net/drsahilthakur/anti-vegfs-in-ophthalmology>

Table 1. Summary of mayor surveys of cardiovascular events following intraocular administration of anti-VEGF agents.

Reference	Type	Eye condition	Agent	N.	Comparator	Cardiovascular outcome
PACORES 2008 (58)	Retrospective	DME	Bevacizumab	1173	Photocoagulation	Similar to comparator
BOLT 2012 (59)	Prospective	CSME	Bevacizumab	80	Photocoagulation	Similar to comparator
Cheng et al 2012 (72)	Meta-analysis	AMD, DR, RVO	Anti-VEGF	4942	No Anti-VEGF	No increase in arterial thromboembolic events
RISE & RIDE 2013 (61)	Multicentre RCT	DME	Ranibizumab 0.3 and 0.5 mg	759	Sham injections	Increased risk of adverse events with 0.5 mg Ranibizumab
CATT/IVAN 2013 (69)	RCT	AMD	Ranibizumab	738	Bevacizumab	More thrombotic events and heart failure with Ranibizumab
RESTORE ext 2014 (62)	Multicentre RCT	DME	Ranibizumab	240	Ranibizumab + laser or laser alone	No report on CV events
Moya et al 2014 (71)	Cochrane	AMD	Bevacizumab	3665	Ranibizumab	More frequent gastrointestinal disorders with Bevacizumab
Thulliez et al 2014 (73)	Meta-analysis	AMD, DME, RVO	Bevacizumab Ranibizumab	9557	No Anti-VEGF	Increased risk of venous thromboembolism with Bevacizumab
Braithwaite et al 2014 (74)	Cochrane	RVO	Anti-VEGF	937	Laser or sham injections	No safety concerns
VIVID and VISTA 2016 (63)	Multicentre RCT	DME	Aflibercept	872 eyes	Laser	Similar to comparator
Avery & Gordon 2016 (75)	Review and Meta-analysis	DME	Aflibercept Ranibizumab	1328	Laser or sham injections	Increased risk of death and cardiovascular incidents
Hanart et al 2017 (60)	Retrospective	AMD	Bevacizumab	>5000	Photocoagulation	Increased risk of death from all causes
Zarbin et al 2017 (76)	Pooled analysis	DME	Ranibizumab	1867	Laser or sham injections	Similar to comparator
Thulliez et al 2018 (77)	Overview	AMD, DME, RVO	Aflibercept Bevacizumab Ranibizumab	>30.000	No Anti-VEGF	Similar to comparator
Virgili et al 2018 (80)	Cochrane	DME	Aflibercept Bevacizumab Ranibizumab	6007	Each other	No difference between the three agents
Maloney et al 2018 (81)	Retrospective	DME	Anti-VEGF	23.348	Laser or intravitreal corticosteroid	More all-causes hospitalization
Starr et al 2019 (78)	Retrospective	AMD, DME, RVO	Anti-VEGF	2541	No Anti-VEGF	Similar to comparator
Dalvin et al 2019 (79)	Retrospective	AMD	Anti-VEGF	504	No Anti-VEGF	Similar to comparator

AMD (Age-related Macular Degeneration), DME (Diabetic Macular Edema), CSME (Clinically Significant Macular Edema), RVO (Retinal Venous Occlusion, RCT (Randomized Controlled Trial), MRCT (Multicentric Randomized Controlled Trial).