

## Blood pressure as a potential biomarker of the efficacy angiogenesis inhibitor

Arterial hypertension is a commonly reported side-effect in all clinical trials testing all inhibitors of angiogenesis and especially inhibitors of vascular endothelial growth factor (VEGF)/VEGFR-2 signaling [1]. Whatever their initial level of blood pressure, every patient receiving antiangiogenic treatment evidenced rapid and large increases in blood pressure; in most cases, the blood pressure values did not reach the levels characterizing clinical hypertension [2]. In the present issue of the *Annals of Oncology*, Scartozzi et al. [3] present a clinical trial suggesting that 20% of patients with metastatic colorectal cancer receiving bevacizumab in combination with irinotecan and 5-fluorouracil developed, as expected from previous trials, grades 2–3 hypertension. Most important: partial remission was observed in 75% of these hypertensive patients versus 32% of patients with no hypertension. These authors suggest that bevacizumab-induced hypertension may represent a predictive marker for antiangiogenic treatment efficacy.

This important result rise several points to be discussed in the present editorial:

1. Does angiogenesis occur in untreated healthy adults?
2. How do antiangiogenic drugs affect the normal (nontumor) microcirculatory network?
3. How is microcirculation affected in hypertension?
4. How to assess and treat hypertension during antiangiogenic therapies?

### angiogenesis in health and diseases

Angiogenesis is a biological process by which new capillaries are formed from preexisting vessels. It is essential in many physiological (embryo development, ovulation, and wound repair) and pathological conditions, such as arthritis, diabetic retinopathy, and tumors.

Angiogenesis is controlled by the net balance between molecules that have positive and negative regulatory activity [4]. Under physiological conditions with a stable microcirculatory network, there is a controlled balance between pro- and antiangiogenic factors. The ‘angiogenic switch,’ depending on an increased production of one or more of the positive regulators of angiogenesis, is likely a key factor initiating tumors and metastasis [5]. Proangiogenic molecules can be exported from tumor cells, mobilized from extracellular matrix, or released from inflammatory cells (e.g. macrophages or lymphocytes). The angiogenic switch clearly involves more

than simple upregulation of angiogenic activity and is thought to be the result of a net balance of positive and negative regulators. Numerous inducers of angiogenesis have been identified, including members of the fibroblast growth factor family, vascular permeability factor (VPF)/VEGF, angiogenin, transforming growth factor alpha and beta, platelet-derived growth factor (PDGF), platelet-derived endothelial cell growth factor, tumor necrosis factor alpha, interleukins, chemokines, and angiopoietins.

Proliferation of new blood vessels is necessary for tumors to grow and contributes to the spread of blood-borne metastases. More than 35 years ago, Judah Folkman [6] proposed to inhibit angiogenesis in order to limit tumor processes. The identification of the major growth factor, the VEGF, occurred later and made the Folkman’s hypothesis much stronger. In 1989, Ferrara and Henzel [7] and Plouet et al. [8] independently reported the purification and sequencing of an endothelial cell-specific mitogen, which they, respectively, called VEGF and vasculotropin. The subsequent molecular cloning of VEGF and VPF [9, 10] unexpectedly revealed that both activities are embodied in the same molecule.

VEGF not only drives angiogenesis but also serves as a survival factor for endothelial cells and contributes to promote the abnormal phenotype of blood vessels in tumors [11]. Unlike tumor vessels that have VEGF as survival factor, the normal adult vasculature is widely regarded as largely independent of VEGF for survival, stability, and normal function [12]. Indeed, the rationale for using VEGF inhibitors on tumors is based on the assumption that tumor vessels can be impacted without harming other vessels.

Preclinical studies of VEGF inhibitors helped to elucidate the mechanism of some adverse events found in the clinic and considered as consequences of blocking actions of VEGF in normal physiology. The essential role of VEGF on vessels was thought not to persist into adult life but to be limited to the fetal development. Yet, actions of VEGF have been identified in normal organs of the adult, especially the role of VEGF in function and survival of normal blood vessels and blood pressure regulation [13, 14]. Studies of the effects of VEGF antibody, analogue to bevacizumab, in mice indicate that VEGF participates in blood vessel survival and plasticity in adult life. Examination of the simple vascular network of the mouse trachea, treated with an inhibitor of VEGF signaling, revealed rapid regression of some normal mucosal capillaries [11, 15]. After only 1 day of treatment, fibrin accumulated and patency was lost in some capillaries. By 2 days, endothelial cells underwent apoptosis and regression. The magnitude of capillary loss after 10-day treatment depended on the age of the mice: up to 39% in young animals and 14% in adult mice. After

inhibition of VEGF signaling for 1–3 weeks, significant capillary regression occurred in pancreatic islets, thyroid, adrenal cortex, pituitary, villi of small intestine, choroid plexus, adipose tissue, and trachea. The amount of regression was dose dependent and varied from organ to organ, with a maximum of 68% in thyroid. Little or no capillary regression was detected in brain, retina, skeletal muscle, cardiac muscle, or lung under these conditions [16].

We recently reported capillary rarefaction in the finger skin in patients with metastatic colorectal cancer receiving bevacizumab treatment; the reduction in capillary density was correlated with the total dose of bevacizumab received by the patients and was closely associated with the rise in blood pressure observed in all patients [17]. Similar results were recently obtained in the mucosal surface of the inner lip of patients with advanced solid tumors receiving telatinib, a small molecule tyrosine kinase inhibitor of VEGF receptors 2 and 3, PDGF receptor, and c-KIT [18]. The mechanisms leading to this increase in blood pressure during antiangiogenic therapy have not fully been elucidated. Proposed mechanisms include reduced formation of nitric oxide (NO) by endothelial cells, a reduced responsiveness of vascular smooth muscle cells to NO, an increased production of or reaction to vasoconstricting stimuli, and microvascular rarefaction.

Both clinical studies suggested a physiopathological link between the microvascular rarefaction and the appearance and the severity of arterial hypertension. It remains unclear whether the key problem is impaired NO synthesis leading to microvascular rarefaction or an imbalance between angiogenesis and endothelial cell apoptosis leading to capillary rarefaction or a combination of both.

### arterial hypertension and microcirculation

A relatively constant finding in both experimental and clinical hypertension has been that of microvascular rarefaction, defined as a reduced spatial density of microvascular networks [19, 20]. Because microvessels (arterioles and capillaries) are a major contributor (>90%) to total peripheral vascular resistance, functional rarefaction (a decrease in perfused microvessels) or anatomic rarefaction (a reduction in capillary density) may play an important role in the development of hypertension. Under physiological resting conditions, a substantial part of microvascular networks of most organs remains closed, constituting a flow reserve for adaptation to increased metabolic needs. It was first noted that hypertensive patients had an abnormally low number of small conjunctival vessel [21, 22]. Using venous occlusion capillaroscopy, the nailfold capillary density was also reported to be significantly lower (by 10%) in nondiabetic patients with never-treated essential hypertension than in healthy normotensive control subjects matched for age, sex, and lipid profile [23]. Analogous results (20% difference) were obtained on the dorsal finger skin [24] and on the forearm skin [25] of hypertensive versus normotensive subjects. Finally, in hypertensive patients, whether treated or not, the Framingham score for cardiovascular risk was negatively correlated to capillary density, evaluated in the dorsal skin of the second phalanx of finger [26]. It is increasingly speculated that

diffuse systemic rarefaction might be a primary defect in essential hypertension. However, the cause and effect relationships of rarefaction and hypertension are still debated. Interestingly, microvascular rarefaction exists in normotensive humans with a familial predisposition to the disease, suggesting a developmental defect, that is, an inability of vascular growth to keep pace with organ growth [27]. Another powerful reason to link abnormalities in the long-term control of angiogenesis and blood pressure is the crucial role played by NO and the renin–angiotensin system in both processes. NO, the bioactivity of which seems deficient in hypertension, as we have seen, is not only a vasorelaxant, but is also required for appropriate vascular budding in wound healing [28] and stimulates the expression of vascular growth factors, notably vascular VEGF [29]. Impaired angiogenesis has been directly demonstrated in experimental hypertension induced by chronic pharmacological inhibition of NO synthesis [30].

Can rarefaction contribute to the increase of peripheral vascular resistance in hypertension? This is a difficult and, in fact, unresolved question. There is uncertainty on the size and anatomic location of resistance vessels because of wild variations of observations made in different organs, species, and experimental conditions and essential issues include the impact of anesthesia and surgically induced disruption of microvascular physiology [31]. Computer simulation of systemic resistances suggested that rarefaction of smaller arterioles can, indeed, augment the global resistance and thus the mean blood pressure by >20% [32]. Other than affecting resistance, rarefaction has the potential to disturb the cellular delivery of nutrients and oxygen, thus contributing to hypertensive end-organ damage.

### antiangiogenic treatments and arterial hypertension

Cardiovascular effects, including hypertension, have emerged as an important toxic effect of antiangiogenic drugs. Wu et al. [33] recently reviewed the incidence of hypertension with sorafenib treatment. An incidence of ~23% for all-grade hypertension and 6% for high-grade hypertension was noted with sorafenib, with a six times greater relative risk for the development of all-grade hypertension compared with controls. This meta-analysis reports incidence and severity of hypertension as a toxic effect of sorafenib very close to those reported with other antiangiogenic molecules.

Actually, ‘serious side hypertensive effect’ does not have the same meaning for oncologists and for cardiologists.

The National Cancer Institute—Common Toxicity Criteria definitions of hypertension, as a toxic effect of oncologic treatment, are:

- grade 1, asymptomatic, transient (<24 h) increase by >20 mmHg (diastolic) or to >150/100 mmHg if previously within normal limits (intervention not indicated);
- grade 2, recurrent or persistent (624 h) or symptomatic increase by >20 mmHg (diastolic) or to >150/100 mmHg if previously within normal limits (monotherapy may be indicated);

grade 3, requiring more than one drug or more intensive therapy than previously;  
 grade 4, life-threatening consequences (e.g. hypertensive crisis).

The definition and grade of hypertension are very different for cardiologists; blood pressure has a unimodal distribution in the population as well as a continuous relationship with cardiovascular risk down to systolic and diastolic levels of 115–110 and 75–70 mmHg, respectively [34, 35]. According to the last guidelines of the European Society of Hypertension, the classification of hypertension is the following [36]:

- Grade 1: systolic blood pressure 140–159 and/or diastolic blood pressure 90–99 mmHg.  
 Grade 2: systolic blood pressure 160–179 and/or diastolic blood pressure 100–109 mmHg.  
 Grade 3: systolic blood pressure >180 and/or diastolic blood pressure >110 mmHg.

The Framingham risk score is a tool used by cardiologists to predict the absolute risk of cardiovascular mortality and morbidity (at 5 and 10 years) in populations free of cardiovascular disease. This score is available in different application formats (e.g. point scoring systems, risk charts, or Web-based calculators) and requires only information from patient history and easily available tests [37]. According to international guidelines, a Framingham risk score >20% is considered to be extremely high and must conduct to specific and strong intervention. The risk level and the scale of time are obviously very different for oncologists.

Thus, when oncologists and cardiologists assess and treat antiangiogenic-induced hypertension, they do not have the same definitions, language, and risk perception.

It is clear that early detection and effective management of hypertension might allow for safer use of the antiangiogenic drugs. The hypertensive and cardiovascular side-effects of antiangiogenic treatments need thorough surveillance and reporting, and future studies will be needed to identify the mechanism and appropriate treatment of treatment-induced hypertension. It is obviously reasonable to recommend to carefully monitor blood pressure and to undertake early and efficient management of hypertension to avoid further toxic complications. Professor Olivier Rixe (National Institutes of Health, Bethesda) is already using the increase in blood pressure as a marker for antiangiogenic treatment efficacy and efficient dosage (personal communication).

When hypertension must be treated, the best therapeutic class of antihypertensive agents has not been yet determined for these patients; however, it appears that, alone or in combination, blockers of the renin angiotensin system, calcium antagonists, diuretics, and/or centrally active antihypertensives allow, in the large majority of patients, to properly control antiangiogenic-induced hypertension [38]. Thus, the decision of stopping antiangiogenic treatment responsible for hypertension must be taken, if necessary, only after trying several antihypertensive drugs and careful and very informed thinking in relation with the benefit to risk ratio for each patient.

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