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Hypothesis

Anemia: A Potential Source of Bias in Clinical Trials of Angiogenesis Inhibitors: A Hypothesis

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Abstract

Although anemia may cause angiogenesis and neovascularization, especially in ocular situations, neither published nonrandomized clinical trials nor registered clinical trials have reported the anemia status as inclusion or exclusion criteria in their design. Increases in the circulating levels of erythropoietin and vascular endothelial growth factor are proportional to the levels of tissue hypoxia, which are influenced by hematocrit. Erythropoietin is a potent retinal angiogenic factor that is independent of endothelial growth factor and is capable of stimulating ischemia-induced retinal angiogenesis. We suggest that clinical trials investigating anti-vascular endothelial growth factor treatment for retinal neovascularization should measure appropriate variables such as serum erythropoietin, vascular endothelial growth factor, hemoglobin, and hematocrit to yield preliminary data for future trials of angiogenesis inhibitors. Ignoring the anemia status, serum erythropoietin, and/or vascular endothelial growth factor levels could create clinical uncertainty and subtle statistical bias in both systematic reviews and nonrandomized clinical trials that aim to evaluate the efficiency of angiogenesis inhibitors in several medical situations, including but not limited to ocular alterations, rheumatoid arthritis, and many types of cancer, just to mention a few. Implications of this type of bias could be involved in other disease situations in which angiogenesis inhibitors are used for medication, such as different carcinomas as well as metastases. In this hypothesis paper, we suggest that clinical trials of angiogenesis inhibitors should measure appropriate variables such as serum erythropoietin, hemoglobin, and hematocrit and match their participants by anemia and its severity to avoid a game-changing bias in their data analysis.

Introduction

Anemia and angiogenesis

Anemia is among the most common hematological manifestations of cancer, and it is estimated that almost 40–64% of patients treated for malignancies are affected by anemia. From a pathophysiological point of view, cancer-related anemia may be categorized into four broad but overlapping classes: hypoproliferative anemia comprising the common anemia of inflammation/chronic disease, miscellaneous etiologies, hemolytic anemia, and anemias with uncertain etiologies. A positive connection is seen between tumor hypoxia and anemia. Hypoxemic situations enhance tumor growth and thereby resistance to medication through stimulating angiogenesis.[1] Erythropoietin (EPO) is considered as a hormone, a cytokine, and a growth factor. Its major function is regulation of erythropoiesis. EPO contributes to angiogenesis and neovascularization in the angiogenic switch of a tumor[2] and vascular endothelial growth factor (VEGF), which is a key dynamic molecule of angiogenesis.[3] The development and application of anti-angiogenesis agents, specifically those targeting vascular VEGF, have become integral components of neovascularization therapies and anticancer regimens for many tumor types.

Anemia and angiogenesis inhibitors

The potential of angiogenesis inhibitors or angiogenesis-modulating agents as therapies for human diseases, namely eye diseases like retinal neovascularization and corneal neovascularization, has increased dramatically over the past 20 years due to newly emerged clinically available agents. Most of the recently published articles benefit from elegant and sophisticated methodology.[4] However, although anemia is causally related to angiogenesis and neovas-

Keywords: Anemia; Bias; Clinical trials; Neovascularization; Angiogenesis inhibitor.
Abbreviations: Anti-VEGFR, anti-vascular endothelial growth factor receptor; EPO, erythropoietin; PDR, proliferative diabetic retinopathy; VEGF, vascular endothelial growth factor.
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cularization,[5] especially in ocular situations,[6] neither the published nonrandomized clinical trials nor the registered nonrandomized clinical trials have reported the anemia status as inclusion or exclusion criteria in their design. The main methodological issue here is whether the iron-deficiency status in enrolled patients confounds the efficacy of angiogenesis inhibitors, since a low hemoglobin level is associated with increased serum levels of vascular endothelial growth factor (VEGF) and it has been suggested that anemia might increase the progression of angiogenesis.[7] Increases in the circulating levels of EPO are proportional to the levels of tissue hypoxia, which are influenced by hematocrit.[8] In addition, there have been several reports that anemic patients have elevated levels of VEGF, which is a marker of tissue hypoxia.[9] Moreover, it has been shown recently that the anemia of newborns induces EPO expression in the developing mouse retina.[10]

EPO and VEGF are independently associated with proliferative diabetic retinopathy (PDR), and EPO is more strongly associated with PDR than VEGF. EPO is a potent retinal angiogenic factor independent of VEGF and is capable of stimulating ischemia-induced retinal angiogenesis in PDR.[11] Additionally, the EPO-independent EPO-receptor signaling pathway has been demonstrated to play a potential role in cell proliferation and angiogenesis in human pterygium.[12] Furthermore, the gene expression levels of EPO and VEGF are upregulated in the murine ischemic retina, and the blockade of EPO inhibits retinal neovascularization *in vivo* and endothelial-cell proliferation in the vitreous of patients with PDR *in vitro*.[13]

Hypothesis

Through alteration of the endogenous EPO and VEGF levels, different types of anemia create a bias in trials of angiogenesis inhibitors. Interestingly, the results from an animal model of cancer showed that the antitumor efficacy of bevacizumab (a well-known anti-VEGF agent) was compromised in an anemic situation. Surprisingly, anti-vascular endothelial growth factor receptor (anti-VEGFR)-2, at the effective dose for normalization of systemic tissues and organs, did not show a significant antitumor effect in that cancer model. This was possibly because the plasma levels of EPO were significantly elevated in VEGF tumor-bearing mice. Also, VEGF-induced anemia was reversed by anti-VEGFR-2 but not by anti-VEGFR-1.[14]

These results may pertain to angiogenesis inhibitors and retinal neovascularization; in the human retina, VEGFR-1 neutralization decreased pigment epithelium-derived factor mRNA and protein expression, whereas anti-VEGFR-2 antibody had no effect.[15] It should be kept in mind that a critical balance between VEGF and pigment epithelium-derived factor is important to prevent the development of retinal neovascularization.

Evaluation of hypothesis

If these findings can be extrapolated to human patients suffering from retinal neovascularization, a loss of anti-VEGF agent efficacy in patients suffering from iron deficiency or elevated plasma EPO levels might be involved. There is indirect evidence from human[16] and direct evidence from animal[14,17] studies that support our idea; for example, in patients with pre-existing higher EPO levels, the efficacy of anti-VEGF treatment was lower than that of patients with lower EPO levels.[18] In addition, in an animal model of musculocutaneous tissue ischemia, flaps treated with EPO and bevacizumab did not show any change in tissue necrosis when compared with animals receiving EPO only,[19] supporting a possible counteraction between EPO and anti-VEGF agents. It might be argued that the interaction between locally administered exogenous anti-VEGF and endogenous intravitreal EPO and/or VEGF levels is complex. It should be kept in mind that there is a direct correlation between serum EPO and intravitreal EPO levels,[20] as well as between intravitreal EPO and intravitreal VEGF levels.[20] Very recently, it was found that the plasma VEGF level could predict the response in non-small cell lung cancer patients treated with bevacizumab.[21] Interestingly, systemic dosing of squalamine lactate, an anti-VEGF agent, has yielded promising results in animal models as well as in humans.[22]

Furthermore, there is strong evidence that changes in endogenous VEGF are related to the therapeutic effect of anti-VEGF agents.[23,24] There are several reports of the high efficacy of systemic steroid treatment for retinal neovascularization, possibly through suppression of intravitreal VEGF. Overall, the results of these studies spark the hypothesis that the correlation between systemic and intravitreal EPO or VEGF levels is more plastic than previously anticipated. An anemic situation might have implications in anti-VEGF dose calculation as well. Such patients suffering from retinal neovascularization may need higher doses of anti-VEGF agents, while administering the same doses to nonanemic patients may increase the side effects of anti-VEGF agents. Further support for our claim comes from findings indicating increased anemia in exudative age-related macular degeneration.[25]

Effects of different types of anemia and supplements on angiogenic/tumorigenic situations

It is noteworthy that different types of anemia and nutrient deficiencies leading to or contributing to anemia may have differential effects on the VEFG and EPO levels or their expression, and thereby on angiogenesis. Both severe and moderate iron deficiency[5–7,26] as well as vitamin B12 deficiency[27–33] lead to higher VEGF levels and angiogenesis, while the opposite is true in the case of folate deficiency anemia.[34–42] Other vitamins have their own mechanism for the regulation of angiogenesis.[43] Recently, Pedrosa and Lemes have reported a significantly enhanced gene expression of VEGF and HIF-1α in patients with zinc deficiency and sickle cell anemia, and they showed an inverse dose-response association with hydroxyurea therapy.[44] Previously, higher serum VEGF levels in beta-thalassemia,[45–48] hemolytic anemia,[49–52] idiopathic aplastic anemia[53–55] and anemia of chronic disease[56–58] have been well established. Etiologically, it is conceivable that different mechanisms are behind different types of anemia. Obviously, the interactions among the severity/types of anemia, nutrient reserves of the body, and the duration of the study/intervention can create an intricate and chaotic pattern, which is difficult to control by usual statistical methods.

There are many examples where the type of anemia and/or the type of nutrient supplementation can alter or modulate the process of tumorigenesis/angiogenesis as well as the therapy. For instance, some people take iron supplements without a medical indication. In a recent randomized controlled trial, it was shown that among comparable patients participating in age-related macular degeneration treatment trials, the use of iron supplements in participants undergoing bevacizumab treatment was correlated with retinal/subretinal hemorrhage in a dose-response fashion.[59] Ample literature exists on the deterioration and/or amelioration of tumorigenesis/angiogenesis following (un)indicated iron, folate, and vitamin B12 supplementation for different angiogenic/tumorigenic situations[31,60–71] Figure 1 shows a simplified diagram of the

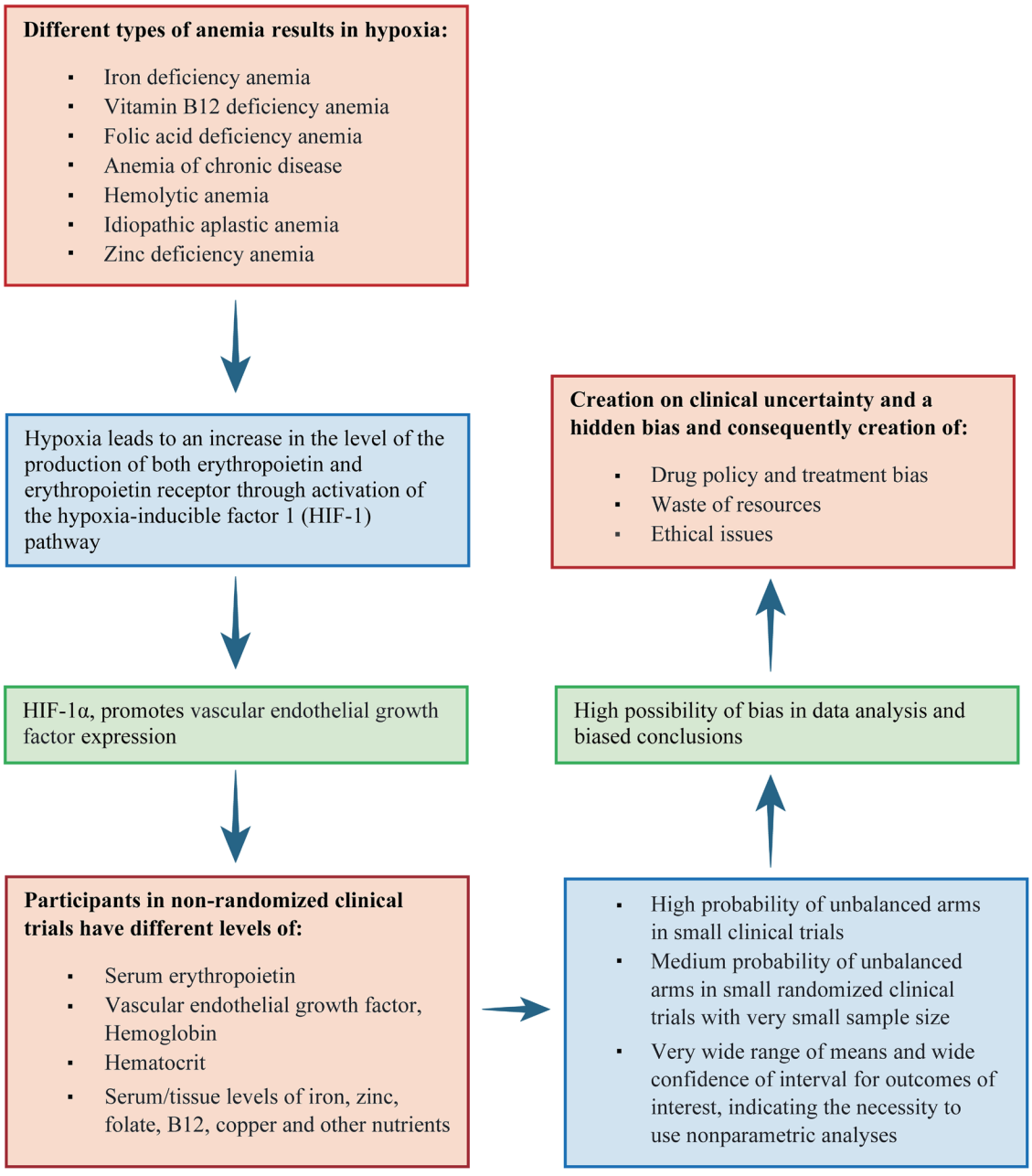

Fig. 1. A simplified diagram of mechanisms by which different types of anemia may create a bias in studies of angiogenesis inhibitors. HIF-1, hypoxia-inducible factor 1; HIF-1α, hypoxia-inducible factor 1 alpha.

mechanisms by which different types of anemia may create a bias in studies of angiogenesis inhibitors.

Discussion

Ignoring the anemia status, serum EPO levels, and/or VEGF levels are likely examples of sources of bias in nonrandomized clinical trials that aim to evaluate the efficiency of angiogenesis inhibitors, especially in eye diseases. It might be argued that in clinical trials that randomly assign treatment or standard care to the study participants, the major advantage is that the treated and the untreated subjects are similar in many aspects because of the power of randomization, provided that a sufficient number of participants are enrolled (adequate power). It might also be argued that randomization is unlikely to lead to bias. However, a simple search of clinical trials registered in the National Institutes of Health database reveals that in almost half of the registered clinical trials, the design of the study is nonrandomized and uncontrolled.

Future directions

We would like to urge that all clinical trials on anti-VEGF agents

include the patient anemia status in their design. Obviously, the VEGF levels depend on the duration of anemia (chronic vs. acute), the definition of anemia, and the metabolic demand of the patients. Thus, it would be more important to measure the VEGF levels to meet the discussed problem more appropriately. To sufficiently randomize patients, data concerning the incidence of elevated EPO levels are necessary to plan a sufficient sample size. If researchers find it difficult to randomly allocate patients to the study arms or to balance the number of anemic/nonanemic patients in the study arms, like most other clinical trials, they can adjust the anemia status, preferably as the serum EPO level, as a potential confounder in the placebo and intervention groups when doing statistics, using proper statistical treatments.

One could argue that besides anemia, we should include genetic analysis, history of smoking, and food frequency questionnaires to measure anti-angiogenic food components into clinical trials. The point is that one should be able to estimate the size of the effect that these factors have based on scientific data prior to the planning of trials and to adjust the power calculation accordingly. The goal is to evenly distribute these factors between the treatment arm(s) and control arm(s) of a randomized controlled trial. So, if it is not possible to correct for all of these factors, the concept of randomization comes into play. Due to the high impact of confounding factors in clinical trials investigating angiogenesis inhibitors, the results of nonrandomized clinical trials might lead to a major bias in reviews and guideline protocols. On the other hand, in randomized clinical trials, the power of the study has to be so strong that all of these confounding factors can be equally distributed between the groups.

Conclusions

We suggest that the significance of systematic reviews could be enhanced by including all factors and giving an estimation of the effect size of the affected group. This could positively impact the design of contemporary clinical trials. Implications of this kind of bias could be involved in other disease situations in which angiogenesis inhibitors/promotors are used for medication, such as different carcinomas as well as metastases. Implications of this kind of bias also could be involved both in situations of higher endogenous EPO production and exogenous EPO administration, just to mention a few examples.

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Conflict of interest

The author declares no conflict of interest.

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