To the editor:

Angiogenesis in acute myeloid leukemia

We have read with interest the report of Padrò et al showing an increased angiogenesis in acute myeloid leukemia. Recently, other studies published in Blood also have shown that angiogenesis in acute myeloid leukemias is higher than in controls and that it has an independent prognostic significance. But measurement of angiogenesis in hematopoietic tumors is still in its infancy, and a consensus on the methodology and criteria of evaluation is still lacking. Some methods employed in solid tumors such as CD34 or CD31 immunostaining cannot be used because of the positivity of leukemic cells for these antigens. In the aforementioned papers, the increase of angiogenesis has been demonstrated by using endothelial cell markers such as immunohistochemistry for von Willebrand factor and thrombomodulin or a monoclonal antibody against endothelial cells (ULEX-E2) or by measuring vascular endothelial growth factor (VEGF3), which is considered the most important soluble mediator of angiogenesis and correlates with microvessel density. On the one hand, the common finding of an increased angiogenesis by using different methods of measurement validates the results of each report. On the other hand, however, the disparity of the methods indicates the necessity for more precise rules in the evaluation of angiogenesis in hematological diseases.

In this regard, it is well known that, in normal conditions, angiogenesis may be driven by hypoxia. VEGF may be released by virtually all normal cells and several neoplastic cell lines in response to hypoxia. Therefore, it is possible that in some anemic patients angiogenesis is increased at least in part for the anemia-related hypoxia. This possible correlation between anemia and angiogenesis is not taken into account in any of the aforementioned papers, although anemia is a common symptom in acute leukemias. Because the hypoxic regulation of VEGF is mediated by the hypoxia-inducible factor 1 (HIF-1) with a mechanism similar to the hypoxic regulation of erythropoietin (Epo), it is possible to use serum Epo concentration (which correlates with the hypoxic stimulus) as an indirect measurement of the amount of hypoxia-related angiogenesis in each patient.

This argument applies also to controls. We think that anemic patients should not be used as controls in evaluation of angiogenesis because of a possible increase of hypoxia-induced VEGF. In contrast, Padrò and colleagues control patients included some lymphoma and other “nonmalignant disorders,” and Hussong and colleagues control patients with “cytopenias.” Although hemoglobin levels were not reported, it is possible that some of these subjects were anemic, which would at least partly explain the increase of angiogenesis observed in some control patients.

Actually, we have studied the VEGF levels in 2 other hematological malignancies, multiple myeloma and idiopathic myelofibrosis, and have found that in both diseases there is an increase of circulating VEGF concentrations (and therefore of angiogenesis) that is not correlated with serum Epo levels. In the same patients, Epo levels correlate with anemia. We concluded that these diseases angiogenesis is increased per se and not as a consequence of anemia.

We are convinced that the same could happen in AML, and the data presented by the various authors are really indicative of a true increase of angiogenesis in acute leukemias. But we think that the simple measurement of serum Epo levels should be included in the evaluation of angiogenesis in all of the diseases where anemia is a common symptom.

Reference:


Response:

Angiogenesis and anemia in acute myeloid leukemia

Dr Di Raimondo and colleagues are concerned about the standardization of the methodology for evaluating angiogenesis in hematological diseases. We fully agree with them that standardization of angiogenesis quantification is desirable to yield reliable results. Thus results obtained at different institutes would be comparable and allow for meta-analyses. But the assessment of angiogenesis by determination of microvessel density as performed in our study is a modification of a well-established method and an international consensus report. To ensure the reliability and reproducibility of this quantification method in acute myeloid leukemia (AML), we adhered to a strict protocol for selection of hot spots and introduced several internal controls. Thus, our investigation has unequivocally demonstrated a significant increase of bone marrow microvessel density in AML.

As Di Raimondo et al correctly point out, the common finding of increased angiogenesis in AML patients in spite of different methods (thrombomodulin/von Willebrand factor staining in our study and von Willebrand factor/ULEX-E staining in the study by...
Hussong et al. underscores the validity of the results obtained in AML. But Di Raimondo and colleagues’ statement that angiogenesis is an independent prognostic factor in AML is not justified. The cited report of Aguayo et al. has shown that cellular vascular endothelial growth factor (VEGF), a potent angiogenic factor, is an independent predictor of outcome in AML. But neither data on bone marrow microvessel density nor on correlations of VEGF levels with microvessel density were provided in this study. Thus, in AML the prognostic value of increased angiogenesis has yet to be demonstrated.

Of course, anemia is a common symptom in newly diagnosed, untreated AML patients, and it has been demonstrated that hypoxia up-regulates VEGF expression in both normal and malignant cells. Accordingly, anemia might contribute to increased bone marrow angiogenesis by aggravating tissue hypoxia. But it is unlikely that the induction of known growth factors for endothelial cells such as VEGF alone is sufficient to induce the angiogenic switch. Therefore, defining more carefully the genes that are in situ up-regulated or down-regulated under hypoxic conditions in AML will be more important than correlating angiogenesis with serum erythropoietin (Epo) levels as a surrogate for tissue hypoxia. Furthermore, factors other than tissue hypoxia have been suggested to be involved in the regulation of Epo production and influence serum concentrations. For example, serum Epo levels have been found to be variable for a given hemoglobin concentration in patients with myelodysplasia. In patients with untreated acute leukemia, Epo levels have been reported to be higher than in controls and have continued to increase following chemotherapy. When assuming that serum Epo concentrations positively correlate with hypoxia-related angiogenesis in AML, it is difficult to reconcile this Epo increase with our observation of a significant decrease in bone marrow microvessel density following chemotherapy.

Indeed, the control patients in our study had lower hemoglobin levels than a healthy population (median [interquartile range], 11.4 [10.8-13.4]). Therefore, we cannot exclude that tissue hypoxia might have caused slightly increased angiogenesis in our control population. If this was the case, however, differences between AML and control patients would be underestimated rather than overestimated in our study. Yet we found no correlation between hemoglobin levels and bone marrow microvessel density within the control group ($r = -0.123$; assessed by the Pearson coefficient). Therefore, we disagree with Dr Di Raimondo and colleagues that serum Epo levels are important for the interpretation of our findings.

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References
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