Hematopoietic Abnormalities in Patients With Cancer

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Understanding the concepts of normal hematopoiesis and the mechanisms of both disease- and treatment-related cytopenias assists in the proper management of patients and rational uses of hematopoietic growth factors, particularly in those cancer patients receiving chemotherapy with or without stem and progenitor cell transplantation.

Introduction

There is a high frequency of abnormalities of peripheral blood hematopoietic cells in patients with neoplastic disorders. The cytopenias, or reductions in erythrocyte, neutrophil, and platelet numbers that are observed in such patients, may be a result of defective hemopoiesis or increased hematopoietic cell consumption or destruction.

Concepts of Normal Hematopoiesis

Hematopoiesis is the process of blood cell production, which involves the maintenance and proliferation of hematopoietic progenitor cells and their differentiation into mature, functional erythrocytes, leukocytes, and platelets. Normal hematopoiesis depends on the presence of (1) normal pluripotent hematopoietic stem cells, which are capable of maintaining differentiated hematopoietic cell production throughout an individual’s lifetime, (2) a bone marrow microenvironment that is supportive of stem cell survival and proliferation and of differentiation of stem cells to committed hematopoietic progenitor cells and mature hematopoietic cells, and (3) a complex system of highly regulated hematopoietic growth factors with overlapping lineage specificities, which are crucial in regulating the proliferation, differentiation, and survival of hematopoietic cells. The central feature of pluripotent hematopoietic stem cells is that they are capable of both self-renewal and irreversible differentiation into mature hematopoietic cells. Although the duration of survival and the function of individual stem cells are controversial, the stem cell pool as a whole has, on average, an approximately equal chance of self-replication or differentiation, thus assuring continuing hematopoietic cell production throughout the life of the individual. Although single stem cells and their direct repopulated progeny may survive for long periods of time, there may be a dynamic process by which some stem cells cease functioning and are replaced by other stem cells that previously had been resting for a prolonged period of time in the G(0) phase of the cell cycle. Regardless of the situation, the purpose of the stem cells is accomplished, and normal individuals have stem cells that maintain hemopoiesis at a stable level throughout their lifetimes, yet retain the ability to respond relatively promptly to stress situations in which marked increases in one or all lineages of mature hematopoietic cells are required.

Hematopoietic stem and progenitor cells are regulated by a combination of random events and permissive or directive effects of a large number of local (within the bone marrow) and circulating hematopoietic growth factors. This complex and carefully regulated system results in the production of 200 to 250 billion erythrocytes, 150 to 200 billion platelets, and 100 to 150 billion neutrophils every day throughout human adulthood. There is an astonishingly tight control over daily hematopoietic cell production in normal circumstances and rapid responses in stress situations. However, a number of disorders that affect any of the components of this production and normal destruction process may result in the development of anemia, neutropenia, thrombocytopenia, or a combination of these cytopenias. Many of these mechanisms of cytopenias occur as direct or indirect results of cancers or the treatments received by cancer patients.

Causes of Cancer-Related Cytopenias

Cytopenias occurring in patients with neoplastic diseases may be directly disease-related or may arise secondary to complications of the cancer. The most common mechanisms of direct and indirect cancer-related cytopenias are listed in Table 1. Metastases within the bone marrow may disrupt hematopoiesis by displacing and destroying stem and progenitor cells, damaging the bone marrow microenvironment, impairing production of hematopoietic growth factors, or inducing production of cytokines that inhibit hematopoiesis. Some types of neoplastic cells may induce extensive bone marrow fibrosis and/or necrosis, which disrupts the marrow microenvironment and results in deficient hematopoiesis. Cancers that do not invade the bone marrow may impair hematopoiesis by inducing the production of circulating direct inhibitors of hematopoietic stem and progenitor cell proliferation and differentiation or by causing the impaired production of hematopoietic growth factors.

<table>
<thead>
<tr>
<th>Cytopenias Directly Related to Cancer</th>
<th>Cytopenias Due to Secondary Complications of Cancers</th>
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<tr>
<td>Metastases to bone marrow</td>
<td>Nutritional deficiencies</td>
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<tr>
<td>Bone marrow necrosis</td>
<td>Blood loss (RBCs)</td>
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<tr>
<td>Bone marrow fibrosis</td>
<td>Chronic disease/inflammation (RBCs)</td>
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<td>Humoral inhibitors of hematopoiesisin</td>
<td>Immune-mediated hematopoietic cell destruction</td>
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<td>Reduced levels of hematopoietic growth factors (EPO)</td>
<td>Hypersplenism</td>
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<td>Microangiopathy (RBCs and platelets)</td>
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Numerous secondary disorders also can occur in cancer patients. These disorders can cause or contribute to the development of one or more cytopenias. Severe malnutrition may result in pancytopenia similar to that which has been observed in patients with anorexia nervosa and in other malnutrition victims. Blood loss can result in acute anemia and in longer-term iron deficiency anemia. The anemia of chronic disease, also called the anemia of inflammation, probably is the result of production of cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-alpha, transforming growth factor (TGF)-beta, and interferon (IFN)-beta and -gamma. These inflammatory cytokines appear not only to reduce production of some hematopoietic growth factors, most notably erythropoietin (EPO), but also to affect hematopoietic progenitor cells directly by impairing their responsiveness to growth factors. Cancer patients may have a more pronounced form of anemia of chronic disease, with lower levels of EPO for a given degree of anemia and with impaired responsiveness to endogenous and exogenously administered EPO. There also are...
several circumstances in which cytopenias in cancer patients are due to increased consumption/destruction of hematopoietic cells rather than to decreased production. Immune-mediated destruction of one or multiple hematopoietic cell lineages occurs predominantly in lymphoid malignancies, but it may occur in other cancers as well. Hypersplenism with cytopenias due to sequestration of hematopoietic cells in the spleen, is also most common in lymphoid malignancies and myeloproliferative disorders, but it may occur in any cancer that invades and enlarges the spleen or in cancers that cause severe hepatic disease or hepatic vascular disease resulting in portal hypertension. Some malignancies, particularly adenocarcinomas and most notably gastric adenocarcinoma, may result in extensive deposition of malignant cells in the distal arterioles and/or tissue factor activation of the coagulation cascade, resulting in erythrocyte and platelet destruction as a result of disseminated intravascular coagulation. Rarely, thrombotic microangiopathy (thrombotic thrombocytopenic purpura), which is not associated with consumption of coagulation proteins, may occur in various cancers.

### Radiation- and Drug-Induced Cytopenias in Patients With Cancer

The cytotoxic chemotherapeutic agents used in the treatment of most cancers have a number of adverse effects on hematopoiesis, which are summarized in Table 2. Ionizing radiation to extensive areas of bone marrow and a large number of non-cell cycle-dependent drugs, of which the alkylating agents are the prototype (eg, cyclophosphamide and its analogues, melphalan, busulfan, chlorambucil, nitrogen mustards, thiota, and nitrosoureas), when administered at high doses or for long periods of time, may lead to progressive depletion of hematopoietic stem cells. Another long-term effect of alkylating agents and topoisoamerase II inhibitors (eg, anthracycines, etoposide, vindesine) is the development of myelodysplasia and acute myeloid leukemias in a small percentage of patients. This frequently results in impaired bone marrow reserve capacity and may cause long-term cytopenias. Drugs that are more effective against actively proliferating cells (eg, cytarabine, methotrexate, anthracycines, etoposide, hydroxyurea) tend to cause earlier and shorter-lasting cytopenias, primarily because they have little effect on the largely quiescent, marrow-repopulating, pluripotent hematopoietic stem cells, but instead they kill committed hematopoietic progenitors and precursors that are proliferating actively. The progression through the cell cycle of some of the cells that are not killed by cycle-active drugs is partially blocked or delayed, resulting in transient cytopenias. Some chemotherapeutic agents (eg, anthracycines) have been reported to have secondary cytotoxic effects by inducing oxidant damage, even in mature hematopoietic cells, although little work has been done on this mechanism of cytotoxicity by chemotherapeutic agents. A rare complication of certain unrelated chemotherapeutic agents (eg, mitomycin C, cisplatin) is the development within one to 12 months after their use of thrombotic microangiopathy, which tends to be relatively refractory to plasma exchange and other usual therapeutic maneuvers. An often ignored or missed cause of pseudoanemia shortly after chemotherapy or biologic therapy administration (eg, IL-2, IL-11, possibly cisplatin) is fluid retention with secondary dilutional anemia. Either dilutional pseudoanemia or hemolysis should be considered in a patient who develops anemia in the first several days after drug administration at a rate of more than the 0.8% to 1% per day decrease in erythrocytes that would occur as a result of complete cessation of new erythroid cell production by the bone marrow.

<table>
<thead>
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<th>Table 2.--Causes of Drug-Induced Cytopenias in Patients With Cancer</th>
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<td>Stem cell death (long-term myelosuppression)</td>
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<td>Committed progenitor cell death (early/short-term myelosuppression)</td>
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<tr>
<td>Blockage or delay in cell cycling of hematopoietic precursors</td>
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<td>Reduced levels of hematopoietic growth factors (especially EPO)</td>
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<td>Oxidative damage to mature hematopoietic cells</td>
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<td>Long-term myelodysplasia</td>
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<td>Fluid retention/plasma volume expansion with dilutional anemia (RBCs)</td>
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### EPO Therapy for Anemia in Patients With Cancer

The anemia of cancer is thought to be due to the increased production of multiple interacting inhibitors of erythropoiesis and to partial inhibition of EPO production. These erythropoietic inhibitory cytokines include IL-1, IL-6, TNF-alpha, TGF-beta, and IFN-gamma. These same cytokines have been implicated in the anemia of chronic disease or inflammation, such as that which occurs in patients with rheumatoid arthritis, chronic infections, or other chronic inflammatory conditions. The anemia of chronic disease/inflammation is associated with a lower than expected plasma concentration of EPO for a given degree of anemia and a blunted response to both endogenous EPO and exogenously administered pharmacologic doses of EPO. These findings are more pronounced in anemia of cancer, and even more prominent after chemotherapy. Doses of EPO of 150 to 300 U/kg three times per week are highly effective in reducing the anemia and requirement for red blood cell transfusions in 30% to 80% of patients with anemia secondary to cancer and postchemotherapy (especially cisplatin).

### G-CSF and GM-CSF Therapy for Neutropenia

Although an uncommon direct complication of cancer, neutropenia is a frequent and potentially serious complication of intensive chemotherapy. Neutropenia results in an increased risk of infections, delays in administration of chemotherapy, and reduced (suboptimal) dose intensity of chemotherapy that is administered. Both G-CSF and GM-CSF administered for approximately 10 to 14 days, beginning shortly after completion of a chemotherapy course, can reduce the duration and, with less intensive chemotherapy, the severity of neutropenia. In several studies, these responses have been associated with reductions in numbers of infections, duration of infections, and days of hospitalization. G-CSF and GM-CSF have little if any efficacy when administered after a patient on intensive chemotherapy develops fever and other signs of infection when the expected duration of neutropenia is less than three weeks.

### G-CSF and GM-CSF in Bone Marrow and Peripheral Blood Stem and Progenitor Cell Transplantation

G-CSF and GM-CSF both are effective in hastening the time of recovery from severe neutropenia after myeloblastic chemotherapy and stem/progenitor cell transplantation. G-CSF and GM-CSF also are effective at "mobilizing" stem and progenitor cells into the peripheral blood to enhance greatly the numbers of stem and progenitor cells that can be obtained by leukapheresis for use in transplantation.

### Thrombopoietin and IL-11 for Treatment of Chemotherapy-Induced Thrombocytopenia

Patients receiving intensive chemotherapy may develop sufficiently severe thrombocytopenia to pose a risk of hemorrhage and to cause delays and reductions in intensity of chemotherapy administration. In phase II and phase III clinical trials, IL-11 reduced the number of platelet transfusions administered post-chemotherapy,
but platelets were required by the study protocol to be given automatically for any platelet count <20,000/µL, regardless of whether the patient was bleeding, had a high fever, or required a procedure. However, none of the patients in either the IL-11-treated or placebo control groups in the studies reported thus far had any significant bleeding complications, which supports the current practice of most hematologists/oncologists of not transfusing platelets prophylactically until platelet counts fall to

Conclusions Significant disruptions of normal hematopoiesis occur in patients with cancer. The mechanisms are related to elimination of pluripotent stem cells, damage to the bone marrow microenvironment that is required for normal hematopoiesis to occur, inhibition of production of hematopoietic growth factors, and/or production of hematopoietic inhibitory cytokines. These adverse effects of cancers on hematopoiesis often are accentuated substantially by ionizing radiation or cancer chemotherapy drugs. Although cytopenias in cancer patients usually are due to impairment of hematopoietic cell production, it is important to be aware of the possibility of dilutional anemia and of excessive sequestration, consumption, or destruction of all lineages of blood cells as a mechanism of anemia, thrombocytopenia, and, less commonly, neutropenia in patients with cancer. EPO is very effective in improving anemia, reducing transfusion requirements, and improving quality of life in over half of cancer patients with significant anemia. G-CSF and GM-CSF can be effective in shortening the duration and sometimes in reducing the nadir of neutropenia in patients receiving chemotherapy if the growth factor treatment is initiated within a day after each course of chemotherapy administration. However, they appear to be of little or no benefit when started in a chemotherapy-induced neutropenic patient who has already developed fever or other signs of infection. The benefits of thrombocytopenic growth factors such as IL-11, TPO, and MGDF are yet to be determined.

Reference

DR SPIVAK

The early literature on dilutional anemia (Nathaniel Berlin at the NCI studying the anemia of cancer in the 1950s) showed that some cancer patients who appeared to be anemic (before IL-11, IL-2 or anything else was available) actually just had an expanded plasma volume. The paper does not state whether these individuals had portal hypertension, congestive heart failure, or some other problem related to plasma volume, but it found that a proportion of anemic cancer patients with an expanded plasma volume will not be truly anemic. If these patients are given EPO, they will not appear to respond.

DR CRAWFORD

Why can EPO and chemotherapy be given concurrently with no negative impact? We have not been successful with other growth factors -- G-CSF and GM-CSF. Do we really know that EPO can be given without a negative impact on other cell lineages? Has a trial been done to determine if chemotherapy blunts the effect of erythropoietin?

DR SPIVAK

When hydroxyurea and EPO were given to patients with sickle cell anemia, the studies were a failure in terms of trying to produce increased hemoglobin F, because timing was everything. Given concurrently, the hydroxyurea tended to blunt the effect of the EPO. On the other hand, there are a number of clinical trials where EPO was given continuously and chemotherapy was given cyclically. In these trials, the effect of EPO was almost as if the chemotherapy was not given. I believe that early cells responsive to erythropoietin are not actively in the cell cycle, and there must be a lot of them.

DR ZUCKERMAN

I am not aware that any trial has addressed whether EPO would be more effective if given in a different schedule, avoiding those few days around chemotherapy, as has been the custom for the other growth factors. The other growth factors that are in current use or in clinical trials have significant effects on at least cells that are as far back to the stem-cell stage, while EPO does not. EPO has some minor effects on early erythroid progenitor cells, but it has no known effect on the very earliest stem and multipotent cells. The majority of its effect is on cells at about the CFU-E stage, which are very mature cells. The maturation time from CFU-E to red blood cell is probably a week at most, although in a stress situation, it is a few days faster than that.

DR SPIVAK

In one study, the late Kurt Reisman gave busulfan to mice and showed he could ablate their stem cell pool. He gave them EPO and could get a response with ablation of the stem cell pool. He was looking at spleen colony-forming units, which today people might not want to consider as stem cells. Nevertheless, in the face of chronic busulfan therapy, where he certainly diminished primitive progenitor and probably stem cells, EPO still worked. There are some agents such as methotrexate and hydroxyurea where you do not get an effect with EPO, but if you use other agents such as BCNU, EPO will work right through it.

The problem is that if you do this long enough, you will eventually deplete your stem cells to such an extent that there is nothing left to come forward for EPO to work on. So it depends on the chemotherapeutic agent. With some, the timing may not be so important.

DR ZUCKERMAN

I would suggest that the chemotherapy schedule probably has something to do with it. If hydroxyurea is given every day and EPO every day, you will see a blunted response to EPO. On the other hand, I would speculate that if hydroxyurea would be given the way that so many other chemotherapy regimens are given -- one to five days at a time and then off for three to four weeks -- the effect might be different. When a cell- cycle-active agent such as hydroxyurea or, I suppose, 6-thioguanine, methotrexate, or cytarabine, is given every day while EPO is being given, it is not surprising that the effect of EPO is diminished. If the cell cycle active agent is given in every three- or four-week cycles, you might see a good response to EPO probably due to that late progenitor effect of EPO and the short maturation time from those cells to a mature red cell.

DR MOSCINSKI

Most of the red cell precursors are in cycle anyway. We do a lot of KI-67 staining of bone marrows, and if you look at regular, normal bone marrow, the vast majority of erythroid precursors are in cycle, which means that they are at G1 or beyond. Does EPO shorten the time of cycle? If most of the red cells are in cycle anyway, I predict that there would be differences between treatment during chemotherapy or afterwards.
There is a narrow window of activity of EPO, actually. There are all the studies that Dr Spivak discussed, and there are the studies in which mice have been made polycythemic by hypertransfusion or by putting them in oxygen deprivation situations until they become polycythemic to suppress endogenous EPO production. If you examine the effects of polycythemia-induced reduction of endogenous EPO levels on erythroid precursors, you find that whenever EPO production is reduced substantially or when EPO is taken away, the cells beyond proerythroblasts, which are slightly more mature than the CFU-E, keep going their merry way, completing their differentiation program in the low EPO environment, and the reduction of EPO levels does not seem to have a substantial effect on them.

If you deprive mice of the EPO effect and then see what progenitors they have, there are approximately normal numbers of BFU-E, the earlier progenitors, and there are no or markedly reduced numbers of CFU-E. Their mature cells in the marrows that already were present before the mice were made polycythemic continue to mature, but these mice do not generate any more maturing erythroid cells until EPO is present again. So it is really a narrow point in erythropoiesis that is the final, single control point of EPO -- immediately prior to and at the CFU-E stage.

Would you guess that maybe it is another effect? Methotrexate and hydroxyurea have profound maturational effects on erythroid and myeloid precursors, as well as affecting them in cycle.

Erythroid cells have been studied at every point in the cell cycle. The simplest way is to get a flow cytometer or, actually, a centrifugal elutriator, and separate erythroid progenitor cells into literally G_0, G_1, S, and G_2/M in high degrees of purity. We have done this and published it. When you put in EPO, the late cells are 90% in cell cycle, and they don’t really care whether EPO is around; they are going to go through the cell cycle, like it or not. They may die before they get too far along if there is no EPO around, because they will apoptosis. On the other hand, EPO neither changes their cell cycle status nor shortens any period of the cell cycle. Late erythroid cells are already in cycle. EPO is just a survival factor. The BFU-E triggers them into cycle, but you do not see those because they are a minority, relatively speaking, they are indistinguishable from any other cell, and they are quiescent. They are in a G_0 state, and it takes a lot of EPO to trigger them into cycle.

The early studies of suppressing EPO production are flawed because the techniques to measure EPO were insensitive to low levels of EPO. You cannot suppress EPO completely. At least some EPO is present in everybody, and we have measured it in people over days, weeks, and months. Even in polycythemia vera with a hemoglobin level of 20 g/dL, you will have perhaps 2 mU/mL of EPO, which is more than enough. EPO is always there, the cells are always viable, but you have to increase the level to get the BFU-E into cycle. So they are protected from cell cycle activations.