Anemia of Malignancy

The Role of the Internist in the Clinical Approach to this Paraneoplastic Lesion

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February 8, 1996

Introduction

Anemia commonly occurs during the course of malignant disease, sometimes as a direct result of the neoplasm or its products and often as a result of therapy. The term "multifactorial" has often been applied to serve as an explanation for the finding, but the use of such nomenclature frequently limits the dissection of the possible mechanisms responsible for the anemia and, thereby, precludes appropriate therapeutic intervention. While anemia often may be a paraneoplastic lesion, the important clinical approach is to work through the full differential diagnosis of anemia, thereby identifying correctable lesions.

Historically, a variety of names have been used to denote the anemia seen in patients with malignant disease: these include Simple Chronic Anemia, the Anemia of Chronic Disease, Iron Re-Utilization Anemia, and more recently, the Anemia of Malignancy. Characterization of the role of cytokine production in neoplasia has now provided the beginning of the delineation of this paraneoplastic lesion, as well as evidence that the relevant mechanism (s) significantly different than those found in the classical "anemia of chronic disease" which occurs during (chronic) infection and inflammatory states (1).

Clinical Features

The clinical symptoms manifest by the paraneoplastic lesion, termed the "Anemia of Malignancy", are in general mild, paralleling the severely of the anemia; it is rare for the hemoglobin to fall below 8 g/dl. In addition, its rate of development is usually slow, generally weeks to months. Although weakness and fatiguability may occur, a severe anemia with altered cardio-pulmonary function is rare. Indeed, the presence of a severe or rapidly developing symptomatic anemia suggests that another mechanism is superimposed upon the anemia of malignancy.

Characterization of the Anemia Associated with Malignancy

Although conceptually, the "Anemia of Malignancy" proposes a paraneoplastic lesion as its basis, its characterization and clinical delineation require that when the anemia is identified, one must first consider that the anemia is due to the direct effects of the neoplasm or that it is due to its known products from the cancer. Only when these are excluded can one define the anemia as the paraneoplastic lesion termed the "Anemia of Malignancy" where the symptoms, signs and pathophysiologic mechanism are not due directly to the tumor, but to products of the malignancy that are still incompletely defined, although currently these appear to be the result of neoplasm-associated cytokine production.

Table 1

Differential Diagnosis of Anemia of Malignancy

1. Anemia Due to Direct Effects of the Neoplasm.
    - Bleeding
    - Bone Marrow Replacement
2. Anemia Due to Known Products of the Neoplasm.
    - Immune Hemolytic Anemia
    - Microangiopathic Hemolytic Anemia
    - Amyloidosis
3. Anemia Due Incompletely Defined Products of the Neoplasm.
    - Pure Red Cell Aplasia
    - Hemophagocytic Syndrome
    - "Anemia of Malignancy"

Anemia Due to Direct Effects of the Malignancy

Two common mechanisms for anemia in patients with cancer that are direct effects of the malignancy are blood loss and bone marrow infiltration by the neoplasm:

Table 2

Anemia Due to Direct Effects of the Malignancy

	Mechanism		Neoplasms
1.	Blood loss: Acute and/or chronic		
	a) Exogenous Blood Loss.	-	Head and neck cancers
		-	Gastrointestinal cancers
		-	Genitourinary cancers
		-	Cervical and vaginal neoplasms
	b) Intra-tumor bleeding.	-	Sarcomas
		-	Bulky melanoma, and hepatoma, ovarian and adrenocortical cancers
2.	Bone marrow replacement	-	Carcinomas (especially breast and prostate)
		-	Leukemia
		-	Lymphoma
		-	Myeloma
	Marrow Replacement:	-	Tumor
		-	Fibrosis
		-	Granulomata
			Lipid Storage
	Severe Marrow Stress		

Blood Loss: Acute or Chronic: As expected, blood loss, both acute and chronic, is most commonly associated and recognized with neoplasms that allow exogenous bleeding. In particular, these include cancers of the gastrointestinal, genitourinary, gynecologic and head and neck sites. Less clinically apparent is hemorrhage within large bulky tumors; this is particularly seen in sarcomas, melanomas, and carcinomas of the ovary, liver and

adrenal cortex. CT imaging has now provided evidence that such intra-tumor hemorrhage is quite common, although it is usually not of great quantity. The laboratory features in the anemia associated with blood loss commonly include a declining mean corpuscular volume (MCV), an increase in the degree of anisocytosis, which may be numerically defined by the red cell distribution width (RDW), and an increase in the reticulocyte count (Table 4). Since the paraneoplastic mechanism of the anemia of malignancy is associated with suppressed erythropoiesis, the changes found in blood loss from cancers are quite different from blood loss from other causes, when erythropoiesis is not suppressed. Acute blood loss usually results in a brisk reticulocytosis; in patients with cancer this may be blunted and reticulocytes may be produced in normal or even reduced numbers. The presence of reactive thrombocytosis commonly seen with the bleeding may help define blood loss as the basis for the declining red cell numbers; again, the platelet increment expected may be less in patients with blood loss from cancer than in other causes of acute blood loss. The changes in the parameters of the body iron compartments are similarly less than those seen when the blood loss is not associated with cancer. While a decline in serum iron occurs, the expected increment in total iron binding capacity, (TIBC) or transferrin is usually not seen with blood loss compounding the anemia of cancer; the TIBC is often below the normal range. Thus the usual 'rule of thumb' of a 10% saturation of iron binding capacity as a way to define iron deficiency is not reliable in this setting (2). Similarly, in simple blood loss the serum ferritin, which in normal individuals provides a measure of body iron stores, can be expected to be progressively reduced, (i.e. to less than 20 ng/ml). In the presence of cancer as with other causes of the "anemia of chronic disease, the serum ferritin declines as in normal individuals, but to a significantly lesser degree. Therefore, in the presence of cancer a serum ferritin below approximately 100 ng/ml provides strong support that iron deficient erythropoiesis has compounded the expected findings in patients with the "anemia of chronic disease" (3, 4, 5). Similarly, the red cell

ferritin is normal in the anemia of malignancy, but will decrease slowly when blood loss is also present (5). Parenthetically, it should be noted that the serum ferritin can be markedly elevated in some neoplasms (especially Hodgkin's disease, hepatoma and leukemia) unrelated to iron stores; this appears to be the result of several different mechanisms, including cytosolic release of ferritin, and ferritin's role as an acute phase protein. Assay of serum human receptor transferrin (T&R) follows a similar pattern. the T&R is proportional to the total body moss of tissue receptors thereby providing a relative measure of the active proliferative portion of the erythron.

Table 3

Serum Transferrin Receptor

Normal: 3.0 to 8.5 μg/ml

↑ receptor indicates increased Erythropoiesis

Also: ↑ in Iron Deficiency

Normal: In Anemia of Chronic Disease

A clinical clue to the presence and even the site of blood loss can be the development of polychromatophilia (or increased reticulocytes) in a patient with known iron deficiency. This finding suggests that the blood loss is proximal to the duodenum, the primary site of iron (or heme-iron) absorption; or, alternatively bleeding into a tumor with (transiently) increased iron re-utilization.

Bone Marrow Replacement by Tumor: Bone marrow replacement by the malignancy provides a second direct mechanism of anemia. This is expected in hematologic malignancies (i.e. leukemia, lymphoma and myeloma), but is often overlooked in other cancers. Prostate and breast cancer, in particular, have a high incidence of bone marrow metastases with direct replacement of the hematopoietic mass. In addition, these

tumors often produce a desmoid (fibrotic) reaction with increased marrow fibrosis that further compromises the marrow space and causes an important alteration of the sinusoidal matrix, thereby affecting the usual orderly release of mature blood cells into the circulation. The primary clue to marrow infiltration with tumor is leukoerythroblastosis (i.e. the presence of immature red and white cells in the circulation) identified on the blood smear (Table 4). The altered release of cells results in nucleated red cells of all stages of maturity in the circulation, an increase the variation in red cell size (RDW) and shape (i.e. poikilocytosis). In addition, immature granulocytes are found in the circulation, often with an associated mild leukocytosis. Finally, an increase in the number of platelets with variation in their size and shape may also be seen. These changes, termed "leukoerythroblastosis" are characteristic enough that it is often possible to make a diagnosis of marrow infiltration (by tumor) simply by examining the blood smear.

Table 4

Laboratory Features of Anemias Associated with Malignancy

	Blood Smear	MCV	RDW	Reticulocyte Count	Serum Iron	Iron Binding Capacity	% Saturation of Transferrin	Serum Ferritin	RBC Ferritin	Erythropoietin Levels
Anemias Related to direct Effects of the Neoplasm										
Blood Loss	Anisocytosis	↓	↑	N to ↑	↓	N to only slightly ↓	+ 10%	< 100	Low N	↑
Marrow Replacement	Anisocytosis, poikilocytosis nucleated RBC's, shift to left WBC, ⇑ Platelets	N	↑	↑	↓	↓	> 10%	N	N	↑
Anemia Due to Known Products of the Neoplasm										
Warm Antibody Hemolytic Anemia	Spherocytosis and Polychromato-philia	N to ↑	↑	↑	N to ↑	N	N	N	N	N
Cold Agglutinin	Clumped ("laked") red cells	Falsely Elevated	↑	↑	N to ↑	N	N	N to ↑	N	N
Microangiopathic Hemolytic Anemia	Red Cell Fragmentation (Schistocytes)	N to ↓	↑	↑	N to ↑	N	N	N	N	N
Amyloidosis	N red cells with Howell Jolly body inclusions	N	N	N	N	N	N	N	N	N
Anemia Due to Unknown Products of the Neoplasm										
Anemia of malignancy	N red cells 30% hypochromic	N 30% microcytic	N to ↑	Markedly ↓	N to ↓	N to ↓	N to ↓	N	N	Slightly reduced
Pure Red Cells Aplasia	N red cells	N to ↑	N	Markedly reduced	N to reduced	N to ↓	N	N	N	↑
Hemophagocytic Syndrome	N red cells	N	N	N	N to ↓	N to ↓	N	N	N	N to ↓

N = Normal

Table 5

"Leukoerythroblastosis"

(Leukoerythropiastranemia)

Features: Nucleated RBC's

Shift to left: Granulocytic Series

Increased and Abnormal Platelets

Diagnostic Considerations:

Marrow Replacement:

- Tumor
- Fibrosis
- Granulomata
- Lipid Storage

Severe Marrow Stress

A somewhat special circumstance of marrow infiltration can be see in patients with malignant plasma cell dyscrasias where the tumor produces amyloid (Table 5). The amyloid can partially replace the marrow, and its perivascular deposition can further reduce red cell release from the marrow. Another further clue to the presence of amyloid is finding the red cell nuclear debris as a form of cellular inclusion. These Howell-Jolly bodies, seen on the blood smear are the result of concomitant splenic infiltration with amyloid with the loss of the normal "pitting" function in this form of "hyposplenic state" (6).

Anemia Due to Known Products of the Malignancy

Cancers are known to produce a variety of proteins that can cause anemia. One such product, amyloid, is known to replace the marrow space as was discussed above. Other mechanisms include antibody-mediated hemolytic anemia, both of the warm-reacting antibody type and the cold agglutinin form, and mechanical (e.g. red cell membrane injury) hemolytic anemias.

Table 6

Anemia Due to Known Products of the Malignancy

Mechanism	Neoplasms
Amyloidosis	Plasma Cell Neoplasms (Myeloma)
Immune Hemolytic Anemia	
Warm Antibody Hemolytic Anemia (AIHA)	Lymphoma Chronic lymphocytic leukemia Adenocarcinomas, mucin producing type
Cold Agglutinin Disease (CAD)	Waldenstrom's macroglobulinemia Lymphoma
Microangiopathic Hemolytic Anemia (MAHA)	Adenocarcinomas, mucin-producing type Prostate Cancer

Warm Antibody-mediated Hemolytic Anemia (AIHA): Approximately 20% of cases of this type of anemia are associated with malignancy; the neoplasms seen are most commonly lympho-proliferative neoplasms, (chronic lymphocytic leukemia and non-Hodgkin's lymphoma usually of the indolent or intermediate types); less commonly it is seen in association with Hodgkin's disease, and quite rarely with adenocarcinomas. The exact mechanism whereby these tumors produce an auto-antibody with red cell specificity is not known. Production of this auto-antibody does not correlate with circulating immunoglobulin concentration, since it can be seen even when hypogammalobulinemia is associated with the lympho-proliferative neoplasm. These auto-antibodies attach to the red cell surface, and are identified by the laboratory evidence of a positive direct antiglobulin test.

The red cells coated with the auto-antibody have a shortened survival in the circulation due to selective sequestration in the spleen, and to a much lesser extent in the liver. Splenic sequestration is then followed by macrophage-mediated destruction of these red cells. Historically great interest has focused on the role of the spleen and liver in the sequestration of antibody coated red cells (7). The monumental studies of Jandl and coworkers at the Thorndike Laboratory (8) carefully examined the quantitative roles of these organs and demonstrated that site of sequestration depended upon the size of the agglutinatis, the relative "pore" size of the splenic and hepatic filters, the pressure gradients, and the "completeness" of the antibody, (in particular the complement "coated" status). Thus, with relatively large agglutinins as occur in cold hemolytic anemias the liver is predominately important, and the spleen relatively unimportant, because of its much smaller blood flow (7,8). In warm antibody mediated hemolytic anemia the agglutinates are smaller and often lack (complete) complement; these pass through the liver sinusoids without further injury and are entrapped by the more sensitive filtering mechanism of the spleen.

AIHA may also, in rare circumstances, occur in the presence of an adenocarcinoma of the mucin-producing type. While this is usually associated with ovarian cancer, it can occur with any mucin-producing tumor. The first case seen in a patient with ovarian cancer was described in 1938 (69); this is now a well recognized circumstance. Laboratory studies are identical to those in other types of AIHA, and it appears that it is free mucin which coats the red cell membrane and produces a finding similar to that seen with true auto-antibodies to the erythrocyte membrane. Characteristically, the AIHA is refractory to the usual therapy (ie steroids, splenectomy, immunosuppressive drugs, etc.), but remission occurs with successful therapy of the cancer (10).

The laboratory features of AIHA of warm antibody type are shown in Table 4 and include spherocytes and increased reticulocytes on the blood smear. The direct antiglobulin test is positive with IgG or complement or both found on the red cell. The eluted or serum antibody usually has pan-agglutinin characteristics; in Hodgkin's disease, the antibody may be directed at a modified I antigen on the red cell - the transitional I or I^t antigen.

Therapy for the warm antibody autoimmune hemolytic anemia is directed at reduction of both antibody production and splenic destruction; adrenocortical steroids (1 -2 mg/kg) are the initial treatment of choice, in addition to treatment of the malignancy itself. Since the hemolysis may be abrupt and precipitous, steroid therapy may be needed on an emergency basis. "Megadoses" of steroids (one or two doses of 500 - 1000 mg. of methylprednisolone) have been utilized at the initiation of therapy if the red cell destruction is rapid; this approach has appeared to be helpful, but the experience has been limited and uncontrolled. The measures used to treat the underlying

neoplasm, if effective, can be expected to control the hemolytic anemia. In general, this is particularly of value when the AIHA is the result of a lympho-proliferative lesion.

Antibody Mediated Hemolytic Anemia of the Cold Antibody Type: 'Cold agglutinin disease (CAD) is only rarely associated with a malignancy. When this association occurs, it is generally with a lymphoproliferative lesion. The classical clinical pattern is the gradual onset of a chronic cold agglutinin hemolytic anemia of mild to moderate severity; then, in ensuing months to years the clinical picture of non-Hodgkin's lymphoma or Waldenström's macroglobulinemia becomes evident. Less common is the presence of an indolent lymphoma occurring with the CAD. Cold agglutinin disease rarely produces a severe anemia; usually the hemoglobin in the 8 - 10 g/dl range. The laboratory features are shown in Table 4. Often, the primary diagnostic clue is auto-agglutination, or "laking" of red cells noted in the blood smear. Clumping of red cells during automated CBC analysis can result in a falsely elevated measurement of MCV. The value declines markedly on warming the blood specimen toward 37°C; this can be done by holding the specimen tube in the hand for a few minutes before allowing blood uptake by the automated counting instrument, a procedure often instinctively performed by the experienced laboratory technologist. In CAD the direct antiglobulin test is positive for complement (C3) alone on the red cell surface; the IgM dissociates from the surface and is not detectable on the red cell. The cold agglutinin in malignant disease has specificity for the I red cell antigen, and has monoclonal characteristics, almost always kappa chain specificity. The anemia of cold agglutinin disease is the result of selective sequestration of the coated red cells. This occurs primarily in the liver, where sensitized cells are removed by Kupffer cells of the reticulo-endothelial system. Careful serologic examination will usually identify C3d rather than C3b on the circulating red cells. This degraded C3d complement is the result of proteolytic inactivation of red cell bound C3b; progression of complement activation through to the formation of the

membrane attack complex, with intravascular hemolysis, does not usually occur in CAD. C3d binds so minimally to the C3 receptors on the phagocytic cells of the reticulo-endothelial system that C3d-coated red cells escape destruction, and circulate with only a modestly shortened survival. In fact, they survive longer than cells transfused into the patient, because the C3d affords a level of protection by limiting subsequent sequestration.

In general therapy for CAD is not very effective. When this anemia occurs in the presence of a neoplasm it is usually mild (hemoglobin in 8 to 10 g/dl) and no specific therapy is needed. Steroids have little effect on the hemolysis. Since red cell sequestration is mostly hepatic, splenectomy is of little value. The primary approach in these patients is to avoid cold exposure when possible; CAD associated with an underlying lympho-proliferative disease often respond to treatment of that lesion, with alkylating agents or purine analogs, especially flutarabine and 2 - chlorodeoxyadenosine.

Microangiopathic Hemolytic Anemia: Microangiopathic hemolytic anemia (MAHA) has a well known, albeit uncommon, occurrence in patients with cancer, particularly with prostate cancer and mucin-producing adenocarcinomas (i.e. ovarian, gastrointestinal). The mechanism which produces MAHA is not certain, but appears to be related to the liberation of procoagulant proteins by the tumor cells, which then activate Factor X inducing a pattern of localized intravascular microthrombosis, a mechanism similar to that in classical disseminated intravascular coagulopathies (11). The laboratory features (see Table 3) which identify this mechanism include evidence of red cell fragmentation and usually an associated thrombocytopenia. The erythrocyte MCV is usually decreased and examination of a red cell mean corpuscular volume distribution graph may show two distinct red cell populations, one made up of the small fragments (schistocytes) and the other a near normal-sized population of red cells.

Evidence of a mild consumptive coagulopathy is frequently identified, with increased fibrin degradation products, such as d-dimers The only effective therapy is that of the successful treatment of the underlying cancer.

Table 7

Anemia Due to Incompletely Defined Products by the Malignancy

The Anemia of Malignancy

	Mechanism	Neoplasm
1.	Anemia of malignancy (anemia of chronic disease; induced iron reutilization defect; cytokine associated anemia).	All cancers
2.	Pure red cell aplasia	Thymoma; Chronic lymphocytic leukemia
3.	Hemophagocytic Syndrome	Gastric Cancer Lymphoma Leukemia

Anemia of Malignancy: It is the "anemia of malignancy" (previously termed simple chronic anemia, the anemia of chronic disease or iron re-utilization [defect] anemia), that is the most consistent, expected association with all cancers. The features that characterize this anemia are:

Table 8

Laboratory Findings in the "Anemia of Malignancy"

Peripheral Blood:	Normocytic anemia; 30% microcytic, hypochromic
	Normal RDW: absence of anisocytosis
	Reticulocytopenia: decreased polychromatophilia
Biochemical Panel:	Serum iron: decreased
	Serum iron binding capacity: normal or decreased
	Transferrin saturation greater than 10%
	Serum ferritin: normal
	Red cell ferritin: normal
	Transferrin Receptor: Normal
Other Studies:	Erythropoietin Levels: decreased for the degree of anemia.
	Reduced re-utilization of 59 Fe from labeled, heat-damaged red cells.
	Shortened red cell survival
Bone marrow Examination:	Relative and absolute decrease in erythroid mass
	Delayed hemoglobinization with increased basophilic nomoblasts
	Decreased marrow sideroblasts
	Normal (or slightly increased) iron content of RE cells

Although the phenomenon was first described more than one hundred and fifty years ago by Andral and Gavarret (12) , and in spite of extensive studies by Cartwright (13) after World War II, the mystery of the pathophysiologic mechanism(s) still remains (14, 15, 16). Cartwright proposed three mechanisms in the pathophysiology of this anemia:

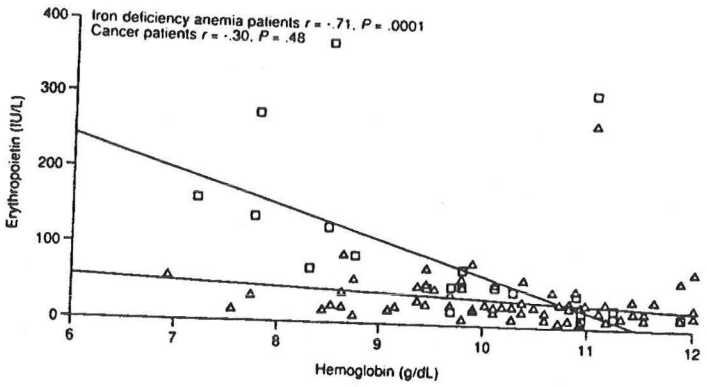
1) shortened red cell survival; i.e. a hemolytic anemia;
2) failure of the bone marrow to increase erythropoiesis to repair the deficiency, i.e. a hypoproliferative state;
3) impaired release of iron from the senescent red cells phagocytosed by the marrow macrophages, i.e. defective iron re-utilization.

Each of these mechanisms are known to be factors in the "Anemia of Malignancy"; it is, however, their specific underlying pathophysiologic basis that is not yet certain.

A decrease in red cell survival has been clearly demonstrated in patients with cancer (15). However, the shortening of the red cell survival is only modest. When examined with radio-chromium labeled - red cells, erythrocyte survival, expressed as half life or $T_{1/2}$ was 20 to 25 days, with normal cells having a $T_{1/2}$ of 29 - 33 days. Parenthetically, the normal red survival measured with radiochromium reflects the approximate 1% per day elution of the label, so that the expected $T_{1/2}$ of 60 days (based on a normal erythrocyte survival of 120 days) is halved. In patients with malignancy the shortening of the erythrocyte survival is found whether the patient's own cells, or compatible donor cells, are given to the patient. However, when the patient's cells are given to a normal recipient, the survival is normal. These are the classical findings of an extra-corpuscular red cell defect as the basis of increased red cell destruction.

Recent studies have identified decreased osmotic resistance of red cells in patients with cancer that appears to be the basis for the shortened red cell survival (17). Honda, et al (18) have now identified a protein (M.W. 50,000) which alters osmotic resistance and has been labeled "anemia-inducing substance". It is of interest, that this "anemia-inducing substance" was not present in the plasma of patients with rheumatoid arthritis, since it is from the studies of such chronic inflammatory disorders that the term "anemia of chronic disease" has been characterized, and it is in this group that most of the pathophysiologic mechanisms have been examined and defined. Whether the anemia of malignancy is fundamentally different from other causes of the anemia of chronic disease (i.e. infection and inflammation) must now be re-examined. Although, it is clear that shortened red cell survival can be found in virtually all patients with cancer, its contribution to the anemia of malignancy must be considered to be very modest at best, since a normal bone marrow can easily compensate for red cell survivals with a $T_{1/2}$ as short as 17 days (19). Thus, the slight degree of hemolysis found in cancer patients should easily be compensated by the expected erythropoiesis of an otherwise uninvolved bone marrow.

The second mechanism of the anemia of malignancy has been the failure of the bone marrow to increase production appropriate to the demand; this hypoproliferative mechanism has been related to decreased erythropoietin (EPO) production or responsiveness. In general, a reduced EPO secretion and response has been identified in patients with the anemia of malignancy (20, 21, 22), but the decrease does not completely correlate with the degree of depressed erythropoiesis (23, 24, 25) that is, the decreased EPO is still relatively elevated above normal, thereby defining a failure of the marrow to respond adequately to that elevated level.



The serum erythropoietin-hemoglobin relationship in patients with uncomplicated iron deficiency anemia and patients with cancer. (21)

However, recent evidence that recombinant EPO can often correct the anemia of malignancy in many cancer patients re-focused interest in the decrease in EPO production as more important than previously considered (26, 27, 28, 29).

The basis of the inappropriate secretion and response of EPO has been extensively studied in in vitro systems. Since the disorders in which the "anemia of chronic disease" develops (chronic inflammation, infection, cancer) circumstances in which macrophage - derived inflammatory cytokines have been noted, these have been measured in cell systems for their effect on EPO production (30). Using hypoxia as stimulus to EPO production by the human hepatoma cell line (Hep 3 B), they examined a large series of cytokines and found that Interleukin (IL- 1) and tumor necrosis factor - ∝ (TNF ∝) inhibited secretion by up to 89%. Inhibition was dose dependent and although IL-l ∝ B were both inhibitory IL-l B was the most suppressive cytokine studied.

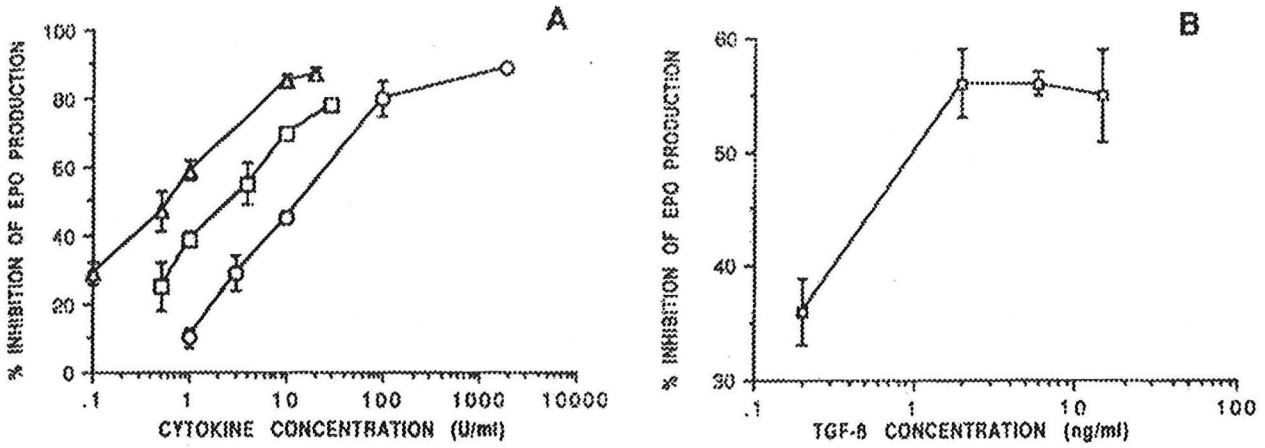


Fig 1. Dose-response inhibition of hypoxia-induced Epo production by IL-1α, IL-1β, TNF-α, and TGF-β. Hep3B cells were grown to confluency in 100-mm tissue culture dishes and incubated under hypoxic conditions (1% O_2) for 24 hours in triplicate with varying concentrations of (A) IL-1α (□), IL-1β (△), TNF-α (○), or (B) TGF-β.

Combinations of cytokines were shown to be additive. By northern blot analysis EPO messenger RNA levels in HEP 3B cells grown in 1% O_2 were decreased when concurrently exposed to either Il-1 or TNF.

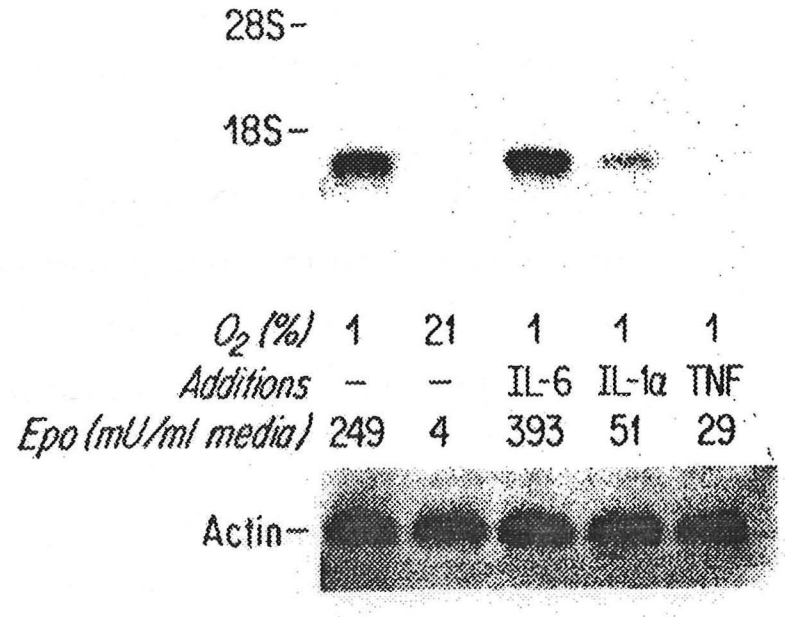


Fig 5. Effect of IL-6, IL-1α, and TNF-α on hypoxia-induced Epo mRNA levels. Hep3B cells grown to confluency in 150-mm plates were incubated either nonhypoxically (21% O_2) or hypoxically (1% O_2) for 24 hours in the presence or absence of IL-6 (1,000 U/mL), IL-1α (20 U/mL), or TNF-α (1,000 U/mL) as indicated. Total cellular RNA was isolated and RNA blot analysis was performed using 20 μg total RNA per lane. The results obtained when the RNA-containing filter was hybridized with ^{32}P-labeled Epo cDNA are shown at the top. As a control, the hybridization to radiolabeled mouse β-actin is presented at the bottom. In addition, the amount of Epo produced by the cells was determined in duplicate by RIA as shown. (30)

That the "cytokine" affect is not a simple one is emphasized by their evidence that the addition of IL-6 resulted in a dose dependent stimulation of hypoxia - induced EPO production by as much as 81% (30).

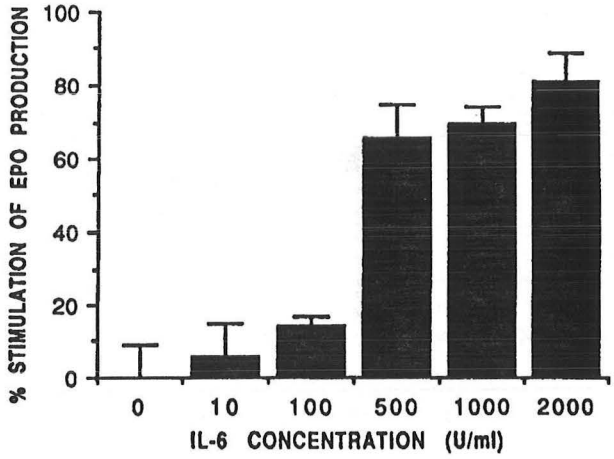


Fig 2. Dose-response stimulation of hypoxia-induced Epo production by IL-6. Confluent 100-mm plates of Hep3B cells were incubated in triplicate for 24 hours under hypoxic conditions with varying concentrations of IL-6.

From these and related studies an attractive explanation for the hypoproliferative response of the marrow appears to be cytokine production by the neoplasm that results in inhibition of EPO production. The mechanism for the decreased responsiveness of the erythroid committed compartment to EPO is less well understood.

The third pathophysiologic mechanism evident in the anemia of malignancy is that of impaired release of iron from senescent red cells that have been engulfed by the marrow macrophages. This is the normal means in the body economy for re-cycling of iron extracted from spent red blood cells. This mechanism has had the greatest research and clinical thrust because of the evident changes in iron metabolism seen in these patients (2, 31, 32) [Tables 3, 4 and 7]. As mentioned above, it must be emphasized that most of the studies of this mechanism have been in chronic inflammatory lesions, especially rheumatoid arthritis, and one must now be more cautious in "lumping" all of the previous mechanisms (infection, inflammation and cancer) as having a single

pathophysiologic mechanism. These studies have demonstrated a decreased re-utilization of iron extracted from senescent red blood cell (2, 33). The physiologic importance of the re-utilization of iron derived from senescent red cells can be defined when one recognizes that approximately 25 mg of iron is needed each day for effective erythropoiesis. Since normal iron absorption is in the range of 1 to 2 mg per day, it's evident that the demands for iron reclaimed from the storage pool (i.e. re-utilization) of iron from dying red cells are great. In terms of "significant" mechanisms, it does appear that the iron re-utilization defect is quantitatively the most important basis for the anemia of malignancy. Again, this mechanism is not completely understood.

It is now well accepted that the impaired iron metabolism and the depressed erythropoiesis are the primary hallmarks of the anemia of malignancy. The mechanism(s) that produce these changes are not entirely clear. The classical historical perspective of the "anemia of chronic disease" is that this anemia occurred in three clinical settings: chronic infection, chronic inflammation, or cancer. The classical models to examine the physiologic changes commonly were turpentine abscesses in animals and rheumatoid arthritis in patients. Recent evidence that some inflammatory cytokines can alter hematopoiesis has led to the conclusion that the anemia of chronic disease is the result of increased cytokine production (25, 34, 35). Animal and in vitro marrow models have shown that the administration of tumor necrosis factor, IL-1 or interferon (IFN) Beta or χ results in an anemia or the changes in erythroid maturation with the features consistent with the anemia of chronic disease (25, 36, 37, 38, 39, 40, 41). A representative result of such a study with TNF:

Table 2. Marrow Progenitors in TNF and Control Mice (37)

Marrow Progenitors	Control		TNF	
	Per 10^5 Cells	Per Femur	Per 10^5 Cells	Per Femur
Cells	—	$6.8 \pm 2 \times 10^6$	—	$2.7 \pm 0.5 \times 10^{6*}$
CFU-E	770 ± 200	$8.5 \pm 0.2 \times 10^4$	$68 \pm 13^*$	$1.2 \pm 0.2 \times 10^{3*}$
BFU-E	180 ± 50	$2.0 \pm 0.5 \times 10^4$	$35 \pm 7^*$	$6.5 \pm 1 \times 10^{2*}$
CFU-GEMM	30 ± 5	$1.9 \pm 0.5 \times 10^3$	43 ± 12	$1.3 \pm 0.4 \times 10^3$
CFU-GM	60 ± 9	$2.4 \pm 1 \times 10^3$	60 ± 20	$1.8 \pm 0.7 \times 10^3$
CFU-Meg	16 ± 3.6	$1.1 \pm 2 \times 10^3$	40 ± 16	$1.1 \pm 4 \times 10^3$

Thus, TNF has been shown to cause in a decrease in erythropoiesis as expressed by a decrease in colony forming activity of both burst and erythroid units (ie CFU - B and CFU - E) in both the liver and spleen. Associated with this is a prompt decline in reticulocytes and a subsequent decline in hematocrit. The addition of IFN, especially IFN - gamma, enhances the suppression.

Subsequent studies in patients have added significant support to the evidence that these three cytokines (TNF, IL-1 and IFNχ). Suppress erythropoiesis (40, 41). Most of the clinical data came from patients with rheumatoid arthritis and the measurements of these cytokines have been used to project parallels between their levels and the degree of suppression of erythropoiesis (42, 43). These observations have led Krantz and coworkers (25) to propound a schema whereby these cytokines induce their hematopoietic suppressive effects inducing the anemia of chronic disease (ACD):

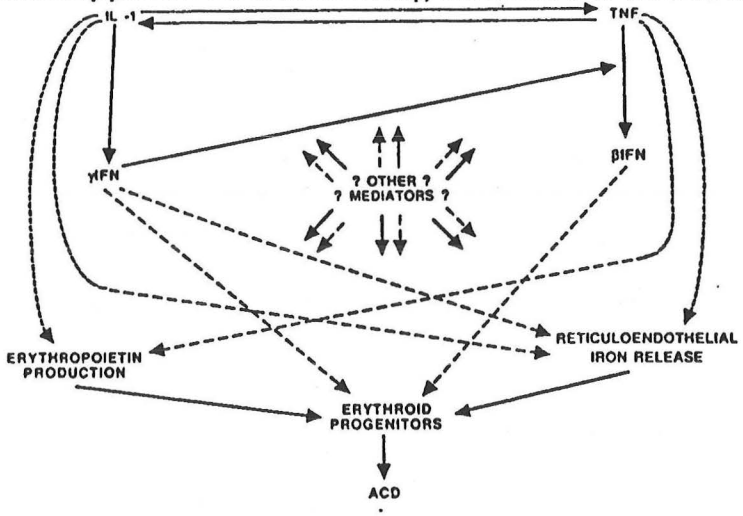


Fig 6. Schematic diagram representing effects of inflammatory cytokines on processes involved in the impairment of erythropoiesis in ACD. Positive regulatory effects are indicated by solid lines and negative effects by broken lines.

Although an interesting way to express these interactions, it is not yet clear how these interactive cytokines actually affect hematopoiesis, and specifically, erythropoiesis in normal physiologic terms. Specifically relevant to the circumstance of the anemia of malignancy, the question must again be asked whether the classical etiologic circumstance (ie infection, inflammation, cancer) induce similar changes resulting in a single conceptual model of the induction of this anemia.

Studies have shown that IL-2 stimulated cells results in increased production of IL-l and TNF both in vitro and in vitro (44).

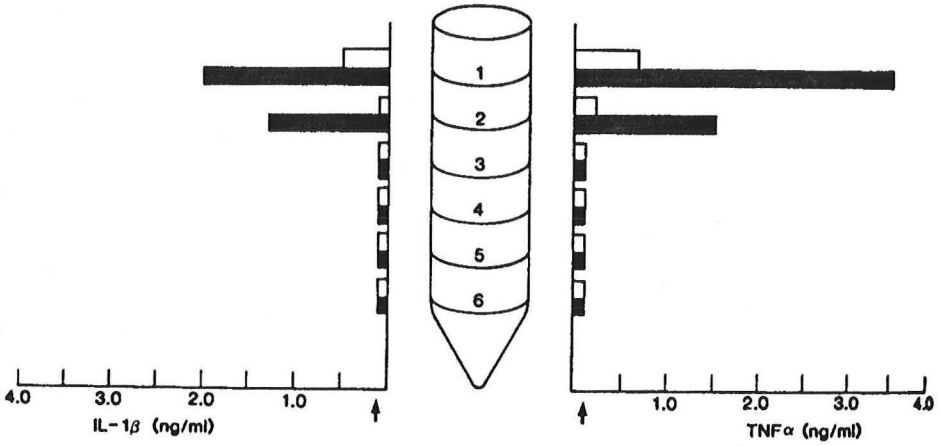


FIG. 1. Constitutive and IL-2-induced production of TNF and IL-1β by nonadherent human PBMC.

Since the cells of the erythron appear to lack IL-2 receptors, the concept is that IL-2 erythropoietic effects are the result of IL-l B and TNF.

Interferon-gamma, like TNF, is a pleiotropic cytokine that is elaborated in a highly regulated fashion. It is now clear that IFN and TNF when released from cells are capable of enhancing the other's production (45); in addition they affect cytokine function and for immature erythroid precursors their individual affects may be synergistic. It is of some interest that mature red cells are the only hematopoietic cells that lack the interferon - receptor (46). In addition, Roodman and coworkers have shown that TNF at picomolar concentrations inhibits erythroid progenitor cells (47), but

with progressive erythroid differentiation the mature erythroid progenitors lose their sensitivity to TNF (48).

A large number of patients primarily with cancer, have been treated with these cytokines thereby providing some clinical physiologic data. In general, intermittent therapy with TNF has not produced significant changes in red cell values regardless of route (49, 50, 51, 52). When TNF was given with Interferon-gamma the serum iron and ferrokinetic features shown in Table 3 and 7 were produced (53).

Thus, there is both in vitro and in vivo evidence that these three cytokines, TNF, IFN-gamma, and IL1, produce the measured changes in erythropoiesis that have characterized the anemia of malignancy. A relevant question exists as to whether these cytokines are produced in patients with cancer? This indeed appears to be the case (54). Recently, Morant and coworkers examined the serum in 201 patients with cancer and demonstrated increased levels of these cytokines (55).

Why the anemia of malignancy does not develop in a cell mass related manner similar to the anemia seen in patients with inflammation where a correlative relationship exists with the extent and severity of the joint disease (for instance)? Other modulators may be critical and different in the circumstance of cancer as has been shown for "hemolytic factor". For instance, Tisdale and coworkers have recently defined a tumor related "cachetic factor" different from TNF (or other known cytokines) that may be both important and interactive with these other cytokines under selected circumstances (56).

From these observations, it appears that the anemia of malignancy is a cytokine-associated syndrome where multiple cytokines interact to produce the defects in erythropoiesis and iron metabolism together with increased fragility of the erythrocytic

membrane. In this respect, the anemia of malignancy then is a true paraneoplastic lesion. It appears appropriate that the term "iron-re-utilization defect" should fall by the wayside, as have the terms "simple chronic anemia" and "anemia of chronic disease", perhaps to be replaced by "cytokine-associated anemia", where the primary involved cytokine can then be delineated by an appropriate subscript. The critical interactions of these cytokines, their sequence of production and release, and their temporal relationships will require further study. However, specific cytokines related to specific neoplasms should provide clearer understanding of the true mechanism of the anemia malignancy.

Therapy

Therapy for the anemia of malignancy focuses on treatment of the underlying neoplasm. Nevertheless, extensive experience with recombinant erythropoietin (EPO) in patients with cancer has clearly documented its ability to repair the anemia and significantly decrease or eliminate the need for red cell transfusions (57 - 61).

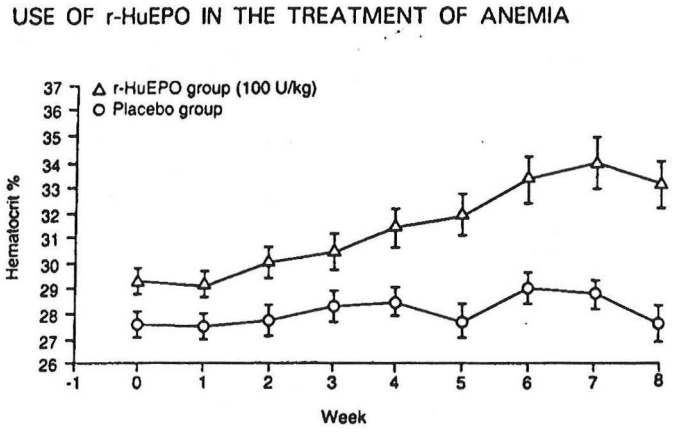


Fig 1. Mean weekly hematocrit values (±SE) for patients administered recombinant human erythropoietin (r-HuEPO) or placebo injections in the population of patients not administered chemotherapy.

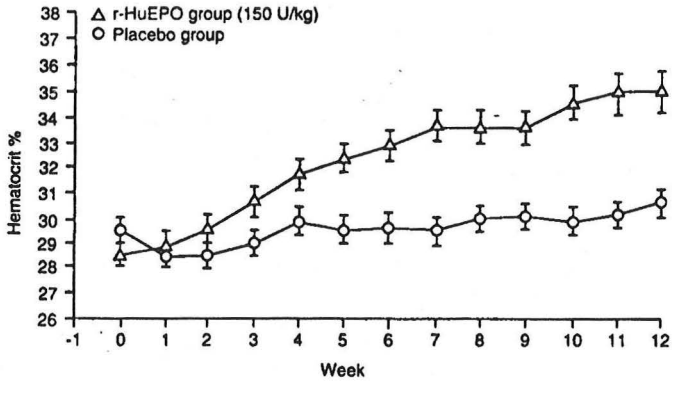


Fig 2. Mean weekly hematocrit values (±SE) for patients administered recombinant human erythropoietin (r-HuEPO) or placebo injections in the population of patients administered chemotherapy that did not include cisplatin.

Thus, when clinically indicated recombinant EPO, in doses of 150 - 300 units/kg subcutaneously three times per week, can be used to increase circulating red cell values. These doses are larger than those needed to treat the anemia of renal disease, because of the reduced responsiveness of the bone marrow to EPO in patients with cancer (61).

Double blind studies have shown that not only does EPO significantly improve hematocrit in these patients, but that this translates into improved well-being and other measures of quality of life (58, 59, 61).

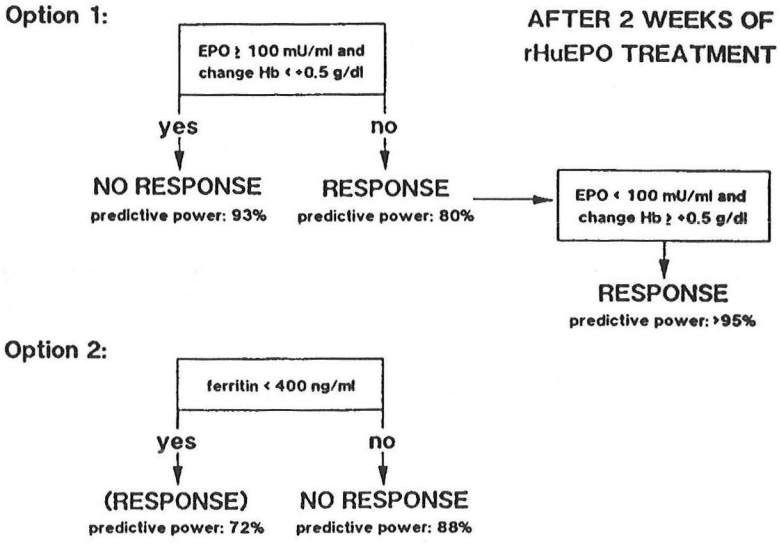
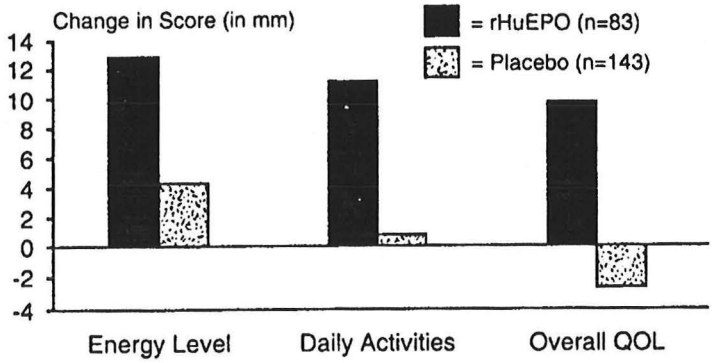


Fig 1. The algorithm to predict response or unresponsiveness to rHuEPO therapy in chronic anemia of cancer is shown. The algorithm offers two options. (1) If, after 2 weeks of therapy, the serum EPO level is 100 mU/mL or more and the Hb concentration has not increased by at least 0.5 g/dL, unresponsiveness of the patient is very likely. Otherwise, response can be predicted with an accuracy of 80%. If the serum level of EPO is less than 100 mU/mL and the Hb concentration has increased by 0.5 g/dL or more, the probability of response is very high. (2) If for some reason option 1 cannot be used, a serum ferritin level of 400 ng/mL or more after 2 weeks of rHuEPO therapy strongly indicates unresponsiveness. A serum ferritin level less than 400 ng/mL suggests response in 3 of 4 patients.

Unfortunately, not all anemic patients with cancer respond to EPO treatment. Ludwig et al (61) used regression analysis to predict which patients will respond to EPO; they found that, even as early as two weeks into EPO therapy, an inadequate increase in the hemoglobin level or persistently elevated ferritin levels predicted a poor response.



Change in quality of life (QOL) measurements (51)

In general it is advisable that oral iron be given during the EPO therapy to help provide the iron needed for accelerated erythropoiesis. We presently favor treatment with iron-polysaccharide compounds (1), which appear to cause much less gastrointestinal distress than the iron salts. This medication is best given 30 minutes before meals, once or twice a day, especially with some orange juice to enhance absorption. The primary indication for EPO include cardiopulmonary symptoms related to the anemia that limit the patient's function. In some circumstances, the anemia could preclude therapy or response to therapy of the neoplasm; for instance, when decreased oxygen delivery will limit the radiation therapy response, or when anemia after chemotherapy, particularly with platinum-containing regimen, delays therapy or necessitates transfusion. The use of EPO can frequently obviate the need for red cell transfusion in cancer patients, which is particularly advantageous because of the possible role that transfusion-associated immune suppression may play in tumor recurrence.

Pure Red Cell Aplasia: Pure red cell aplasia (PRCA) with a progressive normocytic anemia, reticulocytopenia and a decrease or absence of erythroid progenitor cells in the bone marrow is a rare paraneoplastic lesion sometimes is seen in association with malignancy; the neoplasms seen with PRCA include thymoma (62) where it is found in approximately 4% of cases, and less commonly in some hematologic malignancies, particularly chronic lymphocytic leukemia (CLL). PRCA has been seen in both T and B cell CLL and may occur any time during the course of the leukemia (63, 64). The mechanism for the PRCA in malignancy has been thought due to activation of T-gamma cells within the marrow, not to a true humoral inhibitor more commonly seen in cases of autoimmune disease or infection.(64). Whether this is cytokine-related or activated is not known. In general, therapy of the underlying neoplasm has resulted in improved red cell production, but in the short term immune suppression with corticosteroids and sometimes cyclophosphamide are important until definitive treatment of the cancer is completed.

Malignant Diseases and PRCA (63)

- Solid Tumors
  - Gastric adenocarcinoma[41]
  - Breast carcinoma[104]
  - Bile duct adenocarcinoma[74]
  - Bronchogenic carcinoma[12,110]
  - Squamous cell carcinoma[47]
  - Kaposi's sarcoma[103]
  - Thyroid carcinoma[54]
  - Unknown primary[85]
- Hematologic Malignancies
  - Chronic lymphocytic leukemia[1,18,21,51,88,107,114]
  - Chronic granulocytic leukemia[31,61]
  - Idiopathic myelofibrosis[8,22,27]
  - Hodgkins lymphomas[86]
  - Non-Hodgkins lymphomas[2,14,75]
  - Multiple myeloma[43]
  - Acute lymphoblastic leukemia[25,69,98]

Hemophagocytic Syndrome: The very rare hemophagocytic syndrome most commonly seen with viral infections and a variety of related diseases is sometimes seen with malignant lesions, primarily lymphoma, leukemia and metastatic carcinoma of the unknown primary type. It's classical features in patients with associated neoplasms include predominance in men, and presenting features of fever, hepatosplenomegaly, and remarkable depression of all hemotologic parameters. The marrow contains proliferation of mature histiocytes with active phagocytosis. The mechanism for macrophage activation is unknown, but its occurrence has been associated with a poor clinical outcome. (65)

Underlying illnesses in patients with hematophagic histiocytosis (64)

Illness	References and Patients in This Report
Neoplasms	
Acute nonlymphocytic leukemia	Ref. 93, Patients 19, 20
T cell lymphoma	Refs. 37, 68; Patients 16, 17
B cell lymphoma	Ref. 82; Patient 18
Chronic lymphocytic leukemia	Ref. 53
"Histiocytic" lymphoma	Ref. 26
Hodgkin disease	Ref. 46
Multiple myeloma	Ref. 77
Hairy cell leukemia	Ref. 54
Metastatic carcinoma	Ref. 38; Patient 15
Miscellaneous	
Myelodysplastic syndrome	Ref. 71

Anemia Induced by Therapy: The therapy of most cancers invoke varying involvement with surgery, radiation therapy, chemotherapy, and biologic response modifiers. Each of these are known to produce anemia by a variety of mechanisms. This topic is beyond the scope of the current review.

Conclusion

Anemia is a common association of malignant disease. It may be the first diagnostic clue to an underlying malignant disease, it may contribute to the patient's symptoms from the disease, and may affect treatment decisions. It is important to recognize that a number of underlying mechanisms may contribute to the anemia, and to exclude those which are treatable. The recognition that tumor-associated cytokine production is a major factor in the anemia of malignancy, and that recombinant EPO can overcome this suppression are major steps forward.

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