Adverse Effects on Hemostatic Function of Drugs Used in Hematologic Malignancies

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ABSTRACT

The adverse effects of drugs used in the treatment of hematologic malignancies are among the many factors contributing to the increased risk of both thrombosis and bleeding. These effects most often occur when combination of drugs are given. Some, such as L-asparaginase, result in both bleeding and thrombosis. Consideration must be given also to the bleeding or prothrombotic risk of the underlying hematologic disorder. The commonly used drugs with adverse effects on hemostasis include L-asparaginase, corticosteroids, inhibitors of vascular endothelial growth factor, gemtuzumab ozogamicin, thalidomide, and immunomodulatory derivatives of Thalidomide, and the hematopoietic growth factors. In addition, the syndrome of thrombotic microangiopathy may be brought on by several other drugs. Thus, a full understanding of these adverse effects is necessary in treating these disorders.

KEYWORDS: Leukemia, myeloma, thalidomide, L-asparaginase, growth factors, thrombosis

Thromboembolic events (TEEs) are well recognized to contribute significantly to complications during the treatment of hematologic malignant disorders. Several factors must be taken into consideration. The individual disorders per se have characteristics that predispose to TEEs. In addition, many therapeutic drugs have also prothrombotic properties. If the medications are used as a single drug or in combination, the risk of TEEs can be magnified to varying degrees. To add to the complexity, the effect of a particular drug may be sequential, with bleeding risk at one time and prothrombotic risk at another, as exemplified by the case of L-asparaginase. This review discusses both the effects of these combined factors and the specific drug actions.

L-ASPARAGINASE

This antitumor agent has been studied extensively regarding its profound effect on the coagulation and fibrinolytic systems. This drug acts on the essential amino acid L-asparagine, which is essential for cell growth, particularly in lymphocytes, and converts it to aspartic acid. Normal cells contain L-asparaginase synthetase and can be self-supporting. However, tumor cells lack this enzyme and depend on circulating blood to provide this amino acid. Thus, by depleting circulating L-asparagine, tumor growth is inhibited. Adverse effects of this drug include hypersensitivity reactions, ranging in severity from drug rash to anaphylaxis, and impaired protein synthesis, including albumin, procoagulant and anticoagulant factors as well as several components of the fibrinolytic system. The impaired protein synthesis results in the lowering of plasma levels of fibrinogen, factor (F) V, FVII, FVIII, FIX, FX, FXI, histidine-rich glycoprotein, α2-macroglobulin, and α2-antiplasmin. During treatment there is an increased bleeding risk, although the overall incidence of bleeding complications is low. One reason for this low

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incidence is the concurrent impaired synthesis of the naturally occurring anticoagulant proteins, including antithrombin, protein C, protein S, and plasminogen. The balance is tilted further toward a prothrombotic tendency after the cessation of L-asparaginase therapy, when the recovery of the coagulant proteins (fibrinogen, FVII) takes place earlier than the recovery of the anticoagulant proteins. In addition, an increased endogenous thrombin generation has been documented in children with acute lymphoblastic leukemia (ALL) at presentation and throughout the course of therapy.1

Clinically, L-asparaginase is used in several treatment protocols for ALL, and is used in various combinations with prednisone, daunorubicin, vincristine, cytarabine, cyclophosphamide, methotrexate, and thioguanine.2,3 In most treatment protocols, L-asparaginase is administered following several days of prednisone therapy, which has already reduced the fibrinogen level. Thus, the hypofibrinogenemic effect of L-asparaginase can be quite pronounced. Laboratory monitoring of the plasma fibrinogen, prothrombin time, and partial thromboplastin time should be done routinely. Cryoprecipitates are given when severe hypofibrinogenemia, with levels of 100 mg/dL or less, develops. The bleeding risk is accentuated further if there is concurrent thrombocytopenia, which may require additional platelet transfusion. Following this phase of therapy, the prothrombotic risk increases. Earlier studies placed the incidence of thromboembolic complications of L-asparaginase therapy to be approximately 2 to 10%. However, in a recent prospective study of 60 children treated for ALL with L-asparaginase, a much higher rate of 36.7% was found.2,3 Most thromboses were asymptomatic and occurred in the upper extremities, suggesting that the use of central venous access catheters may be a contributory factor. Others have reported intracranial thrombotic and hemorrhagic complications4,5; the former were sinus venous thrombosis and the latter were cerebral hemorrhages. When thrombotic complications occur and require thrombolytic therapy with plasminogen activators, the supplementation of plasminogen may be necessary because the patient’s plasminogen level may be low.6

GLUCOCORTICOIDS
The increased risk for thrombosis has long been known in patients with Cushing syndrome. The effect is likely due to the high plasma levels of glucocorticoids. Earlier studies revealed that prednisone treatment resulted in increased plasma levels of prothrombin, von Willebrand factor, and antithrombin, along with decreased fibrinogen and plasminogen.7,8 These changes have been confirmed recently when prednisone is used during chemotherapy for ALL. In the first phase of therapy, following 5 days of prednisone, there is a decrease in the plasma fibrinogen level.2 Thus, when L-asparaginase is administered in the next phase of the therapy, the hypofibrinogenemic effect of this drug is enhanced. On the other hand, the thrombotic risk is increased when corticosteroids are used in other chemotherapeutic combinations. Among 60 cancer patients who received thalidomide and dexamethasone, including those with myeloma, lymphoma, and renal cell carcinoma, nine (15%) developed thromboembolic complications, including eight with deep venous thromboses and one with a pulmonary embolus. Among 326 cancer patients who received thalidomide alone, 15 (5%) developed thromboembolic complications, including 10 with deep venous thromboses, one with a pulmonary embolus, and four with both a deep venous thrombosis and a pulmonary embolus.9 Similar findings are seen when corticosteroids are used for anticancer treatment with chemotherapy combinations. The thrombogenic effect of dexamethasone in myeloma is discussed in the section entitled “Thalidomide and Immunomodulatory Derivatives.”

VASCULAR ENDOTHELIAL GROWTH FACTOR INHIBITORS
Several antiangiogenic compounds have been developed in recent years. Many of these are inhibitors of vascular endothelial growth factor (VEGF) or its receptors. By blocking VEGF activities, these compounds may interfere with endothelial cell function and thus thrombosis can result. Most reports on increased thrombotic complications are with bevacizumab (Avastin; Genentech, Inc.; South San Francisco, CA), a monoclonal antibody against VEGF. When used in combination with various chemotherapeutic agents in multiple solid tumors, including carcinoma of the colon, lung, or ovary, an increased incidence of TEs was noted: 13 to 26% in the bevacizumab arm versus 9% in the control arm.10,11 In a series of 813 patients with metastatic colorectal carcinoma, roughly half of the patients were treated with chemotherapy (irinotecan, 5-fluorouracil [5-FU] plus leucovorin) alone and the rest received chemotherapy plus bevacizumab. The incidence of all thrombotic events was higher in the bevacizumab plus chemotherapy group (19.4%) when compared with the chemotherapy-alone group (16.2%), although this difference did not reach statistical significance (p = 0.26).12 Thrombotic events noted in several clinical trials have included deep venous thrombosis, pulmonary embolism, catheter-associated thrombosis, cerebralvascular events, and transient ischemic events. Independent risk factors for arterial thrombotic events with bevacizumab and chemotherapy are a history of atherosclerosis or age older than 65 years13; therefore, this agent should be used in caution in this population.

VEGF modulates endothelial cell function including increased permeability, proliferation, and migration,14 and has been shown to induce rapid release of von Willebrand factor; increase the expression of tissue factor
(TF), thrombomodulin, plasminogen activators (tissue-type plasminogen activator and urokinase-type plasminogen activator), plasminogen activator inhibitor type 1, and urokinase plasminogen activator receptor; and to promote adhesion and activation of platelets. Thus, effective inhibition of VEGF activity theoretically could cause both bleeding and thrombosis. In fact, increased bleeding was seen in one phase II trial of bevacizumab in metastatic renal cell carcinoma with statistically higher rates of epistaxis, hematuria, and hemoptysis \( (p \leq 0.05) \). This bleeding risk was also demonstrated in a phase III study of 878 patients with advanced non-small-cell lung cancer who were randomly assigned to receive paclitaxel plus carboplatin alone or with bevacizumab. The group that received bevacizumab had a 4.4% incidence of grade 3 or higher bleeding, whereas the rate was only 0.7% in the chemotherapy-alone group \( (p < 0.001) \). The bleeding events in the bevacizumab group included five fatal pulmonary hemorrhages and two fatal gastrointestinal hemorrhages.

Another anti-VEGF peptide, SU5416, which is a tyrosine kinase inhibitor of VEGF receptor-2, was found to cause endothelial cell activation, and is prothrombotic when administered with cisplatin and gemcitabine. The combination with cisplatin contributes to the thrombogenicity, given that cisplatin can cause platelet activation, an increase in von Willebrand factor, and vasospasm. Because of the double hazard of thrombosis and bleeding with the VEGF inhibitors, the prophylactic use of anticoagulants should be viewed with caution.

**GEMTUZUMAB OZOGAMICIN**

Gemtuzumab ozogamicin (OG; Mylotarg; Wyeth; Madison, WI) is an immunoconjugate consisting of a humanized monoclonal antibody against CD33 conjugated with a cytotoxic agent calicheamicin. It was developed for the treatment of acute myeloid leukemia with CD33-positive myeloblasts. Hepatic veno-occlusive disease (VOD) was reported in 0.9% of patients treated with GO alone for acute myeloid leukemia, but the incidence increased to 5% when administered with other chemotherapeutic agents, and was 19% in those treated after hematopoietic stem-cell transplantation. Other reports showed an incidence of 12.6% in those who did not undergo stem-cell transplantation. Given that hepatic toxicity with GO is quite common, occurring in as many as half of the patients, Nabhan et al. pointed out that VOD may be overdiagnosed if specific imaging studies are not performed. Hepatic vein occlusion (Budd-Chiari syndrome) has also been reported in patients treated with GO. The subject of GO-associated sinusoidal obstruction syndrome, formerly known as VOD, has been reviewed recently. The mechanism of this unusual toxicity is not clear. Several possibilities have been postulated, including free radical damage due to glutathione deficiency, or endothelial cell activation associated with inflammatory cytokines. It has also been shown that CD33 is not expressed in human umbilical vein endothelial cells in culture, but whether this is also the case with hepatic sinusoidal endothelial cells is not known.

**THALIDOMIDE AND IMMUNOMODULATORY DERIVATIVES**

Following the tragic teratogenic effects on newborns, thalidomide was withdrawn in 1962 but was reintroduced into the therapeutic armamentarium in 1998, initially for leprosy and subsequently for various hematologic malignancies, especially multiple myeloma. Among the major adverse effects are TEs. When used alone in myeloma, the incidence is approximately 1 to 2%, but the rate increases to as much as 27% when combined with dexamethasone, and is even higher when an anthracycline drug (doxorubicin) is added to the chemotherapy regimen. This complication also occurs when thalidomide is used in the treatment of mantle cell lymphoma, glioblastoma, melanoma, renal cell carcinoma, hepatocellular carcinoma, carcinoma of prostate or ovary, and for the myelodysplastic syndrome, with rates from 17 to 43% (again higher when given in combination with chemotherapeutic agents). The thromboembolic incidence in the lenalidomide plus dexamethasone combination therapy was reported to be lower (3%) in a small series, but was found to be 10 to 15% in larger clinical trials.

This alarming incidence has led to the use of prophylactic anticoagulation during the treatment using low-dose aspirin or low molecular weight heparin.

A better understanding of the causative factors for thrombosis has been made possible by recent knowledge of the pathogenesis of myeloma as well as the mechanism of action of Thalidomide/immunomodulatory derivatives (IMiDs). In the microenvironment of bone marrow in myeloma, the key players include the myeloma cells, bone marrow stromal cells, microvascular endothelial cells, or CD8+ T cells and natural killer (NK) cells. Myeloma cells adhere to the stromal cells, which prevent apoptosis and secrete the growth stimulatory factor and survival factor interleukin-6 (IL-6). Myeloma cells secrete cytokines including tissue necrosis factor alpha, transforming growth factor beta, and VEGF. These, in turn, affect the stromal cells upregulating IL-6 secretion and promoting angiogenesis and myeloma cell migration. Thalidomide/IMiDs act by interfering the above-described steps, including promoting apoptosis by blocking the adhesion of myeloma cells to stromal cells, inhibiting the myeloma secretion of cytokines, blocking drug resistance, and upregulating the patient's immune apparatus.
(NK cells, CD8+ T lymphocytes) against the myeloma cells and increasing cell kill by antibody-dependent cellular cytotoxicity. The recently approved IMiD, lenalidomide, has a mode of action similar to that of Thalidomide, but is more potent in several respects including augmenting of T-cell activity, inhibiting angiogenesis, and overcoming drug resistance.47 Some of these actions are relevant to thrombogenesis. During apoptosis the procoagulant TF on the cell membrane is activated by phosphorylation,59 rendering apoptotic cells more thrombogenic. The thrombogenic potential of VEGF inhibition is discussed in the section entitled Vascular Endothelial Growth Factor Inhibitors. In addition, the normal regulation of the coagulation system is likely impaired, given that plasma thrombomodulin levels are found to be low during the first month of treatment with thalidomide and dexamethasone.60 Finally, other prothrombotic characteristics in myeloma include newly diagnosed disease, chromosome 13 abnormalities, older age, high lactate dehydrogenase level, and high creatinine level.61

**ALL-TRANS RETINOIC ACID**

In acute promyelocytic leukemia (APL), the chromosomal translocation t(15;17) results in the formation of the fusion proteins PML–RARα or PLZF–RARα.62-68 In the absence of retinoic acid, these proteins bind to the RARα target genes and form heterodimers with RXR and corepressors, leading to failure of differentiation of cells of the myeloid lineage and resulting in the development of APL. All-trans retinoic acid (ATRA) induces the dissociation of the nuclear corepressors and enables the transcriptional activation and induction of differentiation. APL cells expressing (PLZF–RARα) fusion protein are resistant to ATRA but may respond to arsenic trioxide. Treatment with ATRA in APL results in the resolution of the coagulopathy and bleeding induced by disseminated intravascular coagulation that frequently is evident at the time of APL presentation, but paradoxically induces thrombosis in a small number of patients. This was first observed by Schneider69 and Runde et al.70 The prothrombotic complications should be distinguished from retinoic acid syndrome, characterized by hyperleukocytosis, and extravasation of leukocytes especially in pulmonary alveoli, which occurs in 4 to 26% of ATRA-treated APL patients.71-73 In ATRA-associated thrombosis, the complication occurs 1 to 3 weeks following the treatment, at a time when the coagulopathy has been corrected,70,74 and can involve multiple organs including heart, brain, lungs, and spleen.75 Antifibrinolytic agents, such as tranexamic acid, have also been shown to increase the risk of this potentially fatal complication,77,78 and thus are contraindicated. Various thrombogenic factors have been proposed, including the induction of apoptosis by ATRA,79 upregulation of adhesive molecules, and increased production of cytokines.80 It is interesting that arsenic trioxide, which also causes apoptosis and cell differentiation in APL by degradation of the PML–RARα fusion protein, is not associated with significant TEs, although hyperleukocytosis occurs.81-83

**ERYTHROPOIETIN**

Recombinant human erythropoietin is available in the United States as epoetin alfa (Procrit, Epogen) or darbepoetin alfa (Aranesp). Other products that are not available in the United States include epoetin alfa (Eprex) and epoetin β (NeoRecormon). The indications for products approved by the U.S. Food and Drug Administration (FDA) include the treatment of anemia in patients with solid tumors or nonmyeloid malignancies, and the first was approved in 1993. Darbepoetin, initially approved in 2001, has a different amino acid sequence and increased degree of glycosylation, which results in a longer half-life. The erythropoietin products are effective at improving the hemoglobin and decreasing transfusion requirements.84 Epoetin was initially approved for the treatment of anemia in patients with chronic renal disease. In this population early reports suggested that patients who received epoetin had a hematologic improvement but had a higher mortality with a higher target hemoglobin.85 Two recent studies in patients with chronic renal disease were performed to assess optimal target hemoglobin.86,87 There was no evidence that a higher hemoglobin level increased the risk of vascular or thrombotic events; however, overall mortality was higher, with higher target hemoglobin in one of the studies.87 Conversely, a meta-analysis report on 6769 cancer patients in 35 trials treated with epoetin alfa and β (i.e., epoetin) and darbepoetin alfa (i.e., darbepoetin), suggests that the thrombotic risk is increased.84 TEs occurred in 229 of 3728 patients treated with erythropoietin, compared with 118 of 3041 patients who did not.84 This was a statistically significant difference, with a relative risk of 1.67 (95% confidence interval, 1.35 to 2.06).

It is possible that some of the thrombotic risk may be due to a higher hemoglobin level. In data presented to the FDA, three studies of epoetin alfa with a goal to achieve hemoglobin > 13 g/dL were stopped early due to increased incidence of thrombosis. In these studies, 24 of 34 patients with a vascular events while treated with epoetin had a hemoglobin > 13 g/dL in the 28 days prior to the event.88 Conversely, the thrombotic risk may be independent of hemoglobin level. Epoetin alfa administered to normal healthy volunteers three times a week for 2 weeks resulted in a 10 to 20% increase in platelet count on day 5, and elevated P-selectin and E-selectin levels.89 This evidence suggests that erythropoietin alone
may increase thrombogenicity due to increased platelet reactivity or endothelial cell activation.69

The thrombogenic risk is not limited to patients with high hemoglobin levels, but is present in hematologic disorders with high thrombotic risk especially when a drug with thrombogenic potential is administered. In a phase II trial conducted in patients with low or intermediate-risk myelodysplastic syndrome (MDS) treated with a combination regimen of thalidomide and darbepoetin, three patients developed either a deep vein thrombosis or pulmonary embolism, including one fatal event, necessitating the termination of the trial.37 The events occurred 6 to 11 weeks after the start of therapy, and did not occur in association with a high hemoglobin. Prior studies have not found an increased rate of thrombotic events when either erythropoietin90 or Thalidomide91 alone was used in the treatment of MDS.

Because of the potential thrombogenicity of the growth factors, these agents should be used cautiously. An American Society of Hematology/American Society of Clinical Oncology guideline panel suggests the use of recombinant erythropoietin in MDS, whereas in hematologic malignancies the panel recommends that treatment of the underlying malignancy should be foremost, and use of erythropoietin should be considered only if anemia does not respond to tumor-specific treatment.92 If erythropoietin products are used, then careful monitoring of hemoglobin is important, and the dose should be withheld if hemoglobin is > 12 g/dL.

GRANULOCYTE-COLONY STIMULATING FACTOR/GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR

Recombinant granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) are used in cancer patients to shorten the duration of neutropenia and decrease the risk of febrile neutropenia (Amgen, Inc.; Thousand Oaks, CA). In addition, these hematopoietic growth factors are administered to normal healthy donors to facilitate peripheral stem-cell collection. Thrombotic events have been described in patients with malignancy receiving either G-CSF (Neupogen) or GM-CSF (Leukine; BerlexLab; Montville, NJ).93 Barbui et al93 reviewed 52 articles describing 1846 patients who received G-CSF or GM-CSF during treatment for both solid tumor and hematologic malignancies. Thrombotic events, both arterial and venous, occurred in 2.8% of all patients. The incidence was 4.2% with GM-CSF and 1.2% with the use of G-CSF. The primary outcome of these studies was not thrombotic events and therefore it is likely that the incidence was underestimated. The highest risks were found in patients with metastatic gastrointestinal adenocarcinoma who received GM-CSF concurrently with 5-FU, in whom the incidence of thrombotic events was 14%.94 The risk of thrombosis was also increased in patients undergoing chemotherapy for stem-cell transplantation and appeared to be higher with the use of GM-CSF (9.8%) compared with G-CSF (2.3%).95 G-CSF has been found to increase markers of coagulation activation, including FVIII levels, thrombin-antithrombin complexes, and prothrombin fragment F1+2 in normal allogeneic stem-cell donors.95,96 In addition, G-CSF increases both TF antigen and activity in normal donors after 5 days of G-CSF administration.97 It is not clear how much these changes contribute to the thrombogenic mechanism, but clinicians should be aware of the potential for thrombotic complications with these agents.

DRUG-ASSOCIATED THROMBOTIC MICROANGLIOPATHIES

A variety of medications have been associated with the development of thrombotic thrombocytopenic purpura (TTP) or hemolytic-uremic syndrome (HUS; Table 1).98-100 Given the overlap between these clinical entities, particularly when they are secondary to a drug or other condition, the term thrombotic microangiopathy (TMA) has been adopted. The most commonly reported agents associated with TMA include mitomycin-C, quinine, cyclosporine, and ticlopidine.100 Additional drugs used in the treatment of hematologic malignancies that have been implicated include daunorubicin, cytarabine, bleomycin, cisplatin, deoxycoformycin (pentostatin), arsenic, alpha-interferon,101,102 and gemcitabine.103 In addition, patients undergoing hematopoietic stem-cell transplantation (HSCT) can develop a thrombotic microangiopathy, which may be due to exposure to calcineurin inhibitors (cyclosporin or tacrolimus). The two proposed mechanisms by which a drug leads to TMA include immune-mediated effect or a direct toxic effect.104 Recent TTP case series demonstrate that most drug-associated TTP cases are not associated with a severe ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs) deficiency or with the presence of an ADAMTS13 inhibitor.104-106

Table 1 Drugs Associated with TTP/HUS

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<th>Chemotherapeutic Agents</th>
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<td>Mitomycin-C</td>
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<td>Gemcitabine</td>
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<td>Daunorubicin</td>
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<td>Deoxycoformycin</td>
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TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome.


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