Pathogenesis and management of the bleeding diathesis in acute promyelocytic leukaemia

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Life-threatening bleeding, which remains a challenging complication of acute leukaemia, is particularly characteristic of the subtype, acute promyelocytic leukaemia (APL). The clinical picture and laboratory abnormalities are most compatible with the diagnosis of disseminated intravascular coagulation (DIC). Evidence for diffuse activation of the coagulation system, hyperfibrinolysis and systemic elaboration of non-specific protease activity can usually be demonstrated and occurs most commonly during induction chemotherapy. While both host- and tumour-associated mechanisms can be implicated in the pathogenesis of the coagulopathy, leukaemic cell properties appear to be the proximate cause of activation of the haemostatic mechanisms. In this chapter we summarize the current state of knowledge of the pathogenesis of the coagulopathy of APL and the therapeutic approaches that have proved most useful for the management of this complication. Special attention is devoted to the use of all-trans-retinoic acid (ATRA), which has revolutionized the treatment of APL and markedly ameliorated the APL-related coagulopathy.

Key words: acute promyelocytic leukaemia; bleeding diathesis; disseminated intravascular coagulation (DIC); procoagulant activity (PCA); tissue factor; cancer procoagulant; fibrinolysis; proteases; cytokines; platelets; heparin; all-trans-retinoic acid (ATRA).

Patients with malignancy are at increased risk for thrombo-haemorrhagic complications. Venous thromboembolism (VTE) is a frequent complication in patients with solid tumours, whereas life-threatening bleeding due to uncompensated disseminated intravascular coagulation (DIC) is more commonly observed in patients with acute leukaemia. In addition, nearly all patients with malignancy show evidence of subclinical activation of blood clotting, or chronic DIC, in the absence of active bleeding and/or thrombosis.

Most, if not all, patients with acute leukaemias, at diagnosis, present with mild mucocutaneous bleeding symptoms, which readily respond to platelet transfusion. In

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some instances, severe life-threatening bleeding complications can develop and the risk varies according to the type of acute leukaemia. Although the most common cause of bleeding in acute leukaemia is thrombocytopenia, due to either bone marrow invasion by leukaemic cells or the myelosuppressive effect of chemotherapy (or both), underlying DIC can also contribute to the pathogenesis of the bleeding diathesis. Disordered haemostasis is prevalent in patients with acute promyelocytic leukaemia (APL) and other acute hyperleukocytic leukaemias, particularly during induction chemotherapy when large volumes of tumour cells are being destroyed rapidly.

In recent years, DIC complicating the presentation of APL has received new interest, due to several advances in translational research, including: (1) enhanced understanding of the biology of this unique myeloid differentiation disorder; (2) greater sensitivity of diagnostic tests for subclinical DIC; and (3) development of all-trans-retinoic acid (ATRA) for remission induction. ATRA promotes the terminal differentiation of leukaemic promyelocytes and ATRA-induced remission of APL is accompanied by prompt improvement of the attendant coagulopathy typical of this disease.

This chapter focuses on the pathogenesis and proposed treatment of the DIC syndrome occurring in APL, with particular attention to the mechanisms of ATRA. ATRA has profoundly affected APL treatment and approaches to the control of the secondary coagulopathy.

**CLINICAL FEATURES OF THE COAGULOPATHY OF APL AND MODIFICATION BY ATRA**

Acute promyelocytic leukaemia (APL) corresponds to the M3 subtype of acute myelogenous leukaemia (AML), according to the FAB classification. Patients with APL not atypically present with a life-threatening haemorrhagic diathesis, the clinical and laboratory features of which are consistent with DIC. The bleeding disorder is particularly severe in the microgranular variant of APL (M3v), which is characterized by marked hyperleukocytosis. Prior to the introduction of ATRA for the management of APL patients, fatal haemorrhage due to the associated coagulopathy was a major cause of morbidity and mortality, thus contributing to failure of remission induction. In a retrospective multicentre study of 268 consecutive APL patients, the overall remission rate was 62% and the prevalence of haemorrhagic deaths during induction therapy was 14%. No significant difference in remission rate was observed in the groups of patients that received heparin, antifibrinolytic drugs or supportive therapy alone for controlling the coagulopathy. The use of ATRA for remission induction in APL has produced a high rate of complete remission, together with a rapid resolution of the coagulopathy, without causing bone marrow hypoplasia.

As noted, ATRA promotes the terminal differentiation of leukaemic promyelocytes, cells which carry a typical balanced translocation between chromosomes 15 and 17. In these cells, the fusion of the nuclear retinoic acid receptor (RARα) gene on chromosome 17 with part of the PML gene on chromosome 15 results in the expression of a chimeric PML/RARα protein, which is involved in the leukaemogenesis and is the target for the myeloid differentiation effect induced by ATRA. As will be discussed, the PML/RARα fusion protein products may contribute directly to the pathogenesis of the coagulopathy. ATRA also exerts a number of effects on haemostatic functions (see below).

The coagulopathy typical of the onset of APL is a complex disorder and presents with bleeding symptoms of various degrees of severity. Abnormalities of the blood clotting system consistent with the diagnosis of DIC are observed in the majority of
these patients. Severe DIC with life-threatening bleeding involves the very rapid consumption of coagulation factors and platelets in the circulation as a consequence of massive activation of intravascular clotting. Thrombocytopenia, caused principally by bone marrow replacement of megakaryocytes by leukaemic cells and subsequent hypoplasia induced by traditional chemotherapeutic agents, is aggravated by the consumption of platelets during clot formation. Secondary bacterial or viral infections in these susceptible hosts may further complicate the pathogenesis of the thrombocytopenia through direct toxic effects, additional suppression of platelet production by the damaged bone marrow and/or generation of additional stimuli for activation of blood coagulation (e.g. bacterial endotoxin, interleukins, etc.).

LABORATORY FEATURES OF THE COAGULOPATHY OF APL

The most common abnormalities of routine clotting tests in patients with APL include hypofibrinogenaemia, increased circulating levels of fibrinogen-fibrin degradation products (FDPs) and prolongation of both the prothrombin time (PT) and the thrombin time (TT) (Table 1). These abnormalities can be accentuated by the initiation of cytotoxic chemotherapy, resulting in severe haemorrhagic complications. However,

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<tr>
<th>Tests of haemostasis</th>
<th>Anticipated results</th>
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<tr>
<td><strong>A. Routine</strong></td>
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<tr>
<td>Prothrombin time (PT)</td>
<td>Prolonged</td>
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<td>Thrombin time (TT)</td>
<td>Prolonged</td>
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<td>Activated partial thromboplastin time (apt)</td>
<td>Variable*</td>
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<tr>
<td>Fibrinogen</td>
<td>Low*</td>
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<td>Platelet count</td>
<td>Low</td>
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<tr>
<td>Fibrin D-dimer (D-dimer)</td>
<td>Increased</td>
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<td>Fibrinogen degradation products (FDPs)</td>
<td>Increased</td>
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<td><strong>B. Specialized markers of activation of clotting, fibrinolysis and non-specific proteolysis</strong></td>
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<tr>
<td>1. Clotting</td>
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<td>Prothrombin F1 + 2 (Pro F1 + 2)</td>
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<tr>
<td>Thrombin-antithrombin (TAT) complexes</td>
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<td>Fibrinopeptide A (FPA)</td>
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<td>2. Fibrinolysis</td>
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<td>Urokinase-type plasminogen activator (u-PA)</td>
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<td>Plasminogen</td>
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<td>α2-antiplasmin</td>
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<td>3. Non-specific proteolysis</td>
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<td>Elastase-inhibitor complexes</td>
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| * The aPTT may be prolonged in patients with overt DIC, normal or even shorter than normal in those patients with subclinical DIC, while the fibrinogen level is reduced in typical cases associated with symptomatic DIC, it may be normal or elevated in patients with subclinical DIC.
these laboratory findings are not diagnostic of any single coagulation disorder, but instead reflect the interaction of different pathophysiological processes; similar alterations of routine clotting tests may result from the activation of coagulation, fibrinolysis and/or non-specific proteolysis by diverse causes (Table 1). The results of new and more sensitive laboratory tests confirm that activation of all three systems occurs in acute leukaemia. In fact, plasma levels of well known markers of clotting activation, i.e. the prothrombin fragment $1 + 2$ (F1 + 2), thrombin–antithrombin complexes (TAT) and fibrinopeptide A (FPA), are elevated in the overwhelming majority of patients with acute leukaemia. In addition, high levels of fibrinogen/fibrin degradation products (FDPs) and urokinase-type plasminogen activator (μ-PA) associated with low levels of plasminogen and $\alpha_2$-antiplasmin have been described in these patients and provide evidence for activation of fibrinolysis. Plasma levels of leukocyte elastase and fibrinogen split products of elastase are also increased and testify to the elaboration of non-specific proteases. Activation of each of the three cascades (i.e. coagulation, fibrinolysis or non-specific protease) can potentially trigger the bleeding complications of APL. However, the new laboratory tests for the detection of markers of hypercoagulation demonstrate definitively that thrombin generation and fibrin formation are constant events in these patients. Of particular interest is the detection of elevated levels of D-dimer, the lysis product of stabilized cross-linked fibrin. This finding provides strong evidence that the hyperfibrinolysis typical of patients with APL is most likely secondary to the activation of the clotting system.

One laboratory feature that may distinguish the coagulopathy of APL from typical DIC complicating other clinical conditions (e.g. sepsis) is the maintenance of relatively normal levels of the coagulation inhibitors antithrombin (AT) and protein C (PC). Although experimental DIC can occur in the presence of normal levels of AT, such findings are not typical in clinical practice. Of interest, however, is the observation by Rodeghiero and colleagues that reduced levels of AT and PC in patients with acute leukaemia tend to occur in those patients with hepatic dysfunction. Patients with acute leukaemia and DIC with normal liver function in their series usually had normal levels of the inhibitors. Therefore, normal levels of AT and PC in patients with the coagulopathy of APL cannot exclude DIC but may emphasize other features of the coagulopathy.

Some investigators have suggested that primary fibrinolysis may be the major event leading to the bleeding diathesis in APL. On the basis of the laboratory tests currently available, however; it is difficult to prove the existence of primary hyperfibrinolysis in APL and even more difficult to establish the role of excessive fibrinolysis in triggering severe haemorrhage. In fact, while reactive, or secondary, hyperfibrinolysis, in response to clotting activation, can be easily documented in patients with leukaemia, there are no specific tests that define primary hyperfibrinolysis (geno)lysis in vivo. The findings of profound reductions of $\alpha_2$-antiplasmin and plasminogen levels, which can be corrected with the therapeutic use of antifibrinolytic agents, do not allow the distinction between primary and secondary hyperfibrinolysis. Menell and colleagues recently described increased fibrinolytic activity (annexin II-dependent) in vitro in freshly isolated APL cells, when compared to non-APL leukaemic cells. They hypothesized that ‘dysregulated expression of annexin II on the surface of circulating APL cells’ could be responsible for primary hyperfibrinolysis in vivo. However, these authors assessed the activation of the fibrinolytic system in their patients with non-specific tests and demonstrated, among other abnormalities, elevated D-dimer levels in 8/10 patients. These data confirm the presence of
thrombin-mediated cross-linked fibrin in the circulation in the majority of their patients, thus supporting the interpretation that hyperfibrinolysis occurred secondarily to thrombin generation.

The advent of ATRA for remission induction therapy of APL has created a new perspective on the management of the APL-associated coagulopathy. Even the very first reports of clinical experience with this mode of therapy described a rapid resolution of bleeding symptoms in patients treated with ATRA. Thereafter, laboratory studies have confirmed the rapid reversal or normalization of tests of clotting and fibrinolysis during the first 1 or 2 weeks of therapy with ATRA. In our studies of patients before institution of therapy, we found the following: (1) elevated plasma levels of hypercoagulability markers [TAT, F1 + 2, fibrinopeptide A (FPA) and D-dimer]; (2) low mean PC and normal AT levels; (3) normal fibrinolysis proteins in one study with elevated levels of urokinase plasminogen activator (uPA) and mildly elevated plasminogen activator inhibitor (PAI-1) levels in the other study; (4) variable plasma elastase levels. In one of our studies, the median plasma levels of markers of clotting activation (F1 + 2 and TAT) and fibrin degradation (D-dimer) dropped within the first week of the initiation of treatment with ATRA, and fibrinogen levels rose into the normal range. However, F1 + 2 and TAT were not completely normalized by the second week, indicating persistent moderate/low level activation of blood coagulation; levels of PC increased and levels of elastase remained elevated after therapy.

In the study by Tallman and colleagues, some patients retained elevated levels of D-dimer, F1 + 2 TAT and FPA for up to 30 days. The finding of a slower return toward normal of the activation markers of coagulation is consistent with the reports of others, including Dombret and colleagues and Kawai et al. Tallman and colleagues also demonstrated that plasma levels of D-dimer, F1 + 2, TAT and FPA, as well as levels of tissue factor (TF) gene expression in bone marrow cells decreased more significantly over the first 30 days of treatment with ATRA compared with the effects of chemotherapy. The importance of this observation will be reviewed in a subsequent section.

Regarding fibrinolysis, in one of our studies, plasma levels of tissue plasminogen activator (t-PA) and PAI-I antigens, which were normal prior to ATRA therapy, increased during ATRA treatment (Figure 2). The overall plasma fibrinolytic activity, however, as measured by the euglobulin lysis area, was not modified, reflecting a balance between activators and inhibitors. Similarly, in the second study, plasma levels of t-PA were significantly elevated in all patients by day 30 of ATRA therapy. The levels of PAI-I, which were slightly elevated prior to therapy, were within the normal range by day 8 and remained normal through day 30 after initiation of ATRA therapy. In the same study, mean plasma levels of uPA remained elevated throughout the 30 days of observation. In addition, increased proteolysis of von Willebrand factor (vWF), which was observed in most patients with APL prior to therapy, was reduced by treatment with ATRA. These beneficial effects of ATRA on parameters of coagulation, fibrinolysis and proteolysis activation were associated with improvement in clinical signs of the coagulopathy in the same patients. The benefits persisted when ATRA was given in combination with chemotherapy, although the decline in activation markers was more pronounced in patients treated with ATRA alone, suggesting a partial initial exacerbation of the coagulopathy induced by chemotherapy.

A recent report confirms the long-term benefit of induction therapy with ATRA on both disease-free and overall survival in APL and reaffirms the negative prognostic finding of clinical bleeding at the time of presentation.
Figure 1. Panel A. Total procoagulant activity of bone marrow blast cells of APL patients treated with ATRA + / − chemotherapy at diagnosis (n = 9), during ATRA treatment (7 to 10 days of ATRA treatment, n = 7), and at complete remission (n = 9). After starting ATRA, the procoagulant activity significantly decreased and became undetectable upon complete remission. In panels B and C the median plasma levels of thrombin-antithrombin (TAT) complexes and fibrinogen in APL patients (n = 9) at diagnosis (day 0 = before therapy) and on day 8 and 14 of therapy are shown. The dashed lines (−−−−−−) indicate the upper limit of the normal range for each parameter.
the coagulopathy with ATRA, therefore, may improve survival in some of those poor-prognosis patients.

**PATHOGENESIS OF THE COAGULOPATHY OF APL**

Many exogenous factors can interfere with the normal delicate balance between procoagulant and anticoagulant forces in the haemostatic system in patients with APL, including cytotoxic chemotherapy and concomitant infections. However, the major determinants of the coagulopathy in APL are endogenous factors related to properties of the malignant leukaemic cells themselves and their interaction with host defence mechanisms. Illustrated schematically in Figure 3 are some of these key factors, including increased expression of procoagulant activity (PCA), expression of fibrinolytic and proteolytic properties, and the secretion of pro-inflammatory cytokines (i.e. interleukin-1β (IL-1β) and tumour necrosis factor (TNF-α)).

**Procoagulant activity (PCA)**

Leukaemic cells express at least two of the known tumour-associated procoagulants: tissue factor (TF) and cancer procoagulant (CP). They also provide an efficient alternative to platelets as a phospholipid surface for the assembly of the prothrombinase complex. PCA was identified in leukaemic cells as early as 1954,15 but it was not until 1973, that Gralnick and Abrell provided data suggestive that this PCA was related to TF expression.16 Several groups have characterized the expression of TF in APL and APL-like cells in tissue culture17–40 and Falanga and colleagues characterized CP in leukaemic blasts of various phenotypes. These latter investigators found the highest level of CP activity in the APL subtype.41 Levels of CP were elevated in samples obtained at the time of patient presentation, but were low or generally absent from cells obtained from patients at the time of complete remission.42

Both CP and TF have also been identified in the NB4 cell line, the first human APL line containing the typical t(15;17) chromosomal balanced translocation. ATRA-induced APL cell differentiation in vitro is associated with loss of the capacity to express either CP43 or TF.44–46 Further, both procoagulants are progressively reduced in vivo in the bone marrow cells of APL patients given ATRA for remission-induction therapy.22,23 Although this effect parallels the magnitude of the improvement of the coagulation parameters in the same subjects, it usually precedes resolution of the abnormal coagulation studies.23 These findings provide the most compelling evidence for a direct role of tumour cell PCA in the clotting complications of malignancy.

Reduction of leukemic cell PCA by ATRA appears to be one important mechanism involved in the resolution of the coagulopathy (Figure 1). Recent in vitro data also show that, after ATRA treatment, CP activity is virtually abolished only in those NB4 cells that are sensitive to ATRA-induced cyto-differentiation, and not in ATRA-resistant cells that do not differentiate. However, TF activity was significantly reduced in all cell lines in response to ATRA, regardless of sensitivity to ATRA-induced differentiation.47 These data suggest that TF modulation in APL cells, at least in part, occurs independently from the differentiation process. Different mechanism(s) for regulation of these two proteins may exist; this would be consistent with different characteristics of the PCAs. Indeed, while TF expression is common in malignant cells, it is also the PCA found in normally differentiated cells, such as endothelial cells and monocytes/macrophages, and is
Figure 2. Plasma levels of fibrinolytic parameters were measured in three APL patients receiving ATRA as a single agent, at baseline (day 0) and on days 8 and 14 of therapy. The mean plasma levels of both t-PA and PAI-1 antigens (panel A) rose during ATRA treatment compared to baseline (day 0). The t-PA specific activity (panel B) tended to increase, whereas, the overall plasma fibrinolytic activity (euglobulin lysis area) was not modified during ATRA.
the major physiological activator of mammalian blood coagulation. In contrast, CP, with
the exception of its presence in products of conception (e.g. chorion/amnion tissue),
has been described only in neoplastic cells, and particularly in patients with acute
leukaemia. CP can be detected in leukaemic blast cells at the onset of the disease, but
not during complete remission. These observations support the hypothesis that
expression of CP in malignant cells is repressed once normal differentiation occurs. TF
expression can be down-regulated by ATRA in both APL cells and in other types of
leukaemic cells and also in normally differentiated cells—all cells that do not
express the PML/RARα fusion protein and which, therefore, might not be expected to
be sensitive to ATRA-induced differentiation. It is possible that, in APL cells, TF
down-regulation under the influence of ATRA may involve mechanisms both dependent
upon and independent of cell differentiation.

Nuclear run-on experiments in human monocytes and monocytic leukaemia cells
support the concept that ATRA inhibits induction of TF expression at the level of
transcription, but independently of the common transcription factors AP-1 or
NF-κB. Zhu et al demonstrated destabilization of TF mRNA induced by ATRA in NB4
cells, which is partially dependent upon protein synthesis, and Raelson and colleagues
showed that ATRA induces synthesis of a protein in NB4 cells that selectively degrades
PML/RARα. Therefore, one or more proteins induced by ATRA in leukaemic cells
may also destabilize TF mRNA. The observations of Zhu and colleagues, which
were based on experiments in cells obtained from patients with APL and from
leukaemic cells lines, are consistent with this interpretation—reduced TF expression
correlated with the degradation of the PML-RARα in the cells. Furthermore, this group
has also reported recently that bone marrow cells from mice transgenic for the fusion
genes PLZF-RARα or NPM-RARα expressed the TF gene, whereas the TF gene was
absent from cells from those mice who exhibited transgene integration but without
fusion gene expression. These data, therefore, link directly for the first time the
regulation of TF gene expression in APL cells with the malignant transforming event and
provide strong support for the hypothesis that down-regulation of TF gene expression
is a direct result of the mechanism of the ATRA effect on oncogene expression.

In the Intergroup study, we measured TF mRNA in bone marrow cells from APL
patients treated either with ATRA or chemotherapy. The expression of TF, which was
uniformly measurable in bone marrow cells from all but 1 of 9 patients with APL at the
time of diagnosis, was reduced rapidly over 2 weeks following ATRA therapy in 3 of 4
patients. Chemotherapy was associated with a reduction of TF mRNA expression in
three patients but an increase in TF mRNA in one patient. These results are consistent
with the clinical observation that the coagulopathy tends to resolve rapidly in those
patients whose bone marrow cell TF levels were reduced by ATRA therapy.

Fibrinolytic and proteolytic properties
Leukaemic promyelocytes contain both the urokinase-type plasminogen activator
(u-PA) and the tissue-type plasminogen activator (t-PA). In addition, granulocytic
proteases, such as elastase and chymotrypsin, are found in the granules of myeloid
blasts and are released into the bloodstream, where they can be bound and neutralized
by inhibitors. Increased plasma levels of elastase-inhibitor complex are indeed
described in patients with acute leukaemia. These enzymes can interfere in several
ways with the haemostatic system, i.e. by degrading clotting factors (extrapolated from
in vitro studies) and cleaving inhibitors of fibrinolysis. Elastase can degrade fibrinogen, producing a pattern of fibrin degradation products (FDPs) different from
those produced by plasmin cleavage. A variety of proteases, exemplified by elastase, which can be elaborated by APL cells, have been implicated in the pathogenesis of the bleeding syndrome. However, in a recent study, freshly isolated APL blasts expressed lower fibrinolytic and proteolytic activities compare with mature neutrophils. With the exception of an increase in u-PA levels, granulocytic differentiation induced by ATRA was not associated with significant changes in the protease profile of these cells. Similar results were reported previously by Wijermans and colleagues, who studied the M-2 myeloid leukaemia cell line HL60.

As noted in a previous section, APL blasts express increased levels of annexin II-associated fibrinolytic activity, compared with other less differentiated leukaemic subtypes. To our knowledge, however, a comparison has not been made between the levels of cell-surface, t-PA-dependent, annexin II-associated plasmin in APL cells with levels in normal, mature granulocytes. In NB4 cells this enzymatic activity is sensitive to ATRA in vitro, which reduces the rate of plasmin production by approximately 68% over 7 days in tissue culture. In another study of NB4 cell fibrinolytic activity, retinoids induced a prompt rise in u-PA activity on the cell surface, which was promptly down-regulated by an increased production of PA inhibitors, including PAI-1 and PAI-2. Thus, various mechanisms can contribute to a reduction in fibrinolytic activity in APL cells in response to ATRA, as demonstrated in in vitro studies. These in vitro results agree with our finding in vivo that the overall, ‘global plasma fibrinolytic response’, as measured by the ‘euglobulin lysis area’, is normal in APL patients receiving ATRA (Figure 2). As stated previously, we believe that the initial hyperfibrinolysis observed in patients with APL is most likely secondary to DIC, as indicated by elevated circulating levels of fibrin D-dimer. Hyperfibrinolysis may reflect activation of the fibrinolytic system on the surface of the malignant cells, where specific receptors favour the assembly of all the fibrinolytic components. ATRA acts initially to enhance this fibrinolytic activity by increasing synthesis of u-PA. Thereafter, however, ATRA-induced synthesis of PA inhibitors and/or inhibition of annexin II synthesis may be favoured, contributing to the down-regulation of receptor-bound plasminogen activators, as described for in vitro cell culture models. On balance, therefore, no change in total fibrinolytic activity occurs in most patients in response to ATRA. Because chemotherapy given without ATRA has been reported to depress PAI-1 levels in some, but not all, patients with APL, with a resulting significant increase of plasma fibrinolytic activity, these data provide support for the now common practice of combining ATRA with chemotherapy.

Plasma elastase levels, measured as a complex between elastase and α1-proteinase inhibitor, are elevated at the time of diagnosis of APL, most likely as the result of cell degranulation and lysis; ATRA therapy does not appear to affect these levels. Furthermore, no relation was observed between plasma elastase concentration and the levels of D-dimer or other haemostatic variables during treatment with ATRA. These data, together with the data of De Stefano et al, cast doubt on the earlier hypothesis of Egebrin and colleagues that elastase makes an important contribution to the bleeding disorder of patients with APL or other myeloid leukaemias.

Cytokine release

Leukaemic blasts produce inflammatory cytokines such as TNF-α and IL-1β. Cozzolino and colleagues distinguished leukaemic promyelocytes from patients with DIC from patients without DIC by demonstrating increased secretion of IL-1β from the former compared to the latter. These investigators suggested a role for leukaemic cell
cytokines in the pathogenesis of the coagulopathy of APL. Cytokines such as TNF-α, IL-1β, as well as the Gram-negative bacterial product, lipopolysaccharide (LPS), or endotoxin, can down-regulate the anticoagulant properties of the normal vascular endothelium and up-regulate the procoagulant properties. These cytokines induce the expression of TF in endothelial cells (ECs) and down-regulate the expression of EC thrombomodulin (TM), the surface high-affinity receptor for thrombin. The effect of cytokine release by leukaemic cells is a reduction in the ability of the TM–thrombin complex to activate the protein C system. In addition, TNF-α and IL-1β can stimulate the EC to produce the fibrinolytic inhibitor PAI-1, thus further contributing to the prothrombotic potential of ECs.

ATRA up-regulates cytokine production by leukaemic cells, which could theoretically worsen the coagulopathy in APL. However, the prothrombotic potential of the endothelium does not appear to be enhanced, most probably due to the relative protective role of ATRA on the endothelium. ATRA prevents both the down-regulation of TM and the up-regulation of TF induced by TNF-α and by IL-1β produced by NB4 cells. Therefore, although ATRA increases cytokine synthesis by APL cells, it also
appears to protect the endothelium against the prothrombotic stimulus of these mediators through a complex set of interactions, detailed as follows.

Activation of the endothelium by IL-1β or TNF-α also leads to an increase in the expression of EC surface adhesion molecules, such as ICAM-1 or VCAM-1, which among other functions, serve as the counter-receptors for leukaemic cell membrane adhesion molecules (i.e. integrins, such as LFA-1 and Mac-1). Some cytokines mediate tumour cell adhesion to the endothelium and to the subendothelial matrix. Attachment of leukaemic cells to the vessel wall via these adhesion molecules (with special emphasis on so-called junctional adhesion molecules or JAM), with subsequent trans-endothelial migration represents one potential mechanism to explain the higher incidence of vascular complications in acute leukaemia in association with high white blood cell (WBC) counts. Indeed, both early mortality and the so-called retinoic acid syndrome (RAS), which is characterized by unexplained fever, weight gain, respiratory distress, interstitial pulmonary infiltrates, pleural and pericardial effusions, episodic hypotension and acute renal failure, have been correlated with the de novo WBC count, as well as the expression of one or more adhesion molecules and/or cytokines that promote cell–cell interaction. Both clinical and experimental evidence, therefore, supports the concept that in patients with high WBC counts, leukaemic cells (particularly APL cells) promote both localized clotting activation and WBC aggregation by adhesive interactions and subsequent activation of ECs.

An analogy has been drawn between the RAS and the early events in patients with acute myelogenous leukaemia (AML) with hyperleukocytosis and pulmonary compromise. However, as pointed out by Tallman and colleagues, differences observed in the histopathology of the two conditions suggest that the pathogenesis may be different. Pulmonary infiltrates in patients with AML and hyperleukocytosis appear to result from the formation of leukoaggregates in the circulation, which are believed to damage pulmonary vasculature, whereas in the pathology of the RAS, leukocyte infiltration in the lungs has been seen in the absence of leukoaggregates. Although ATRA increases the adhesion capacity of APL cells to the endothelium in vitro, pre-treatment of ECs with ATRA reverses this effect and actually results in impaired adhesion of APL cells to ECs. This anti-adhesive effect may be explained by the down-regulation of EC surface-specific counter-receptors by ATRA. Perhaps ATRA is unable to exert this same protective effect on the specialized endothelium of the lung, thus explaining the unusual features of the RAS. It seems likely that a further understanding of the pathogenesis of the RAS and its prevention, as well as better strategies for the treatment of the consumptive coagulopathy of APL, will evolve from an improved appreciation of the biological properties of the fusion proteins of RARα.

MANAGEMENT OF THE COAGULOPATHY IN PATIENTS WITH APL

Disseminated intravascular coagulation, which complicates a wide variety of disparate conditions linked principally by damage to the vascular endothelium, is best approached by interventions aimed at cure of the underlying disease, together with supportive care. Aggressive attention to the early initiation of supportive measures is particularly important in the management of acute leukaemia, because effective chemotherapy often exacerbates the DIC and accentuates the bleeding syndrome by worsening the thrombocytopenia. The most important supportive tool, therefore, is the judicious use of platelet transfusion. The use of anticoagulants and antifibrinolytic agents, on
the other hand, remains a hotly debated issue. The current standard of care for induction therapy, which virtually always calls for the use of ATRA, has ushered in a new era in the management of the coagulopathy of APL.

**Platelet transfusions, heparin, and antifibrinolytic agents**

Platelet transfusions represent an essential part of the modern supportive care for all patients with acute leukaemia. Prophylactic transfusion of platelets has resulted in a significant decrease in the incidence of fatal bleeding and, therefore, a prolongation of survival.89,90 While the threshold for platelet transfusion has changed recently for patients with other forms of acute leukaemia, prompting clinicians to withhold the prophylactic use until the platelet count reaches 5–10 000/μl91–93, in patients with APL, the bleeding risk and platelet transfusion requirements remain higher in spite of the introduction of ATRA therapy.5 In APL the correction of thrombocytopenia with platelet transfusion is of special importance and may obviate the need for heparin therapy.94–96 In a series of 65 adults with APL, the complete remission (CR) rate was higher in patients transfused intensively with platelets and not given heparin.96 Therefore, our current recommendations for supportive care for patients with APL are as follows. In patients who are not actively bleeding, platelets should be transfused to maintain the platelet count above 20 $\times 10^9/l$; in patients who are actively bleeding, platelets should be transfused to maintain the platelet count above 50 $\times 10^9/l$.3,5

The role of heparin therapy in the treatment of the coagulopathy complicating APL is uncertain. The aim of heparinization is to inhibit intravascular fibrin formation and reduce the consumption of clotting factors and platelets, hence limiting the bleeding tendency. Although a compilation of reported series of patients with APL suggested a statistically significant benefit associated with the use of the anticoagulant therapy,3 the majority of the studies involved a small number of patients, were retrospective and were not controlled. In several studies a historical control group was used, thus not taking into account the rapid induction of remission accompanying more intensive chemotherapy regimens utilized in contemporary protocols nor the increased availability of blood products. Indeed, the benefit of heparin therapy has never been proven in large, prospective, randomized controlled trials. In a large, retrospective analysis of 268 consecutive patients with APL,13 no significant benefit was demonstrated in those patients who received heparin with respect to the incidence of early haemorrhagic deaths, CR rate, or overall survival. Thus, the routine use of heparin in the management of the coagulopathy in APL patients cannot be recommended.

Therapeutic regimens, including antifibrinolytic agents, such as epsilon-aminocaproic acid (EACA, Amicar®) or tranexamic acid, and/or protease inhibitors, such as Aprotinin (Trasylo®), have also been suggested for the management of the coagulopathy, based on the potential role of fibrinolytic activators and other proteases in the pathogenesis of the coagulopathy. However, as discussed above, even the most recently published studies have failed to document adequately the existence of primary hyperfibrinolysis in vivo. Thus, we are not able to provide data from well-designed studies to support the use of antifibrinolytic drugs for the treatment of the bleeding diathesis of APL. The efficacy of tranexamic acid was suggested by two small series of patients,30,31 both of which were published prior to the introduction of ATRA for induction therapy. We would be particularly cautious in the use of inhibitors of fibrinolysis or non-specific proteolysis in view of more recent reports of the occurrence of thromboembolism, when antifibrinolytic agents are given during the course of ATRA induction therapy.97
ALL-TRANS-RETINOIC ACID (ATRA)

The development of ATRA for remission induction therapy of APL has opened new perspectives in the management of the consumptive coagulopathy that often accompanies APL. Since it was first introduced, reports of the use of ATRA have emphasized that the drug produces a high rate of CR with rapid resolution of the coagulopathy but without inducing bone marrow hypoplasia.1 As discussed above, ATRA induces the differentiation of leukaemic promyelocytes by a mechanism involving the expression in APL cells of chimeric PML/RARα fusion proteins.49,50,56,88,98 In earlier non-randomized studies in which historical controls were utilized, APL patients treated with ATRA achieved a 9–20% improvement in CR rate and a 5–6% reduction in early haemorrhagic deaths, as compared to patients treated with conventional chemotherapy.5,98–102 These preliminary findings have been confirmed in more recently published randomized, controlled, clinical trials.103–108 The prevalence rate of early haemorrhagic deaths in patients with APL has been reduced to 2.4–6.5% when patients are treated with a variety of combinations of ATRA with chemotherapy. The standard of care for patients with APL is now ATRA and chemotherapy for induction treatment, although in the most recently published series comparing ATRA with chemotherapy for induction therapy, no difference was observed in early death rates between the two arms.104,108 The importance of ATRA therapy, however, cannot be overemphasized in view of the most recent data, which indicate that long-term (5-year) disease-free survival (DFS) rates approach 75% for those patients who receive ATRA for induction therapy as well as ATRA maintenance therapy.108

As discussed above, several studies have documented the rapid improvement or normalization of clotting and fibrinolytic parameters within a short time of initiation of therapy with ATRA.22–26 Some of the mechanisms by which ATRA can interact with the haemostatic system have been elucidated and were reviewed in a previous section. ATRA can effectively modulate all of the principal haemostatic properties of leukaemic cells, including the expression and/or the effect of APL cell procoagulant activity, profibrinolytic activity, inflammatory cytokines and adhesion molecules. In addition, as indicated, ATRA also modulates the haemostatic properties of normal cells—in particular, the vascular endothelial cell, ameliorating to some extent the procoagulant effect of released cytokines and directly stimulating the synthesis of thrombomodulin109 and t-PA.110 Taken together, we interpret the published data as consistent with a balanced antithrombotic effect of ATRA, with the important caveat that the RAS may have a thrombotic component in some patients. While the preliminary data from studies of arsenic trioxide (As2O3) is also very encouraging with regard to the overall effects of this new agent on the coagulopathy of APL53, it is too early to assess whether As2O3 will prove as efficacious as ATRA. This answer should be forthcoming as the results mature of randomized, controlled trials of arsenic trioxide therapy in patients with APL.

CONCLUSIONS

Nearly all patients with malignancy manifest various abnormalities of blood clotting at the time of clinical presentation, which can be interpreted as most consistent with the syndrome of DIC—along a clinical spectrum from low-grade activation of the haemostatic system to the more rarely observed consumptive coagulopathy. In patients with acute leukaemia, systemic bleeding manifestations tend to predominate over
localized thrombosis of large vessels. The risk of bleeding, due to thrombocytopenia and/or massive blood clotting activation with defibrination, varies according to the type of leukaemia and is most commonly observed in patients with APL. The coagulopathy of APL is characterized by low fibrinogen levels, prolongation of the PT and TT, along with elevated plasma levels of markers of hypecoagulation, hyperfibrinolysis and non-specific proteolysis. The levels of the primary endogenous coagulation inhibitors, AT and PC, are often normal, raising some questions about the diagnosis of DIC. However, the nearly ubiquitous presence of elevated levels of fibrin D-dimer strongly favours the hypothesis of secondary or reactive hyperfibrinolysis occurring in response to activation of blood coagulation.

The pathogenesis of the coagulopathy in APL is most closely linked to the intrinsic procoagulant properties of APL cells. Recent successful efforts to duplicate the syndrome in experimental animals who express the products of transfected APL fusion genes should provide definitive evidence that the malignant transformation event is responsible for the coagulopathy. The APL cellular procoagulants activate the coagulation cascade and stimulate the prothrombotic properties of other blood cell components, including endothelial cells.

Bleeding complications in patients with APL carry a high risk for mortality and, therefore, the use of prophylactic platelet transfusions is highly recommended. Although not discussed, aggressive management of infections is also very important, because viruses, Gram-negative and Gram-positive organisms can contribute to the development of DIC. In contrast, the routine use of anticoagulants and/or antifibrinolytic agents in the control or prevention of DIC cannot be recommended. The advent of ATRA for induction and maintenance therapy of APL has profoundly modified the outlook for patients with this disease and significantly reduced the early mortality rates from bleeding in most series. The use of ATRA fulfils the requirement for an optimal treatment of DIC in APL, because it treats the underlying disease.

**Practice points**

- the bleeding diathesis of APL is most consistent with the diagnosis of DIC
- primary fibrinolysis is hard to document in APL and probably does not exist-excessive fibrinolysis is probably secondary to excessive thrombin generation
- ATRA rapidly reverses the coagulopathy in most patients with APL
- rapid reversal of the coagulopathy by ATRA may improve survival statistics in poor-prognosis patients with APL
- the use of chemotherapy in combination with ATRA is justified on several grounds, including the balanced effects of these two treatment modalities on the coagulopathy of APL
- in patients with APL, prophylactic platelet transfusions should be utilized to maintain the platelet count above 20 000/µl; in patients who are actively bleeding, the platelet count should be maintained above 50 000/µl.
- the role of heparin therapy in the treatment of DIC in APL has not been established in randomized, controlled trials; similarly, the use of antifibrinolytic therapy cannot be recommended in the absence of compelling evidence for primary fibrinolysis in APL patients
Research agenda

- further work is needed to establish definitively the role of the fusion genes PLZF-RARα and NPM-RARα in the pathogenesis of the coagulopathy of APL
- the mechanism for the rapid reversal of the coagulopathy in APL in response to ATRA needs further elucidation to link directly suppression of tumour procoagulant expression with oncogene expression
- a comparison should be made between the levels of cell-surface, t-PA-dependent, annexin II-associated plasmin in APL cells with levels in normal, mature granulocytes, in order to determine the potential significance of this interesting fibrinolytic mediator in APL
- among the non-specific proteases released by APL cells in tissue culture, further work is necessary to establish whether any pathophysiological role exists for elastase in the coagulopathy of APL (doubtful)
- an improved understanding is needed of the role of junctional adhesion molecules (JAM) and trans-endothelial leukocyte migration in the pathogenesis of RAS and the local activation of clotting in the lung of patients with APL
- in view of the probable role of adhesive cellular interactions in the pathogenesis of both the coagulopathy of APL and the RAS, experimental approaches to inhibition of β1-integrins (e.g. VLA-4 or α4β1) with endothelial counter-receptors (e.g. VCAM-1 and ICAM-1) should be tested as adjunctive therapy during remission induction
- experiments should be performed to determine whether specialized pulmonary vascular endothelial cells are resistant to the protective effects of ATRA

REFERENCES


The bleeding diathesis 481


