Coagulopathy in Acute Promyelocytic Leukemia: Can We Go Beyond Supportive Care?

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Acute promyelocytic leukemia (APL) is characterized by frequent complications due to a distinct coagulopathy. While advances in treatments have improved long-term survival, hemorrhagic and thrombotic complications remain the most common causes of death and morbidity. Improved understanding of the mechanisms of the coagulopathy associated with APL may lead to therapeutic interventions to mitigate the risk of hemorrhage and thrombosis.

Keywords: APL, ATRA, hyperfibrinolysis, hemorrhage, thrombosis, delayed bleeding

INTRODUCTION

Acute promyelocytic leukemia (APL) is caused by a translocation of the retinoic acid receptor alpha (RARα) on chromosome 17, most commonly with the promyelocytic leukemia gene (PML) on chromosome 15, which leads to clonal proliferation of promyeloblasts (1). The specific focus of this review is APL with PML-RARα, classified by the 2016 World Health Organization (WHO) criteria as a distinct entity apart from rare variants of promyelocytic leukemia (2).

Long-term survival outcomes for APL are now higher than any other acute leukemia as a result of advances such as all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) (3, 4). For APL patients who survive the first 30 days, over 90% are cured of the disease (4, 5). The increasing use of combined ATRA and ATO for patients with low and intermediate-risk APL has improved long-term cure rates in the disease (6). Nevertheless, death within the first 30 days after diagnosis remains the most common cause of treatment failure (7). Indeed, an updated analysis from the Swedish Acute Leukemia Registry revealed a 25% mortality rate in the first 30 days of therapy, with no improvement from 1997–2008 compared with 2009–2013 (8). Hemorrhage, particularly intracranial hemorrhage, is the most common cause of early death (7–9). The highest risk period for early death and hemorrhagic complications is in the first 4 days of therapy, though almost 50% of early deaths and hemorrhagic complications occur between day 5 and 30 (8, 10–12). Additionally, venous and arterial thrombosis occurs in up to 20% of patients with APL (13–15). Common thrombotic events include deep vein thrombosis, pulmonary embolism, myocardial infarction, and ischemic cerebrovascular events (13–15). The increased risk of both hemorrhagic and thrombotic complications in APL highlights the unique mechanisms that govern the coagulopathy of these patients. Recent work allows us to better understand how current anti-leukemia treatments impact the coagulopathy associated with APL.
At steady state, blood flow is maintained in the face of vascular injury via complex interactions that balance primary and secondary hemostasis (mitigating blood loss) with antithrombotic mechanisms (preventing obstruction of flow and loss of coagulation factors) (Figure 1A). Our understanding of the molecular processes that govern hemostasis and thrombosis have significantly improved during the last three decades. To this end, platelets play a central role not only during primary hemostasis but also in the cellular model of secondary hemostasis (16). More so, it is now clear tissue-specific differences in basal fibrinolytic capacity and endothelial cell expression of thrombomodulin (TM) and endothelial protein C receptor (EPCR) contribute to different patterns of bleeding and thrombosis (17). Lastly, the past 30 years have seen rapid development and FDA approval of a number of drugs meant to mitigate uncontrolled bleeding (i.e., recombinant FVIIa, tranexamic acid, and ε-aminocaproic acid) and clotting (direct thrombin inhibitors, direct factor Xa inhibitors) (18–20). Improved access to supportive measures (replacement of platelets and coagulation factors) together with better understanding of the molecular mechanisms of action of unfractionated and low molecular weight heparins provide a vast armamentarium to approach patients with impaired hemostasis and thrombosis. Yet, progress in the management of these complications in patients with APL lags behind. This lag in therapeutic advancement may be related to difficulties in studying a rare disease, the need for therapeutic interventions to begin very early to mitigate the early complication rate, and lack of commercial support for clinical trials of approaches that utilize blood products or generic medications.

This review highlights the unique pathophysiology of hemostasis and thrombosis in APL and the major clinical reports on the frequency and nature of hemorrhagic and thrombotic events. We will emphasize the commonly used interventions in the management of complications in patients with APL. This lag in therapeutic advancement may be related to difficulties in studying a rare disease, the need for therapeutic interventions to begin very early to mitigate the early complication rate, and lack of commercial support for clinical trials of approaches that utilize blood products or generic medications.

BLEEDING DIATHESIS IN APL

Mechanisms of Hemorrhage in APL

Patients with APL have a distinctively low fibrinogen and increased fibrinogen degradation products and fibrin degradation products. In fact, fibrinogen levels < 150 mg/dL in a patient with acute myeloid leukemia and relatively low white blood cell count raise the clinical suspicion for APL (21). Even though secondary fibrinolysis contributes to the coagulopathy seen in patients with APL, primary hyperfibrinolysis dominates the pathophysiology and drives the increased risk of major bleeding in these patients (22–24). Primary hyperfibrinolysis in APL relies on tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), and annexin II, while secondary hyperfibrinolysis relies on systemic breakdown of clots and microvascular clotting/lysis typically seen in disseminated intravascular coagulopathy (22, 23). Promyeloblasts may interact with fibrinolysis at multiple points of the pathway (Figure 1B) but of particular interest is their expression of annexin II, the receptor for uPA and tPA (23, 25). High levels of annexin II on APL cells contribute to abnormal activation of plasmin and subsequent degradation of fibrin and fibrinogen, resulting in critically low levels of this product (23). Once released, plasmin binds, inactivates, and is inactivated by α2-antiplasmin—an inhibitor of the fibrinolytic pathway (23, 26). In addition to high expression of annexin II on APL cells, serum from these patients has reduced levels of α2-antiplasmin, contributing to further uncontrolled fibrinolysis (25). Recent studies have called into question the role of annexin II in hyperfibrinolysis seen in APL and reported low circulating levels of this protein. In these studies, it was the increased urokinase-type plasminogen activator receptor (uPAR) that claimed a role in fibrin and fibrinogen degradation (27). Since cellular annexin II levels were not assessed in these studies, the contribution of this mechanism cannot be refuted. Nevertheless, further studies may clarify the differential contribution of uPAR and annexin II mechanisms to the hyperfibrinolysis present in patients with APL.

In addition to the annexin II-plasmin axis, other pathways triggered by presence of abnormal myeloid cells could set the stage for the dramatic drop in fibrinogen seen in APL. Abnormal elastase activity and activation of matrix metalloproteinases may contribute to the initial bleeding risk of patients with APL, though more research is needed to define their role (28–31). In addition, a number of investigators have reported increased levels of tissue factor pathway inhibitor (TFPI) in patients with APL. Further studies are necessary to define not only the pathophysiological role of TFPI in the hemorrhagic events seen in APL but also the therapeutic potential of recombinant human TFPI in these patients (32).

Beyond systemic markers of hyperfibrinolysis and coagulopathy, local cell-based factors may play a role in the distribution of hemorrhagic events in APL. The increased expression of annexin II on central nervous system endothelial cells may contribute to the high incidence of intracerebral hemorrhage in APL (33). Thrombocytopenia is a common finding in APL. Nevertheless, the degree to which thrombocytopenia leads to hemorrhagic events remains unclear (11, 34). Coagulopathy in APL shares some characteristics with DIC in sepsis, but the quantitative and qualitative platelet defects in APL are more profound (35). While antithrombin III levels are reduced in DIC, they are usually preserved in APL (36). The combination of decreased capacity to activate platelets and lower levels of platelet concentration does provide further physiologic rationale for the hemorrhagic tendency in APL.

Clinical Bleeding

Hemorrhagic events in APL follow a characteristic clinical pattern. The most frequent cause of fatal bleeding is intracranial hemorrhage (8, 37, 38). Among 22 early hemorrhagic deaths in the Swedish Acute Leukemia Registry between 1997 and 2013, 21 were due to intracranial hemorrhage (8). A report from the PETHEMA Group of patients treated on clinical
FIGURE 1 | Mechanisms of coagulopathy in APL. (A) Physiologic mechanisms of coagulation and anticoagulation. Tissue factor (TF) released by trauma to the vascular wall activates factor VII. Small amounts of activated factor VII (VIIa) activates factor X which in turn activates factor V. Activated factor X (Xa) together with
trials with ATRA and idarubicin found 37 of 66 deaths during induction were related to hemorrhage, 24 intracranial and 12 intrapulmonary (39). In this report, while most hemorrhagic deaths occurred in the first 10 days, fatal bleeding events continued until day 23 of induction (39). Table 1A details clinical reports of hemorrhage and associated risk factors.

One extensive report of intracranial hemorrhage in 12 patients with APL and 39 with other AML showed various locations of bleeding (intraparenchymal, subarachnoid, subdural, epidural, and intraventricular) (37). Most of the intracranial hemorrhages in this study, except those with subdural hemorrhage, resulted in death (37).

Higher white blood cell (WBC) count and decreased fibrinogen at presentation is associated with increased risk of severe or fatal bleeding (12, 39, 41–43). Notably, the association between fibrinogen level at diagnosis and increased hemorrhagic events was not found in other retrospective studies (39, 44). Even though thrombocytopenia is common in APL, reports vary on the association of baseline thrombocytopenia with increased hemorrhage (39, 42–44). More so, prothrombin time (PT) and activated partial thromboplastin time (aPTT) are not consistently associated with bleeding risk (11, 45). Thus, standard coagulation and laboratory assessments of patients with APL do not provide a complete understanding of the risk of hemorrhage in these patients. We and others have used specialized tests that capture a more comprehensive picture of coagulopathy such as thromboelastography (TEG) in order to better understand the coagulopathy in certain patients with APL as well as other types of leukemia (46). Prolonged R time and decreased angle and maximum amplitude have been observed amongst patients with clinical bleeding (46). The interpretation of these tests in the context of APL remains investigational and their restricted availability outside of large academic centers makes them impractical for most patients with this disease.

In addition to typical coagulopathy assessments, other factors are associated with increased incidence of hemorrhagic events in APL. The PETHEMA Group report found a strong association on multivariate analysis of abnormal renal function and fatal hemorrhage, a metric not universally analyzed in other reports (39). While one could hypothesize that abnormal renal function may result in impaired platelet activity, this causative relation has not been formally proven. Similarly, impaired functional status, as measured by the Eastern Cooperative Oncology Group Performance Status, is associated with increased risk of hemorrhage or early death (11, 47). The mechanism responsible for increased hemorrhage or early death in patients with poor performance status is unknown, limiting the ability to adjust therapy to mitigate this risk.

More so, while the first 7 days of therapy are associated with the highest incidence of bleeding events and early death, hemorrhagic risk and fatal bleeding events persist through the first month of therapy (10, 11, 42). Thus, bleeding complications during induction but past the first few days from diagnosis may have divergent pathophysiology and depend more on treatment response and fibrinogen consumption, and less on factors such as WBC at diagnosis (9, 10, 42).

**HYPERCOAGULABILITY IN APL**

**Mechanisms of Thrombosis in APL**

Malignant cells in APL, like those from solid cancers, induce a procoagulant state. This thrombotic tendency is mostly mediated by expression of cancer procoagulant and tissue factor, though additional mechanisms are also involved. For instance, microparticles generated by various cells are elevated in the plasma of patients with APL compared with normal individuals (48, 49). These microparticles express tissue factor and induce widespread thrombin generation (48–50). Compared with other acute leukemias, levels of cancer procoagulant are more elevated in APL, leading to activation of factor X and increased propensity for thrombosis (51, 52).

In addition, APL promyeloblasts overexpress various cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor α (TNF-α) (50). IL-1β and TNF-α augment the activity of tissue factor and plasminogen activator inhibitor-1 (PAI-1) and may contribute to hypercoagulability (53, 54). TNF-α downregulates transcription of thrombomodulin, with an in vivo study demonstrating >80%
TABLE 1A | Incidence of fatal hemorrhage and risk factors in published reports.

| References | Years | Incidence of early death | Incidence of early hemorrhagic death | Risk factors for early death |
|------------|-------|--------------------------|------------------------------------|-----------------------------|
| Lehmann et al. (8) | 1997–2013 | 49/195 (25.1%) | 22/195 (11.3%) | Age >55 years, Worse performance status |
| Xu et al. (9) | 2003–2013 | 49/212 (23.1%) | 37/212 (17.5%) | ECOG PS 3–4, High-Risk Sanz, Increased creatinine, Increased LDH |
| Park et al. (7) | 1992–2007 | 242/1,400 (17.3%) | N/A | Age >55 years |
| Mantha et al. (11) | 1992–2010 | N/A | 37/995 (3.7%) | WBC ≥ 20 × 10^9/L, ECOG PS 3–4 |
| Asou et al. (40) and Yanada et al. (12) | 1997–2002 | 13/283 (4.6%) | 9/283 (3.2%) | Fibrinogen < 1.0 g/L, WBC ≥ 20 × 10^9/L |

Incidence of fatal hemorrhage and risk factors in published reports.

Incidence of early death
Incidence of early hemorrhagic death
Risk factors for early death

Notes:
1. Includes all early deaths associated with bleeding, including causes of death where bleeding and other events such as leukostasis were deemed combined causes of death.
2. Clinical trial population rather than registry/database of all patients with APL.
3. Risk factors for hemorrhagic death rather than all early death.

TABLE 1B | Incidence of thrombosis and risk factors in published reports.

| References | Years | Incidence of thrombosis | Location of thrombosis |
|------------|-------|--------------------------|------------------------|
| Breccia et al. (13) | 1993–2001 | 11/124 (8.9%) | DVT (5), Cardiac (4), Intracranial (2) |
| Rashidi et al. (14) | | N/A<sup>a</sup> | DVT (27/84), Cardiac (25/84), Intracranial (27/84) |
| Mitrovic et al. (15) | 2004–2010 | 13/63 (20.6%) | DVT (7), Cardiac (2), Intracranial (2), Budd-Chiari (1), Retinal vein (1) |
| Montesinos et al. (60) | 1996–2005 | 39/759 (5.1%) | DVT (17), PE (5), Cardiac (4), Intracranial (10) |
| Bai et al. (63) | 2013–2018 | 6/33 (18.2%)<sup>b</sup> | DVT (2), Intracranial (4) |

Incidence of thrombosis and risk factors in published reports.

Incidence of thrombosis
Location of thrombosis

Notes:
<sup>a</sup>Literature review.
<sup>b</sup>Only included patients with WBC at diagnosis > 10 × 10^9/L.

Reduction in thrombomodulin levels after treatment with TNF-α (55, 56). Thus, low levels of thrombomodulin are thought to play a central role in the coagulopathy associated with APL. Initial clinical trials testing the use of recombinant human soluble thrombomodulin (rTM) to mitigate the hypercoagulability of APL have been published (57, 58). The use of rTM for patients with APL has become common in Japan, though a recent retrospective report did not find evidence of improved clinical outcomes (59).

Clinical Thrombosis
While bleeding is the most feared complication of APL, thrombosis is a common but under-recognized presentation. Multiple reports reveal a 5–20% risk of thrombotic events during induction therapy for APL, with the largest PETHEMA report finding 39/759 (5.1%) with a thrombotic event (15, 60–63). The most common locations of thrombosis are cerebral infarction, deep vein thrombosis, pulmonary embolism, and acute myocardial infarction (60–63). Table 1B details clinical reports of thrombotic complications and their incidence in patients with APL. Small sample sizes of the available retrospective reports have hampered the efforts to define risk factors for thrombosis in APL. Interventional research to guide management of thrombosis, particularly life-threatening thrombosis such as cerebral infarction, is currently lacking. A 2019 European LeukemiaNet (ELN) panel recommended the consideration of heparin with dose modifications based upon degree of thrombocytopenia for severe thrombosis (64). The approach to thrombosis remains a difficult decision for the bedside clinical team.

IMPACT OF THERAPY ON COAGULOPATHY
Reports of the ongoing risk of thrombosis and hemorrhage during the initial 30 days of treatment for APL underscore the pathophysiologic importance of the interaction of therapies with the promyeloblasts and resulting alterations in coagulopathy (Figure 1C).

Promyeloblasts secrete high amounts of proteases which degrade collagen and thereby activate enzymes of the coagulation cascade (65). Conventional chemotherapy, including anthracyclines, may lead to a flare-up of coagulopathy and later...
increased risk of developing a hemorrhagic syndrome. Studies of anthracycline-free regimens using arsenic trioxide (ATO) showed less early death and severe thrombocytopenia, with fewer reported cases of hemorrhagic death (4, 66). One report of two patients treated with gemtuzumab ozogamicin (GO) identified marked increase in fibrinogen degradation products after initial dose, however the impact of GO on coagulopathy in APL remains understudied (67).

ATRA's impact on coagulopathy in APL is more thoroughly studied and better understood compared with other therapies. ATRA-based regimens lead to less expression of tissue factor and annexin II by APL promyeloblasts, with a subsequent decrease of the dynamic coagulopathy after treatment initiation (68, 69). ATRA leads to a downregulation of tissue factor expression and cancer procoagulant activity in APL (51, 70, 71). A study using NB4 APL cells that were either sensitive or resistant to ATRA-induced differentiation revealed that ATRA decreases the activity of cancer procoagulant as the blasts differentiate (51). In contrast, ATRA downregulates tissue factor activity independent of pro-differentiation effects (51). More so, ATRA-exposed NB4 cells demonstrate increased thrombomodulin expression while annexin II levels are reduced within 24 h of initiation of ATRA treatment (23, 69, 70). In fact, even though studies evaluating clinical outcomes stratified by time to ATRA initiation are limited by their retrospective nature and small sample size, they confirm the pathophysiologic rationale for rapid treatment initiation and have led most centers to expedite the initiation of treatment with ATRA at first suspicion of APL (64).

Nevertheless, the effects of ATRA on the coagulopathy of APL are complex and not fully elucidated. For instance, by inducing differentiation of malignant promyelocytes, ATRA could enhance ETosis in APL. This newly recognized form of cell death allows the nuclear chromatin to come into direct contact with intracellular enzymes and be released outside the cells (72). Outside the cells, chromatin is organized into NETs (neutrophil extracellular traps) physiologically designed to catch bacteria; however, in patients with APL this may cause endothelial damage and not only further activate the coagulation cascade but also trigger intracranial bleeding, alveolar hemorrhage and differentiation syndrome (73–75). The hypothesized ATRA-induced ETosis may contribute to the delayed bleeding found in patients with APL though further research is necessary to establish to what extent this mechanism is clinically significant.

**PRACTICAL STRATEGIES TO MITIGATE COAGULOPATHY IN APL**

**Transfusion**

Standard supportive care to mitigate the hemorrhagic complications of APL includes platelet transfusion to goal $>30–50 \times 10^9/L$, cryoprecipitate to maintain fibrinogen $>100–150$ mg/dL, and fresh frozen plasma to maintain INR $<1.5$ (64). Limited prospective data exist to define optimal transfusion thresholds. Guidelines and clinical practice have grown to follow those used in the protocols for major trials in APL, such as platelet transfusion to maintain $>30 \times 10^9/L$ and plasma to maintain fibrinogen $>150$ mg/dL in the 2013 ATRA/ATO trial published by Lo-Coco et al. (4).

**Antifibrinolytics**

Given the predominant role of hyperfibrinolysis in APL, antifibrinolytics were evaluated to decrease bleeding risk. A retrospective study of 268 patients in the pre-ATRA era showed no significant difference in bleeding between those treated with antifibrinolytics or supportive care alone (76). Additionally, the PETHEMA group's LPA96 and LPA99 trials had similar rates of early death (5.1 and 5.0%, respectively) despite the use of prophylactic tranexamic acid in LPA99 and not LPA96 (39). More so, in these studies, there was an increased risk of thrombosis associated with the use of tranexamic acid without any reduction in hemorrhagic events (77). The use of antifibrinolytics in specific clinical situations, such as in patients with hyperfibrinolytic coagulation profiles or actively bleeding patients, has not been systematically studied. Thus, at this point we avoid the routine use of antifibrinolytics to mitigate the coagulopathy in patients with APL. A potential avenue of investigation would be the use of additional coagulopathy testing such as TEG to determine the patients most likely to benefit from antifibrinolytics, however this approach requires clinical investigation before incorporation in standard care.

**Heparin**

Heparin's ability to decrease intravascular fibrin formation and coagulation factor consumption due to disseminated intravascular coagulation has been the subject of much speculation and some investigation. Low doses of unfractionated heparin or low molecular weight heparin act on endothelial cells to protect them from unwanted interactions with malignant promyeloblasts (78). By interfering with excess clotting activation, low dose heparins (in the range of 5–10 units/kg/h) may decrease need of platelet and cryoprecipitate transfusions (79). Nevertheless, a retrospective study of 268 patients in the pre-ATRA era found no difference in early hemorrhagic death for those treated with prophylactic heparin compared with supportive care alone (76). In addition, more recent studies have raised the concern of increased hemorrhagic events, particularly delayed hemorrhage, with exposure to heparin (10). Thus, further research is necessary, and at this time, we do not recommend indiscriminate use of heparin in patients with APL.

Some centers have used recombinant factor VII for APL patients with an intracranial hemorrhage, but there is limited literature to support standard utilization in life-threatening hemorrhage at this time (80, 81). The use of recombinant thrombomodulin has been reported and was well-tolerated, though small patient populations limit the ability to judge clinical effectiveness (57, 59, 82).

Coagulopathy in APL shares some characteristics with DIC in sepsis, but the quantitative and qualitative platelet defects in APL are more profound (35). Agents such as antithrombin have shown no clear benefit in DIC, though the mechanism is an appealing target for study in APL-particularly in patients at highest risk for thrombotic events (83). Importantly, recombinant human activated protein C (APC) was removed...
from the marked after initial use for severe sepsis and DIC, in part due to risk of hemorrhage-a particularly concerning risk in patients with APL (84).

DISCUSSION

APL, more than any other acute leukemia, is marked by a characteristic coagulopathy at diagnosis. Hemorrhagic syndromes vary from easy bruising or purpura to hematomas and even intracranial hemorrhage, the most serious complication and the main cause of early death.

Since 1990, an immense body of basic science research has improved our understanding of the pathophysiology of coagulation in APL. Despite these advances in knowledge, interventional studies to prevent or manage hemorrhagic and thrombotic complications are infrequent and not powered to drive clinical practice forward. These failures to advance our management of coagulopathy in APL are underscored by the persistently high rates of early hemorrhagic and thrombotic death. While long-term outcomes have improved with the use of ATRA and ATO, the last major hurdle in APL is to prevent early hemorrhagic and thrombotic deaths.

Multiple questions face clinicians aiming to decrease hemorrhagic and thrombotic risk in APL. Are there markers which can reliably and reproducibly identify degree of endothelial damage in an individual patient? Can we identify patients more prone to hemorrhage vs. thrombosis, and tailor risk mitigation strategies based on individual coagulopathy profile? Are there any biomarkers or risk factors that would be particularly helpful in making these decisions? Moreover, as the risk for bleeding and clotting events is dynamic during induction therapy, how can we assess these changes in individual patients? The dynamic nature of coagulopathy in APL suggests that risk mitigation strategies should vary depending on time in therapy. Such an approach may transfuse platelets earlier in induction, shifting to more aggressive use of cryoprecipitate for a higher fibrinogen goal during the second week of therapy.

In addition to its role in APL, ATRA is able to differentiate non-APL myeloblasts. The relative contributions of annexin II, tissue factor, and cancer procoagulant to coagulopathy in non-APL acute leukemias are less well-understood, and further studies are needed to define if ATRA is able to mitigate the coagulopathy in other acute leukemias.

Coagulation remains profoundly altered despite the administration of ATRA, even if much improved in comparison with the pre-ATRA era. Interventions such as recombinant human soluble thrombomodulin to mitigate the risk of coagulopathy remain understudied. Moreover, the role of recombinant factor VII in life-threatening hemorrhage requires additional research. Likewise, the approach to anticoagulation after confirmed thrombosis remains a clinical challenge with little data to guide bedside clinicians. To adequately study the aforementioned interventions, many centers will have to collaborate prospectively and/or pool their retrospective experience in this relatively rare malignancy.

Such an effort will pose significant logistical challenges, but may finally leverage our pathophysiologic understanding into improved outcomes for patients with APL.

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All authors contributed to the design, writing, and critical review of the manuscript.

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