7th INTERNATIONAL SYMPOSIUM ON
ACUTE PROMYELOCYTIC LEUKEMIA
ROME, September 24-27, 2017

APL fatal bleeding and thrombosis in the ATRA era

Anna Falanga

Department of Immunohematology and Transfusion Medicine & Hemostasis and Thrombosis Center

ASST Papa Giovanni XXIII, Bergamo

The coagulopathy of Acute Promyelocytic Leukemia (APL): A thrombo-hemorrhagic syndrome

- The onset of APL is characterized by a **severe coagulopathy** responsible for a high rate of hemorrhagic deaths (mainly in brain and lung).
- ***Bleeding*** can occur concomitantly to ***thrombotic manifestations***.
- Simultaneous bleeding and thrombosis are part of the same clinical picture, which reflects the complexity of the coagulopathy of APL.

An imbalance between procoagulant, anticoagulant, and profibrinolytic forces occurs in APL patient hemostatic system

- A hemorrhagic phenotype prevails when the **consumption of clotting factors and platelets**, and **activation of fibrinolysis** dominate the picture.
- This coagulopathy may occur to different extent in all types of acute myeloid leukemia.
- However, in patients with APL, **hemorrhage** is usually predominant and is relevant for mortality rates.

Laboratory signs of the coagulopathy

- Coagulation test abnormalities include:

  Thrombocytopenia (mainly due to bone marrow failure)

  +

  - Hypofibrinogenemia
  - Increased FDPs and D-Dimer
  - Prolonged prothrombin and thrombin times
  - Increased hypercoagulation markers, i.e. Thrombin-Antithrombin complex (TAT), prothrombin fragment 1+2 (F1+2)

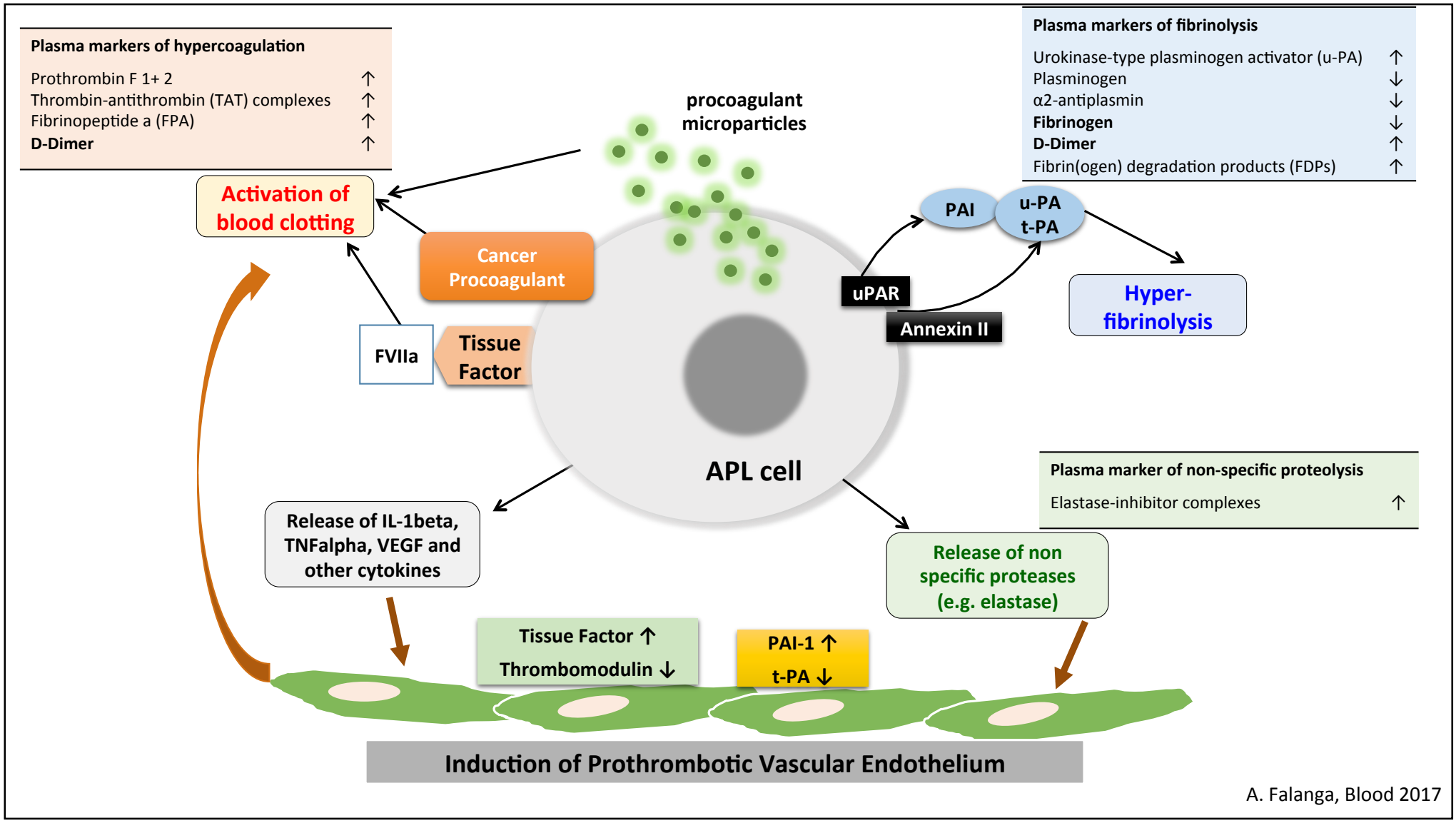
- These abnormalities are consistent with the diagnosis of disseminated intravascular coagulation with excess hyperfibrinolysis.

Pathogenesis of the APL coagulopathy

- At least three processes are involved:
  1. disseminated intravascular coagulation
  2. fibrinolysis imbalance
  3. direct proteolysis of several coagulation proteins including fibrinogen and von Willebrand factor

- **All three of them can be triggered by circulating APL cells**

Falanga A, Blood 2017

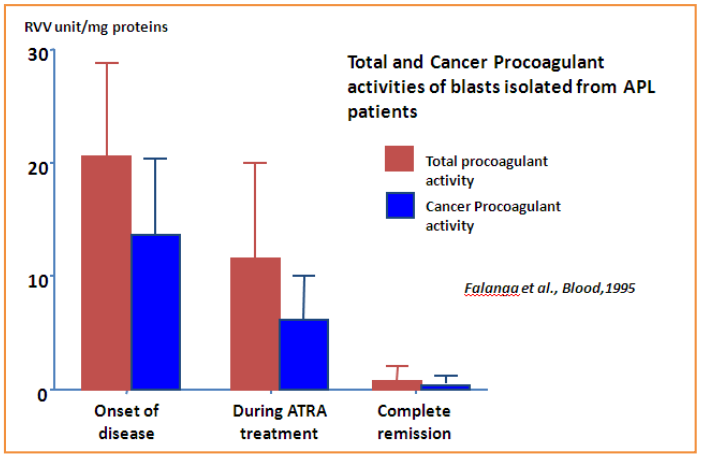

Plasma markers of hypercoagulation
Prothrombin F 1+ 2 ↑
Thrombin-antithrombin (TAT) complexes ↑
Fibrinopeptide a (FPA) ↑
D-Dimer ↑
Plasma markers of fibrinolysis
Urokinase-type plasminogen activator (u-PA) ↑
Plasminogen ↓
α2-antiplasmin ↓
Fibrinogen ↓
D-Dimer ↑
Fibrin(ogen) degradation products (FDPs) ↑
procoagulant microparticles
Activation of blood clotting
Cancer Procoagulant
FVIIa
Tissue Factor
PAI
u-PA
t-PA
uPAR
Annexin II
Hyper-fibrinolysis
APL cell
Release of IL-1beta, TNFalpha, VEGF and other cytokines
Plasma marker of non-specific proteolysis
Elastase-inhibitor complexes ↑
Release of non specific proteases (e.g. elastase)
Tissue Factor ↑
Thrombomodulin ↓
PAI-1 ↑
t-PA ↓
Induction of Prothrombotic Vascular Endothelium
A. Falanga, Blood 2017

The advent of *all-trans* Retinoic Acid (ATRA) differentiation therapy has been a landmark in APL treatment

ATRA

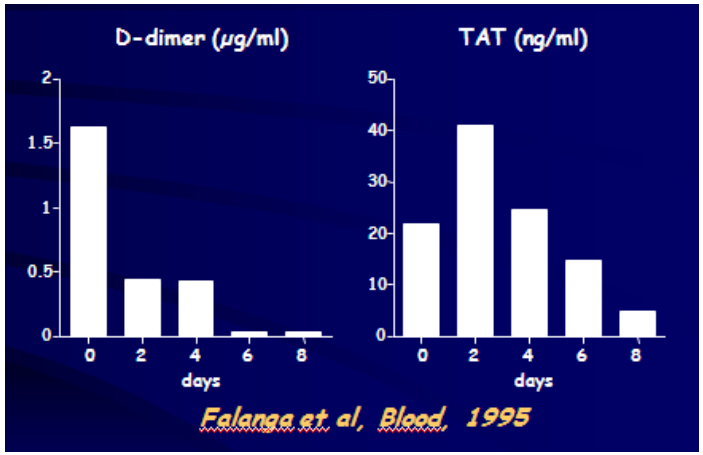
Differentiation of leukemic blasts

↓ procoagulant activity of APL cells



Correction of the coagulopathy

↓ plasma hypercoagulation markers



Remission Induction

Falanga A, Blood 1995

However ATRA's effect on the coagulopathy is slow

It may take 2 to 3 weeks to normalize coagulation.

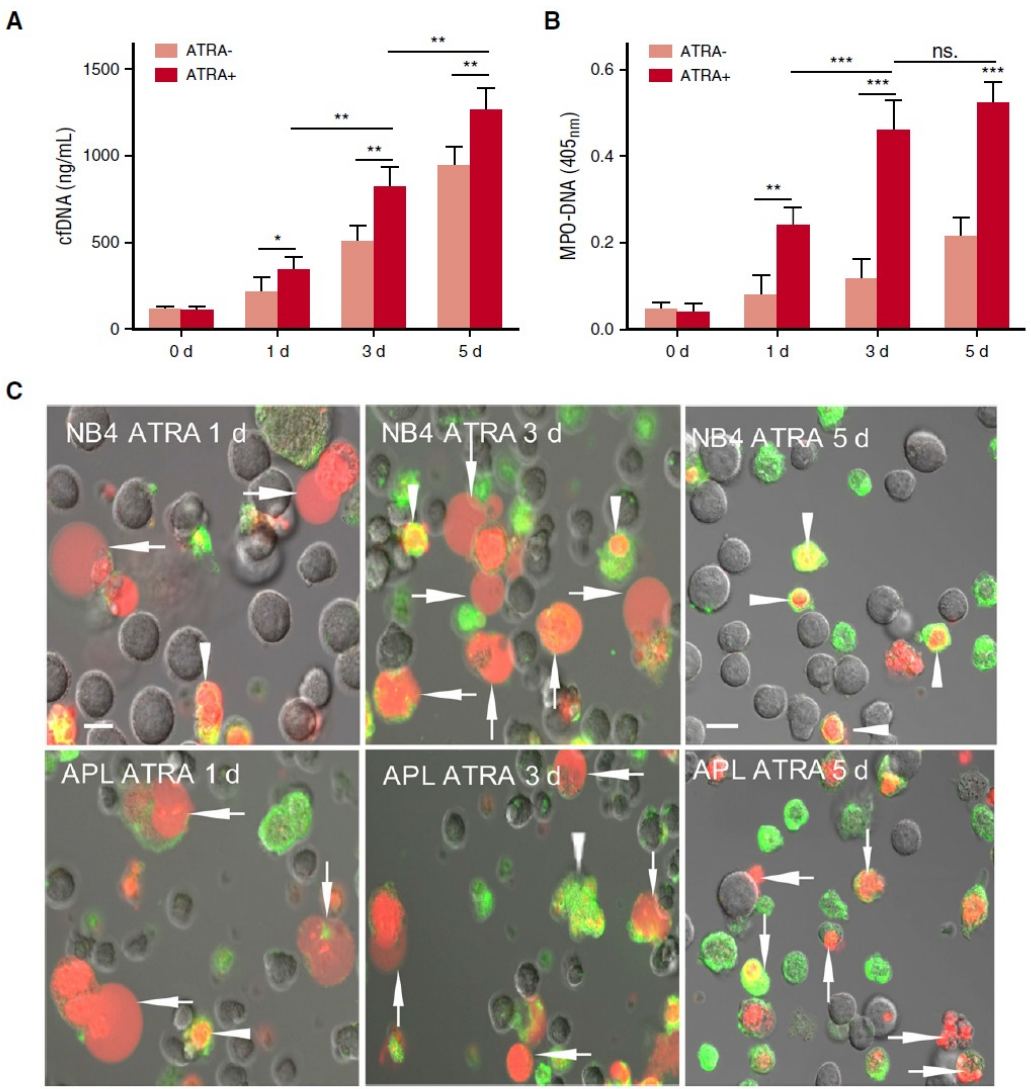
A novel procoagulant mechanism induced by ATRA:

Extracellular chromatin release (Etsosis) from malignant promyelocytes

- ATRA potentiates and induces extracellular chromatin and cell-free DNA (cf-DNA) generation by ETsosis, which correlates with thrombin generation and strong procoagulant effect.
- Thrombin generation is inhibited by DNAse (by degrading cf-DNA), but not by anti-Tissue Factor antibody.
- Promyelocytic extracellular chromatin (ETs) induces fibrin deposition, plasmin generation, and fibrinolysis, and produces cytotoxic effects on endothelial cells, which shift to a procoagulant phenotype.
- The authors suggest that this novel mechanism of coagulopathy in APL, that is exacerbated on initiation of treatment with ATRA, may contribute to early hemorrhagic deaths during ATRA.

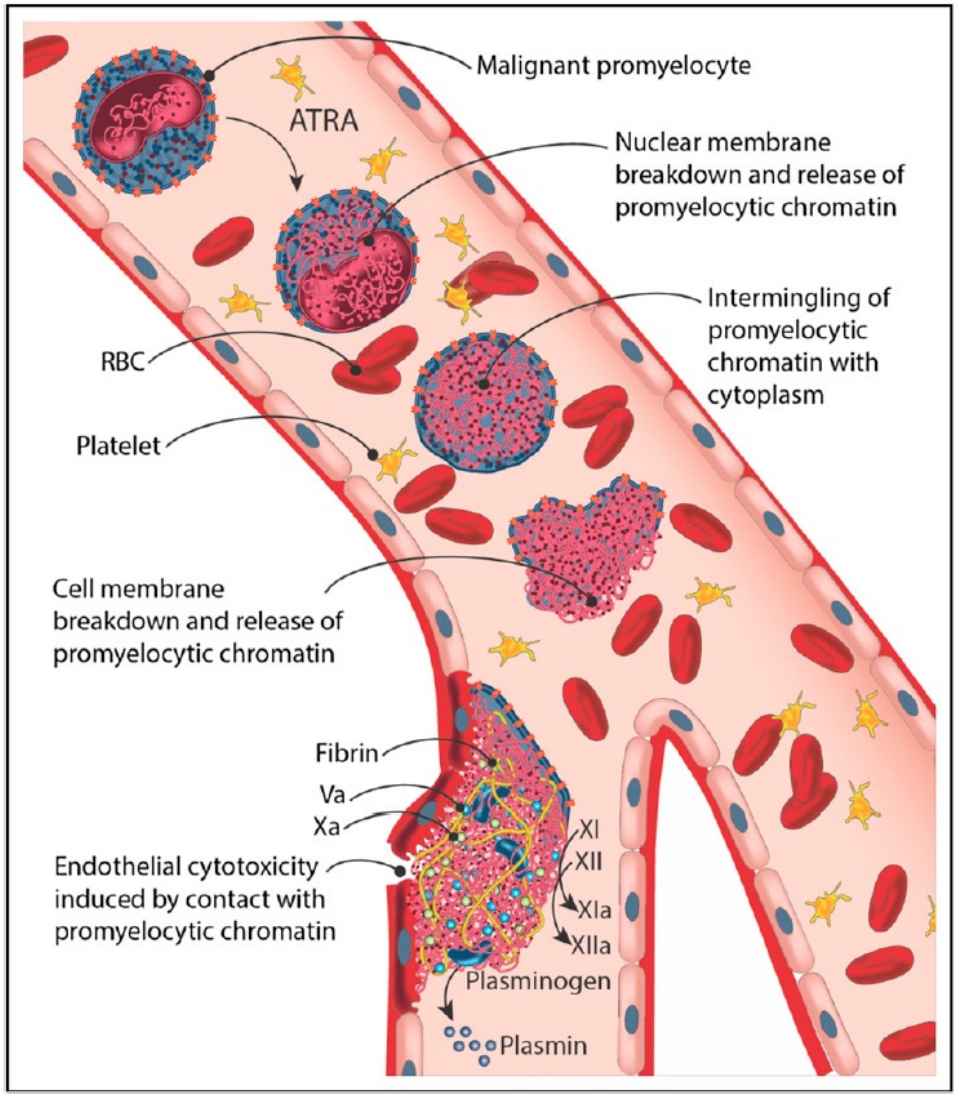
Cao et al., Blood 2017

Promyelocytic extracellular chromatin exacerbates coagulation and fibrinolysis in acute promyelocytic leukemia



- **A) ATRA treatment induces markedly increased cell-free DNA (cf-DNA) release in a time dependent manner compared with the untreated group**.
- **B) MPO-DNA, a marker of ETosis, is higher in the ATRA-treated cells than in controls**.
  No significant increase from day 3 to day 5 is seen anymore, indicating that the increase in cell-free DNA (cf-DNA) during this time is mainly from apoptosis.
- **C)** APL/NB4 cells were stained with lactadherin (green = apoptosis) and PI (red = ETsosis) and analyzed by confocal microscopy. **ETosis was the major cell death pattern seen in the ATRA-treated group up to the third day, indicating that the increase in cf-DNA triggered by ATRA is mainly from ETosis.**

Cao et al., Blood 2017



APL: Oh! What a tangled web we weave

- Malignant promyelocytes on exposure to ATRA undergo nuclear and granule membrane breakdown, with a subsequent mixing of chromatin and cytoplasmic contents within the cell.

- Then, there is swelling, further weakening, and final breakdown of the cell membrane with release of promyelocytic chromatin, which forms a NET-like structure and binds to other cells and endothelial cells.

- The surface of the promyelocyte extracellular chromatin (ETs), along with the cell surface membrane, concentrates procoagulant factors and fibrin.

- The promyelocyte ETs and cell-free DNA (cf-DNA) also facilitate increased generation of plasmin and activate the intrinsic coagulation cascade.

- Finally, promyelocytic ETs damage endothelial cells with which they come into contact, leading to a procoagulant phenotype, and provide additional surface area for clot formation and fibrin deposition. Ensuing endothelial cytotoxicity probably also leads to loss of endothelial cell integrity.

Vikram Mathews. Blood 2017

Fatal bleeding

- Before the ATRA era, early hemorrhagic death (HD) occurred in up to 20% of new APL patients.
- Currently, the standard of care regimens based on ATRA and arsenic trioxide (ATO) provide >90% complete remission rates together with amelioration of the coagulopathy.
- However, data from clinical trials show that a **3-10% risk of early HD remains during ATRA**, peaking in the first 2 weeks of treatment.
- Rates are as high as 30% in population-based studies.
- **Fatal bleeding remains a major cause of treatment failure and is one of the main obstacle to final cure of APL.**

The characterization of the coagulopathy and the identification of predictive markers remain a critical issues in the ATRA era

- Today, early death rather than resistant disease represents the major cause of treatment failure in APL.
- The main cause of early death in these patients is bleeding, often occurring at the intracranial level.
- Still efforts are needed to **decrease the early death rate**, which is the primary cause for treatment failure.

Supportive measures are important

- ATRA and ATO ameliorate the bleeding syndrome. Indeed, experts recommend ATRA be started as soon as the diagnosis of APL is suspected.

- Unfortunately, it takes 1 to 3 weeks for ATRA treatment to resolve the APL coagulopathy, therefore additional measures to prevent bleeding are often required.

Aggressive supportive therapy

- This includes:
  - platelet concentrates,
  - cryoprecipitate or fibrinogen,
  - Fresh frozen plasma
  - and, still controversial, treatments with anticoagulants or antifibrinolytics.

- None of these measures have been evaluated for efficacy and safety in prospective randomized trials.

- There are no data-driven algorithms available to guide blood product support for the coagulopathy.

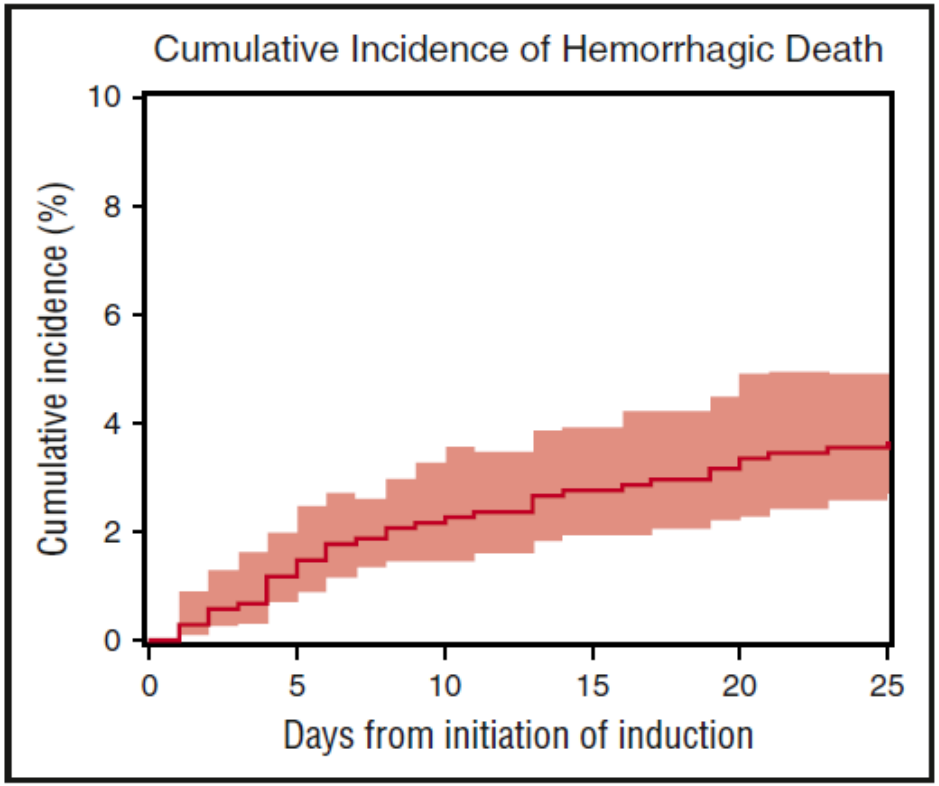
- Similarly, no trial data exist to demonstrate the utility of low molecular weight heparins (LMWH) or new oral anticoagulants (DOACs).

Identifying patients who are at greatest risk of fatal bleeding is very important for the design of prospective clinical trials to decrease early HD

- Published reports provide conflicting results on which patient characteristics are predictors of early HD.
- Some of the risk factors for hemorrhage that have been suggested include:

    - age >60 years
    - high WBC count
    - high peripheral blast cell count
    - Low fibrinogen levels (<10 g/L)
    - poor performance status
    - elevated creatinine
    - elevated lactate dehydrogenase
    - prolonged prothrombin time and partial thromboplastin time
    - low platelet counts

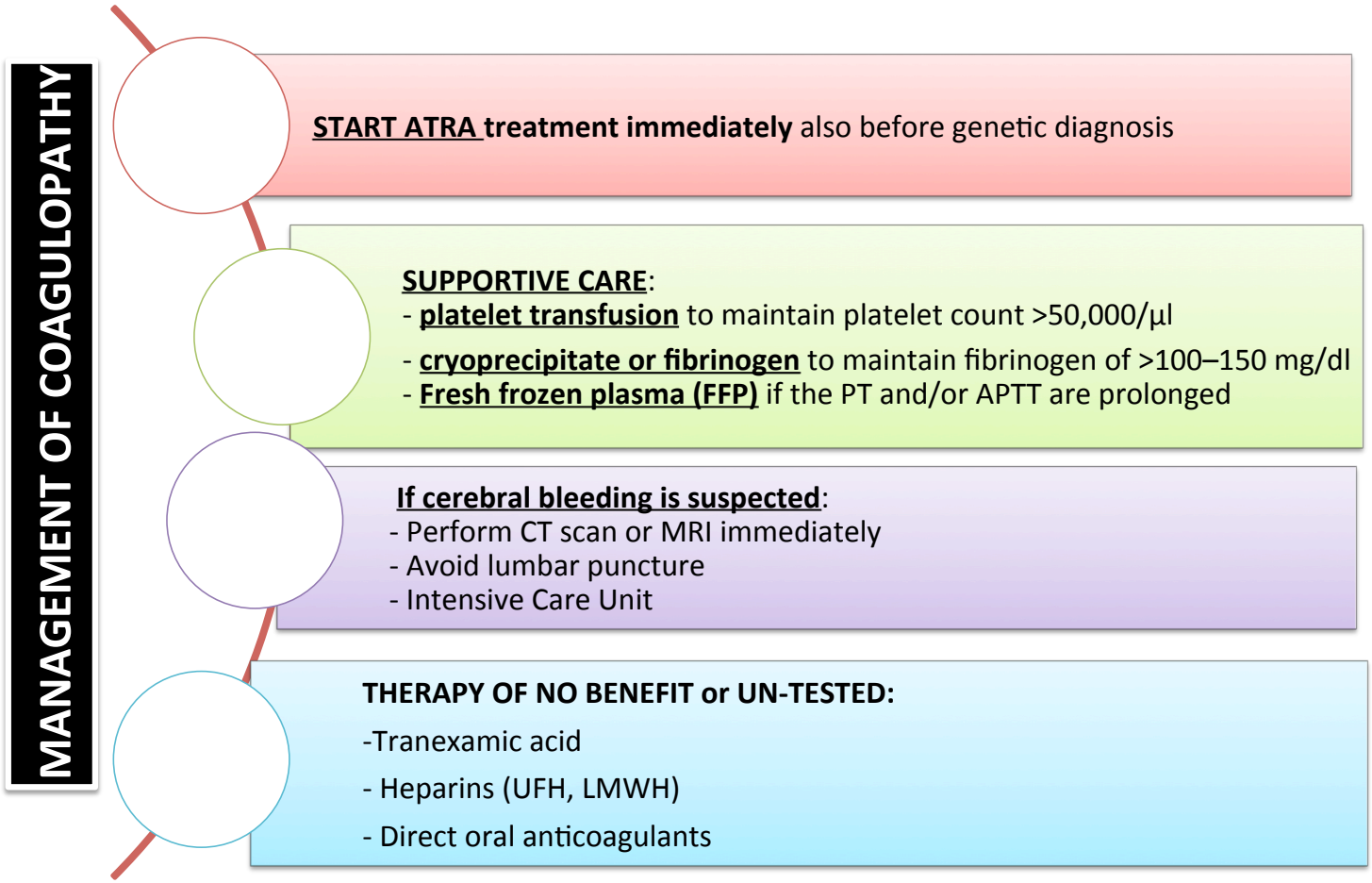
Determinants of fatal bleeding during induction therapy for acute promyelocytic leukemia in the ATRA era



- Data on most of the identified risk factors in patients enrolled in 5 major clinical trials of APL that included ATRA in the induction regimen.
- The risk factors are considered at baseline in 995 evaluable patients, the largest cohort examined so far, and the potential predictive value on the occurrence of fatal bleeding within 30 days of treatment is estimated .
- ***At 30 days, the incidence of hemorrhagic death was 3.7% (95% CI, 2.6% to 5.0%).***
- ***At multivariate analysis, a high total WBC count ≥20x10^9/L emerged as an independent predictor of early HD.***

Mantha et al. Blood 2017

Schematics of the current approaches to treatment of APL coagulopathy



Treatment of VTE in hematologic malignancies (1)

- No *ad hoc* studies or guidelines are available for patients with hematologic malignancies
- **Guidelines for patients with solid tumors**:
  - Initial treatment: low molecular weight heparin (LMWH) full dose (100 U/Kg x 2/d or 200 U/Kg/d) for 1 month
  - Long-term treatment: 70-80% of the initial dose for at least 5 months
- **Adapted to hematologic malignancies**:
  - Reduce the initial dose to 70-80% if platelets ≤ 70 X 10^9 /L
  - Reduce the initial dose to 50% if platelets ≤ 50 X 10^9 /L
  - Stop therapy if platelets ≤ 20 X 10^9 /L

Falanga A, Montesinos P (in press 2017

Treatment of VTE in hematologic malignancies (2)

- LMWH for 6 months is the minimum treatment
  - ✓ Dose adjustments vs. platelet count
  - ✓ Frequent measurement of anti-factor Xa levels
  - ✓ Role of fondaparinux, idraparinux, direct thrombin inhibitors and new oral anti-Xa inhibitors unknown
  - ✓ Bleeding complications may be responsive to recombinant factor VIIa (rVIIa) (unknown)
- Central venous catheter related thrombosis may not always require treatment
- Role of inferior vena cava filters?
  - Removable filters in select patients (e.g. platelet count $\leq 30 \times 10^9$) for short-term use

Future perspectives

- Future studies should:
  - identify whether circulating plasma biomarkers of hypercoagulation and/or hyperfibrinolysis or global rapid coagulation assays (i.e., thrombin generation, or thromboelastography) can add value to predictive models of early HD.
  - test by means of RCT the efficacy and safety of supportive measures (including LMWHs and DOACs) to reduce HD.