Pulmonary Thromboembolus: What Electron Microscopy Teaches Us

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Introduction

In patients surviving pulmonary embolism (PE), .4-8.8% of patients develop pulmonary hypertension (CTEPH) and 30-50% sustain long-term pulmonary disability (CTED).(1,2) PE treatment options have expanded in recent years, with the availability of new percutaneous embolectomy devices. These specimens of thrombus, appearing of various ages are available for visual (macroscopic) and microscopic examination.

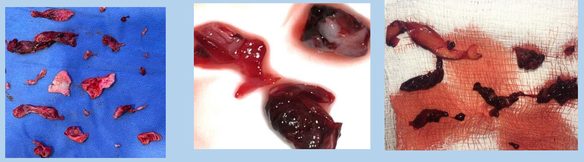
This investigation was undertaken to examine emboli to learn why some pulmonary emboli resolve with anticoagulation or plasminogen activator and why others fail to resolve.

Materials and Methods

Samples obtained from 20 pulmonary embolectomy patients using the Inari FlowTriever were inspected and the most chronic (pale color, firm texture) were sent in glutaraldehyde for transmission electron microscopy. In some specimens, dark red, presumed fresh thrombus was also processed separately. Percentage fibrin content was calculated with Adobe Photoshop using area determination to calculate a relative percentage, in fresh and intermediate thrombus. Images were reviewed by authors 1-3.

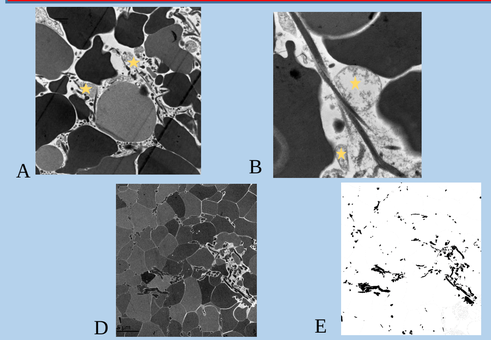
Results

All patients demonstrated heterogeneity in the macroscopic appearance of the thrombus. Over 80% of the patients had thrombi demonstrating areas of light red, pink or tan-white thrombus. 3 illustrative samples are displayed below:

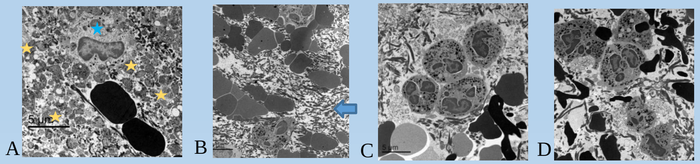


Macroscopic appearance: Note the differences in the color of emboli. In all cases, ranging from dark red, soft fresh thrombus to white/pale or pink thrombus (arrows). The lighter emboli were more firm and rubbery consistency.

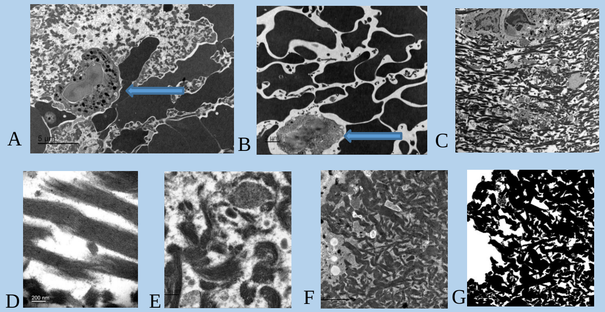
1. Early/ Induction: 1-2 days; Thin fibrin, trapped RBCs and platelets
2. Acute fibrin Mesh:2-7 days: Platelets, RBCs, Neutrophils — At 7 days: Thick fibrin bundles, polyhedrocytes, early collagen
3. Intermediate/Organizing: 2 weeks: Thick fibrin, RBC stretching (polyhedrocytes), NETS, Macrophages, phagocytosis, collagen
4. Chronic:2-4+ weeks: Endothelializaton/recanalization, densely organized confluent fibrin, collagen, liposomes



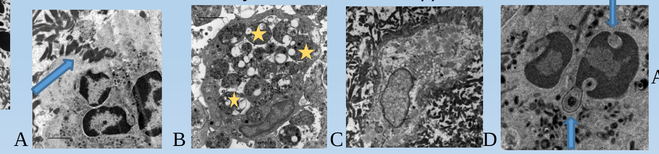
Stage 1:Induction- Fresh Thrombus: Thin fibrin, RBCs and Platelets (*) (A, B). Note thin fibrin strand in B and crosslinking of fibrin (C), due to parallel-aligned fibrin monomers and COOH bonds. Sparse fibrin and densely compacted RBCs.(D) Fibrin is 4.6% area calculated from Photoshop, in (E).



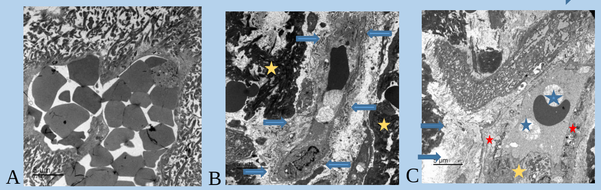
Stage 2: Acute fibrin dense mesh: (A) < 7 days; numerous platelets (Yellow *) (plug?) with a neutrophil (*), two trapped RBCs and thin strand fibrin.
(B) Denser fibrin (arrow), neutrophils, and trapped RBCs.
(C, D) Numerous neutrophils, with dense fibrin and senescent RBCs and degranulated platelets.



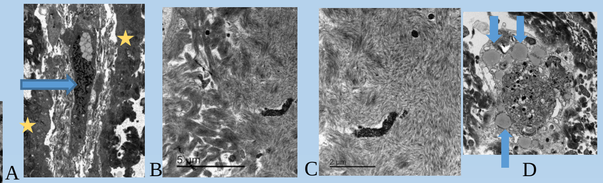
Stage 3: Intermediate/Organizing: Polyhedrocytes (irregularly shaped RBCs) from platelet induced clot contraction and stretching with fibrin mesh (A,B). Note: Monocytes (B, arrows), precursor of macrophage, which can degrade fibrin without plasmin. (C,D,E) Thicker bundles of Fibrin with partly degraded, denser sheets of fibrin. Notice abundance of fibrin in (C). Fibrin area percentage has increased to 60%, as contrasted with stage 1 (F) and (G, reformatted to show only fibrin on Photoshop).



Stage 3 Organizing: Increased cellularity: (A) Neutrophil phagocytizing fibrin (arrow), (B) Neutrophil which engulfed many platelets (yellow *), and a macrophage/early foam cell of increased phagocytosis(C). Neutrophil Extracellular Trap (NET) in action with budding extrusion of DNA from the cell's nucleus, arrows (D).



Stage 4: Chronic: recanalization/endothelialization: (A) Tightly packed RBCs in new vessel. Recanalization, (B) arrows, with flowing blood elements of RBC, degranulated platelets and a neutrophil. Note dense, organized fibrin (*). (C) A channel with blood elements: RBC, senescent platelets (blue *), and neutrophils (yellow *) lined by endovascular cells (red *)(C). Note collagen, (arrows) as fine wavy lines located to the left of this recanalized vessel.



Stage 4 : Chronic: Collagen formation as demonstrated in older thrombus samples. (A) Hyalinized thrombus with fibroblast (arrow) and very dense organized collagen (middle), compared with densely compacted fibrin both sides (*). (B) Old compact fibrin (left) and collagen (right), showing chronicity changes in thrombus. Magnified image shows fine, swirling collagen (C).
Macrophage/Foam cell with lipid droplets (blue arrows), indicating chronic thrombus (D).

Discussion and Conclusions

PE thromboemboli have developed and propagated in the venous circulation from a few days to many weeks. Thrombus resolves by: (1) Plasmin lysis of fibrin and (2) phagocytosis/lysis by neutrophils and macrophages in older thrombi (3). Transition from early to organizing, and then, chronic thrombus may explain the discrepancy in lytic susceptibility of older, organized thomboemboli compared with new thrombus.

Our study provides compelling explanation for plasmin resistance of PE, namely significant aging beyond early or acute thrombus:

1.Thin, sparse fibrin strands transition to dense, compacted, abundant thick bundles of "cross-linked insoluble fibrin".

2. Cellular evolution from early platelets and neutrophils to macrophages and fibroblasts; abundant phagocytosis.

3. NETS composed of DNA chromatin material, further consolidate the clot. (2)

4. Insoluble collagen synthesized by fibroblasts.

5. Recanalization/endothelialization promoting a return of blood flow.

Ultimately, pulmonary artery wall thickening, webs etc. occur late in the course of unresolved PE and may manifest as pulmonary artery stenosis and hypertension. (4,5,6,7,8)

Long-term disability, (CTEPH and CTED) of PE survivors should be taken into account as we develop new ways to treat acute PE. This nascent research of the EM appearance of PE illustrates much still needs to be learned. A re-assessment of treatment algorithms incorporating endovascular embolectomy, trans-catheter thrombolysis and anticoagulation-only protocols seems justified given the heterogenous thrombus we observed using EM. Could current PE management algorithms be optimized towards embolectomy, in lieu of thrombolysis and anticoagulation alone? We also propose a need for real-time discriminating pre-treatment assessments, to determine which patents are more likely to benefit from embolectomy alone or thrombolytic therapy.

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