More Evidence for Inflammatory Thrombosis in Chronic Thromboembolic Pulmonary Hypertension

Is the Embolic Hypothesis Losing Ground?

Chronic thromboembolic pulmonary hypertension (CTEPH) is a subset of pulmonary vascular disease that is characterized by obstruction of pulmonary arteries with fibrotic thrombus, with layers of fresh thrombus in about 40% of cases, that is amenable to cure by surgical pulmonary endarterectomy (PEA). In recent years, multimodality treatments, including balloon pulmonary angioplasty and medical treatments, have made CTEPH one of the best treatable forms of pulmonary vascular disease. CTEPH has been reported as a long-term complication of acute pulmonary embolism (PE), with estimated cumulative incidences between 0.1% and 9.1% within the first 2 years after symptomatic PE (1). Both recurrent venous thromboembolism and unprovoked PE are associated with a higher risk of CTEPH, with odds ratios of 3.2 and 4.1, respectively (2). These observations are confirmed by data from the European CTEPH registry (3), lending support to the concept of a thromboembolic origin of CTEPH. However, CTEPH pathogenesis is still not easily explained. Only recently, research has underpinned the concept of "inflammatory thrombosis" as a trigger for abnormal propagation of fresh thrombus on the endothelial surface and the transition of fresh thrombus to fibrotic tissue (4).

In this issue of the *Journal*, Manz and colleagues (pp. 806–818) (5) performed meticulous studies using endothelial cells isolated from patients during PEAs and modeled flow of whole blood and platelets *in vitro* to study platelet-endothelial cell interactions. The authors show that vWF (von Willebrand factor) is increased in plasma and the pulmonary endothelium of CTEPH. CTEPH patient-derived pulmonary artery endothelial cells show increased platelet adhesion compared with control, which is abrogated by a monoclonal antibody directed against the A1 domain of vWF. Increased platelet adhesion is mediated by enhanced vWF gene expression and by an increase of endothelial nuclear factor (NF)-κB2. Increased histone acetylation of the vWF promotor in CTEPH endothelium and reduced histone trimethylation (H3K27me3) facilitate binding of NF-κB2 to the vWF promotor during vWF transcription. Genetic interference of NF-κB2 normalized high vWF RNA expression levels and reversed the prothrombotic phenotype.

This work provides powerful confirmation of an important role of inflammatory thrombosis in the pathogenesis of CTEPH. The mammalian Rel/NF-KB family of transcription factors, including RelA, c-Rel, RelB, NF-KB1 (p50 and its precursor p105), and NF-KB2 (p52 and its precursor p100), is a central regulator of inflammation. The five members of the NF-KB family are normally kept inactive in the cytoplasm by interaction with inhibitors called IκBs or the unprocessed forms of NF-KB1 and NF-KB2. Antigen receptors, pattern-recognition receptors, receptors for the members of TNF-α and IL-1 cytokine families, and others induce differential activation of NF-KB heterodimers. NF-KB activation induces adhesion molecules promoting binding and transmigration of leukocytes (6). Recently, neutrophil activation and neutrophil extracellular traps (NETs), which are in sequence with NF-KB activation, were shown to increase clot size and enhance transforming growth factor β signaling, which leads to fibrotic thrombus remodeling in CTEPH (4). Neutrophil activation is a hallmark of CTEPH (4). A majority of CTEPH risk factors (e.g., Crohn's disease, ulcerative colitis, chronic osteomyelitis, Behcet's disease, *Staphylococcus*-infected intravenous lines including infected ventriculoatrial shunts, chronic leg ulcers, Familial Mediterranean Fever, and cancer classify as NETs-driven diseases) (7).

Despite the demonstration of a contribution of platelets to CTEPH thrombosis (5), the precise role of platelets in CTEPH is still unclear. For example, the proportion of platelets in fresh CTEPH thrombus is unknown. What we know is that platelet counts and global platelet function tests in CTEPH are usually normal. Furthermore, venous thrombi and pulmonary emboli contain fewer platelets than arterial thrombi (8). While Yaoita and colleagues (9) reported highly activated platelets in CTEPH that were hyperresponsive to thrombin stimulation, it was recently reported that in patients with phospholipid antibodies, a risk factor for CTEPH, platelets show increased baseline activation but decreased reactivity, presumably as a consequence of inflammatory preactivation (10). In the experiments of Manz and colleagues (5), platelet adhesion was only 1.5-fold higher in CTEPH than controls. This observation constitutes a weak signal but is of interest as new data suggest that NF-KB signaling is a modulator of platelet function (6).

Over more than 20 years, the "embolic hypothesis" in CTEPH pathogenesis has been in scientific competition with the hypothesis of "thrombosis *in situ* of the pulmonary arteries" (11), and with their work, authors stimulate a new discussion of this question. The assumption of a thromboembolic origin of CTEPH is based on an association with large and recurrent venous thromboembolism (12), venous malformation (13), right atrial thrombi, coagulation factor deficiencies, and circulating phospholipid antibodies. The role of vWF in venous thrombosis, thrombosis recurrence, and CTEPH (14) is well established and further supports the thromboembolic hypothesis. However, vWF is also a component of platelet alpha granules and an important endothelial cell protein stabilizing factor VIII, which is in linkage equilibrium with blood groups, that

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represent another risk factor for CTEPH (14). The *in situ* thrombosis concept is in line with old studies showing the expression of plasminogen activator inhibitor type 1 in endothelial cells lining CTEPH thrombus channels (1). However, epigenetic modifications of the vWF promotor and increased binding of NFκB2 with enhanced platelet aggregation on endothelial cells represent a novel mechanism of *in situ* thrombosis. We agree with CTEPH basic scientists that *in situ* thrombosis remains an amplifier of pulmonary vascular disease in CTEPH, but the data of Manz and colleagues (5) do not speak against thromboembolism, which appears evident for the CTEPH clinician. ■

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Smriti Sharma, Ph.D.
Irene M. Lang, M.D.
Department of Internal Medicine II (Cardiology)
Medical University of Vienna
Vienna, Austria

ORCID IDs: 0000-0003-4206-8815 (S.S.); 0000-0003-0485-2692 (I.M.L.).

References

1. Lang IM, Madani M. Update on chronic thromboembolic pulmonary hypertension. *Circulation* 2014;130:508–518.
2. Ende-Verhaar YM, Cannegieter SC, Vonk Noordegraaf A, Delcroix M, Pruszczyk P, Mairuhu AT, *et al.* Incidence of chronic thromboembolic pulmonary hypertension after acute pulmonary embolism: a contemporary view of the published literature. *Eur Respir J* 2017;49:1601792.
3. Lang IM, Simonneau G, Pepke-Zaba JW, Mayer E, Ambrož D, Blanco I, *et al.* Factors associated with diagnosis and operability of chronic thromboembolic pulmonary hypertension. A case-control study. *Thromb Haemost* 2013;110:83–91.
4. Sharma S, Hofbauer TM, Ondracek AS, Chausheva S, Alimohammadi A, Artner T, *et al.* Neutrophil extracellular traps promote fibrous vascular occlusions in chronic thrombosis. *Blood* 2021;137:1104–1116.
5. Manz XD, Szulcek R, Pan X, Symersky P, Dickhoff C, Majolée J, *et al.* Epigenetic modification of the von Willebrand factor promoter drives platelet aggregation on the pulmonary endothelium in chronic thromboembolic pulmonary hypertension. *Am J Respir Crit Care Med* 2022;205:806–818.
6. Mussbacher M, Salzmann M, Brostjan C, Hoesel B, Schoergenhofer C, Datler H, *et al.* Cell type-specific roles of NF-κB linking inflammation and thrombosis. *Front Immunol* 2019;10:85.
7. Ondracek AS, Lang IM. Neutrophil extracellular traps as prognostic markers in COVID-19: a welcome piece to the puzzle. *Arterioscler Thromb Vasc Biol* 2021;41:995–998.
8. Chernysh IN, Nagaswami C, Kosolapova S, Peshkova AD, Cuker A, Cines DB, *et al.* The distinctive structure and composition of arterial and venous thrombi and pulmonary emboli. *Sci Rep* 2020;10:5112.
9. Yaoita N, Shirakawa R, Fukumoto Y, Sugimura K, Miyata S, Miura Y, *et al.* Platelets are highly activated in patients of chronic thromboembolic pulmonary hypertension. *Arterioscler Thromb Vasc Biol* 2014;34:2486–2494.
10. Hell L, Mauracher L, Roiß J, Krall M, Gebhart J, Ay C, *et al.* Increased baseline platelet activation and decreased platelet reactivity in patients with the antiphospholipid syndrome [abstract]. *Res Pract Thromb Haemost* 2020;4.
11. Egermayer P, Peacock AJ. Is pulmonary embolism a common cause of chronic pulmonary hypertension? Limitations of the embolic hypothesis. *Eur Respir J* 2000;15:440–448.
12. Pengo V, Lensing AW, Prins MH, Marchiori A, Davidson BL, Tiozzo F, *et al.*; Thromboembolic Pulmonary Hypertension Study Group. Incidence of chronic thromboembolic pulmonary hypertension after pulmonary embolism. *N Engl J Med* 2004;350:2257–2264.
13. Seferian A, Jaïs X, Savale L, Jevnikar M, Ghigna MR, Weatherald J, *et al.* Klippel-Trenaunay syndrome as a rare cause of chronic thromboemboembolic pulmonary hypertension. *Respir Med Res* 2019;76:48–53.
14. Bonderman D, Turecek PL, Jakowitsch J, Weltermann A, Adlbrecht C, Schneider B, *et al.* High prevalence of elevated clotting factor VIII in chronic thromboembolic pulmonary hypertension. *Thromb Haemost* 2003;90:372–376.

Is There a Role for Using Race-Specific Reference Equations? Yes and No

We know that normal lung function is determined by age, sex, and height, but what is the role of race? Race is considered a socially defined construct and not a biological one (1, 2). One way to assess the contribution of self-reported race or ethnicity to lung function is through statistical modeling. Even though much of the regression error can be accounted for by a variety of anthropomorphic, environmental, nutritional, and socioeconomic factors, small differences in lung function across different racial or ethnic groups remain (3–5), indicating that we need to learn more about the role of race and ethnicity in determining lung function. Another approach is to relate lung function interpreted with and without race-specific equations to important clinical outcomes. This method has suggested that mortality in African Americans is more closely linked to lung function interpreted according to the Third National Health and Nutrition Examination Survey (NHANES-III) White (6) or race nonspecific Global Lung Function Initiative (GLI)-Other (7) reference equations rather than equations specific to African Americans (8–10), questioning the utility of race-specific reference equations.

In this issue of the *Journal*, Baugh and colleagues (pp. 819–829) examine how race influences the association of lung function to chronic obstructive pulmonary disease (COPD) outcomes in African Americans in the SPIROMICS (SubPopulations and InteRmediate Outcome Measures In COPD Study) dataset (11, 12). Lung function

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