Original Research Article

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Pulmonary arterial hypertension patients display normal kinetics of clot formation using thrombelastography

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Abstract

Pulmonary arterial hypertension is characterized by endothelial dysfunction and microthrombi formation. The role of anticoagulation remains controversial, with studies demonstrating inconsistent effects on pulmonary arterial hypertension mortality. Clinical anticoagulation practices are currently heterogeneous, reflecting physician preference. This study uses thrombelastography and hematology markers to evaluate whether clot formation and fibrinolysis are abnormal in pulmonary arterial hypertension patients. Venous blood was collected from healthy volunteers ($n = 20$) and patients with pulmonary arterial hypertension ($n = 20$) on stable medical therapy for thrombelastography analysis. Individual thrombelastography parameters and a calculated coagulation index were used for comparison. In addition, hematologic markers, including fibrinogen, factor VIII activity, von Willebrand factor activity, von Willebrand factor antigen, and alpha2-antiplasmin, were measured in pulmonary arterial hypertension patients and compared to healthy volunteers. Between group differences were analyzed using t tests and linear mixed models, accounting for repeated measures when applicable. Although the degree of fibrinolysis (LY30) was significantly lower in pulmonary arterial hypertension patients compared to healthy volunteers ($0.3\% \pm 0.6$ *versus* $1.3\% \pm 1.1$, $p = 0.04$), all values were within the normal reference range (0–8%). All other thrombelastography parameters were not significantly different between pulmonary arterial hypertension patients and healthy volunteers ($p \geq 0.15$ for all). Similarly, alpha2-antiplasmin activity levels were higher in pulmonary arterial hypertension patients compared to healthy volunteers ($103.7\% \pm 13.6$ *versus* $82.6\% \pm 9.5$, $p < 0.0001$), but all individual values were within the normal range (75–132%). There were no other significant differences in hematologic markers between pulmonary arterial hypertension patients and healthy volunteers ($p \geq 0.07$ for all). Sub-group analysis comparing thrombelastography results in patients treated with or without prostacyclin pathway targeted therapies were also non-significant. In conclusion, treated pulmonary arterial hypertension patients do not demonstrate abnormal clotting kinetics or fibrinolysis by thrombelastography.

Keywords

anticoagulation, thrombosis, fibrinolysis

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Introduction

Pulmonary arterial hypertension (PAH) is a progressive disease characterized by endothelial dysfunction and pathologic vascular remodeling that can lead to the development of right heart failure and death. Despite the development of pulmonary vasodilator therapy, there is still no definitive

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cure that can stop or reverse the progression of disease and the mortality rate remains high. The use of anticoagulation to treat PAH was proposed after pathology studies dating back to the 1960s showed microvascular thrombi in the pulmonary arterioles of patients with both idiopathic and other forms of PAH.[1–3] In 1992, a clinical trial studying the effects of high-dose calcium channel blocker therapy in idiopathic PAH (IPAH) patients showed that the use of concurrent anticoagulant therapy with warfarin improved overall survival, although the effect was primarily apparent in the patients who did not respond to calcium channel blocker treatment.[4] Since then, a number of prospective and retrospective cohort studies, observational studies, and meta-analyses have revealed conflicting results on whether long-term anticoagulation improves mortality in PAH patients.[5–9] The controversy surrounding anticoagulation in PAH patients was recently highlighted by analyses from two large PAH registries. Data from the European-based Comparative, Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension study showed a significant survival benefit in IPAH patients receiving anticoagulation while data from the U.S.-based Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL) cohort did not show a survival benefit for IPAH patients.[5,6] The role of empiric anticoagulation remains controversial and current clinical practice is highly variable, largely depending on physician preference. There is no standardized recommendation for patients with IPAH, heritable PAH, and PAH due to anorexigens.[10–12]

Though abnormal hematologic parameters have been described, traditional blood coagulation tests like prothrombin time (PT) and activated partial thromboplastin time (PTT) have not shown differences between PAH patients and healthy controls.[13–15] Whereas traditional PT and PTT primarily focus on the initiation of coagulation, thrombelastography (TEG) is argued to offer a more comprehensive evaluation of the entire hemostatic process by examining multiple aspects of the kinetics of clot formation.[16,17] Our study uses TEG to evaluate whether the processes of clot formation and fibrinolysis are abnormal in PAH patients. To our knowledge, this is the first use of a viscoelastic coagulation test comparing treated PAH patients to healthy volunteers. In addition, we also examined hematology markers that have been shown to correlate with either thrombotic risk or fibrosis including fibrinogen, factor VIII level, von Willebrand factor (vWF) activity, vWF antigen, and alpha2-antiplasmin. Fibrinogen, factor VIII, and vWF play key roles in coagulation and hemostasis, while alpha2-antiplasmin levels have been correlated with vascular remodeling, thrombosis, and fibrosis.[18–20]

Methods

Patients and healthy volunteers were recruited from February 2018 to February 2021 and enrolled in protocols entitled "Natural History Study of Biomarkers in Pulmonary Arterial Hypertension" (PAH Natural History Study) and "Samples from Human Subjects to Facilitate Basic, Translational and Clinical Research," respectively. Our institutional review board approved these protocols and all participants provided written informed consent.

Patients were diagnosed with PAH according to consensus guideline recommendations, including right heart hemodynamics and the exclusion of other contributing pulmonary hypertension etiologies.[11] PAH was defined as a mean pulmonary artery pressure ≥ 25 mmHg, a pulmonary vascular resistance > 3 Wood units, and either a left ventricular end diastolic pressure ≤ 12 mmHg or pulmonary artery occlusion pressure ≤ 15 mmHg. Consecutive patients enrolled in the PAH Natural History Study were assessed for eligibility ($N = 41$). Subjects were excluded if they had a diagnosis other than PAH ($n = 1$), an acute inflammatory illness ($n = 1$), were receiving an anti-inflammatory therapy known to effect hemostasis ($n = 1$), or currently on anticoagulation ($n = 11$). The indication for anticoagulation was either PAH ($n = 10$) or atrial fibrillation ($n = 1$). Subjects currently on aspirin ($n = 6$) were eligible if therapy could be stopped a week prior to blood collection ($n = 1$). Two eligible subjects were not approached for sample collection (Fig. 1).

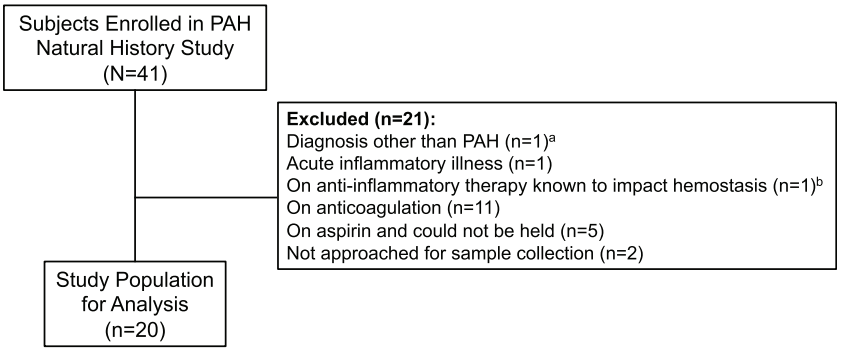

Fig. 1. Study population flow diagram.
[a]Pulmonary venoocclusive disease/pulmonary capillary hemangiomatosis; [b]Subject treated with mycophenolate mofetil and hydroxychloroquine. PAH: pulmonary arterial hypertension.

Current PAH medication regimen and use of supplemental oxygen were recorded at the time of blood sampling in PAH patients. Clinical data necessary for calculating REVEAL 2.0[21] scores were available for all subjects. The majority of the variables for REVEAL were obtained on the same day as TEG and hematologic marker assays. When not available on the same day, the most recent results were used. For all patients, at least 10 variables were used to calculate REVEAL 2.0 scores. Healthy volunteers did not have any history of hemostatic disorders, cardiopulmonary disease, hepatic and renal insufficiency, active malignancy within the past five years, active infections, or other severe or uncontrolled comorbid conditions. Healthy volunteers also had a documented normal hemoglobin and platelet count prior to TEG and hematologic marker assessments. All subjects were instructed to hold nonsteroidal anti-inflammatory drugs for 48 h prior to blood collection.

TEG

During a TEG assay, blood is heated to 37°C and oscillated in a cup with or without the addition of an activating agent to promote coagulation. Formation of a clot is detected by torsion on a pin suspended in the cup, and the magnitude of torsion is used to quantify the speed and strength of clot formation as well as fibrinolysis as the clot breaks down. Venous blood was collected from healthy volunteers ($n = 20$) and patients with PAH ($n = 20$) on stable medical therapy for TEG analysis. Peripheral venous whole blood was collected in a 3.2% buffered sodium citrate tube with a citrate-to-blood ratio of 1:9. A TEG 5000 Hemostasis Analyzer® (Haemonetics Corporation, Braintree, MA, USA) was used to perform the kaolin-activated assay. Each analyzer was maintained and calibrated according to the manufacturers' recommendations and two control samples were run in each machine channel to confirm that the output results matched the expected normal range. After blood collection, 1 mL of whole blood was pipetted into a vial containing 40 µL of kaolin (using a proprietary concentration) and mixed by gentle inversion. $CaCl_2$ (0.2 M, 20 µL) was added to the pre-warmed cup and pin chamber, followed by 340 µL of kaolin-treated blood. All samples were analyzed according to manufacturer protocols. TEG parameters included R (reaction time; time to initiate clot formation, defined by an amplitude of 2 mm), K (kinetic time; additional time after R to reach a clot amplitude of 20 mm), alpha angle (angle between the horizontal axis and a line drawn between R and K on the TEG tracing, representing the speed of fibrin cross-linking), MA (maximum amplitude of clot strength), and LY30 (degree of fibrinolysis at 30 min). Another parameter, G (shear elastic modulus strength), was calculated using the equation provided by the manufacturer

$$G = (5000 * MA/(100 - MA))/1000$$

In addition to comparing individual TEG parameters, for ease of comparison in subgroup analyses, we also calculated a combined score called the coagulation index.[22] The coagulation index was calculated from a linear combination of R, K, MA, and alpha angle, using the equation provided by the manufacturer for kaolin-activated whole blood

$$\begin{aligned} Coagulation\ index = {} & -0.6516(R) - 0.3772(K) \\ & + 0.1224(MA) + 0.0759(alpha) \\ & - 7.7922 \end{aligned}$$

Hematology markers

Clinical Laboratory Improvement Amendments approved assays of fibrinogen, factor VIII activity, vWF activity, vWF antigen, and alpha2-antiplasmin were performed by the Department of Laboratory Medicine at our institution.

Statistical analysis

Demographic variables were compared between PAH patients and healthy volunteers using t tests for continuous variables, and Chi-squared tests or Fisher exact tests for categorical variables. T tests were used to compare TEG parameters and hematology markers between two groups. Normality assumption was checked by visually inspecting histograms, boxplots, and Q–Q plots for each group. As the study took place over multiple years, different TEG analyzers and technicians were utilized to measure TEG results in healthy volunteers. We measured nine samples in duplicates across analyzers and technicians. To assess and adjust for rater effects when analyzing TEG parameters, we used linear mixed models to account for repeated measures within each subject using a random subject effect, and a fixed rater effect. Standard residual diagnostics were used to check model assumptions. Pearson correlation was calculated between TEG parameters and REVEAL 2.0 scores, and between hematology markers and coagulation index. SAS version 9.4 (Cary, NC) was used for all analyses. All p-values were two-tailed and considered significant if $p \leq 0.05$. Figures were created in GraphPad Prism 9.0.1 and data are presented as mean ± standard deviation (SD).

Results

Demographics and patient characteristics

Demographic variables were not significantly different between healthy volunteers and PAH patients (Table 1). Likewise, baseline mean (±SD) hemoglobin (13.0 ± 1.1 versus 13.3 ± 2.4 g/dL, for healthy volunteers and PAH patients, respectively; $p = 0.62$) and platelet count (248.1 ± 54.2 versus $227.1 \pm 62.0 \times 10^3$/µL, respectively; $p = 0.26$) were also similar between the two groups. Median

Table 1. Demographics of healthy volunteers and PAH patients.

	Healthy volunteers	PAH patients	*p*-Values
Age, years (mean ± SD)	45.8 ± 9.0	49.7 ± 16.0	0.35
Female, *n* (%)	19/20 (95%)	20/20 (100%)	1.00
Race or ethnic group, *n* (%)			0.60
Non-Hispanic white	11/20 (55%)	11/20 (55%)	
Non-Hispanic black	5/20 (25%)	5/20 (25%)	
Hispanic	0/20 (0%)	2/20 (10%)	
Asian	4/20 (20%)	2/20 (10%)	

PAH: pulmonary arterial hypertension.

Table 2. Characteristics of PAH patients.

PAH etiology, *n* (%)	
IPAH	12/20 (60%)
CHD-PAH	4/20 (20%)
CTD-PAH	3/20 (15%)
Drug-induced PAH[a]	1/20 (5%)
Functional status	
NYHA/WHO class, *n* (%)	
I	5/20 (25%)
II	9/20 (45%)
III	6/20 (30%)
6MWD, meters (mean ± SD)	467.2 ± 132.8
REVEAL 2.0 score (mean ± SD)	4.5 ± 2.5
PAH therapy,[b] *n* (%)	
Prostacyclin infusion[c]	4/20 (20%)
Oral prostacyclin or prostacyclin receptor agonist	3/20 (15%)
Phosphodiesterase type 5 inhibitor	15/20 (75%)
Endothelin receptor antagonist	18/20 (90%)
Soluble guanylate cyclase stimulator	3/20 (15%)
Calcium channel blocker	3/20 (15%)
Diuretic(s)	12/20 (60%)
Oxygen use, *n* (%)	
Continuous	3/20 (15%)
Intermittent	4/20 (20%)
None	13/20 (65%)

[a]Drug exposures included methamphetamine, anorexigen, cocaine, and interferon.
[b]Two patients were on monotherapy, one on subcutaneous treprostinil, and the other on a calcium channel blocker.
[c]Includes intravenous and subcutaneous prostacyclin.
PAH: pulmonary arterial hypertension; IPAH: idiopathic PAH; CHD-PAH: congenital heart disease associated PAH; CTD-PAH: connective tissue disease associated PAH; NYHA: New York Heart Association; WHO: World Health Organization; 6MWD: six-minute walk distance; REVEAL: Registry to Evaluate Early and Long-Term PAH Disease Management.

(interquartile range) time between complete blood count and TEG was 0 (0–0) days for PAH patients and 60.5 (6.25–145.8) days for healthy volunteers.

The majority (60%) of patients were diagnosed with IPAH while PAH due to congenital heart disease (CHD-PAH) was the second most common etiology (Table 2), followed by PAH due to connective tissue disease and drug-induced PAH. The majority of PAH patients were NYHA/WHO functional class II (45%) or III (30%) and on combination pulmonary vasodilator therapy (90%).

TEG comparing PAH patients and healthy volunteers

Except for the degree of fibrinolysis (LY30), individual TEG parameters were similar in both PAH patients and healthy volunteers after adjusting for rater effect (Fig. 2). Notably, even though LY30 values were lower in PAH patients (i.e. less fibrinolysis) compared to controls, all of the individual patient values were within the normal reference range (Table 3). Likewise, the mean value of each of the other TEG parameters in the PAH cohort were also within the normal range (Table 3).

The manufacturer's suggested reference range for the coagulation index is –3 to +3, where a higher value corresponds to a more hypercoagulable state. Consistent with the comparisons for each individual parameter, the coagulation index was also not significantly different in PAH patients compared to healthy controls ($p = 0.48$ after adjusting for rater effects; e-Figure 1).

TEG subgroup analysis

Next, we investigated whether TEG results were influenced by prostacyclin pathway-directed treatment, since these therapies are known to inhibit platelet aggregation.[13,23] Despite potential antiplatelet effects, TEG profiles were similar among PAH patients treated with either prostacyclin analogs or prostacyclin receptor agonists compared to those patients not receiving prostacyclin pathway-directed treatment (Fig. 3 and e-Figure 2). Finally, there was no significant correlation between TEG parameters and risk of death as determined by REVEAL 2.0 scores ($p > 0.20$ for all).

Hematology markers comparing PAH patients and healthy controls

Each of the five hematologic markers of interest were measured concurrently with TEG in healthy volunteers and PAH patients. Although all results were within the normal reference range, alpha2-antiplasmin activity levels were significantly higher in PAH patients compared to healthy volunteers ($p < 0.0001$). There was also a trend toward higher fibrinogen ($p = 0.07$), vWF activity ($p = 0.10$), and vWF antigen ($p = 0.13$) levels in PAH patients compared to healthy volunteers (Fig. 4 and Table 4).

Correlation between hematology markers and TEG

There was a significant positive correlation between the coagulation index and factor VIII activity, vWF activity, and vWF antigen. Factor VIII and vWF antigen had the strongest correlation with the coagulation index ($R = 0.42$ and 0.51, respectively; Table 5). These results are consistent with the role these factors play in the coagulation process and their expected effect on TEG.

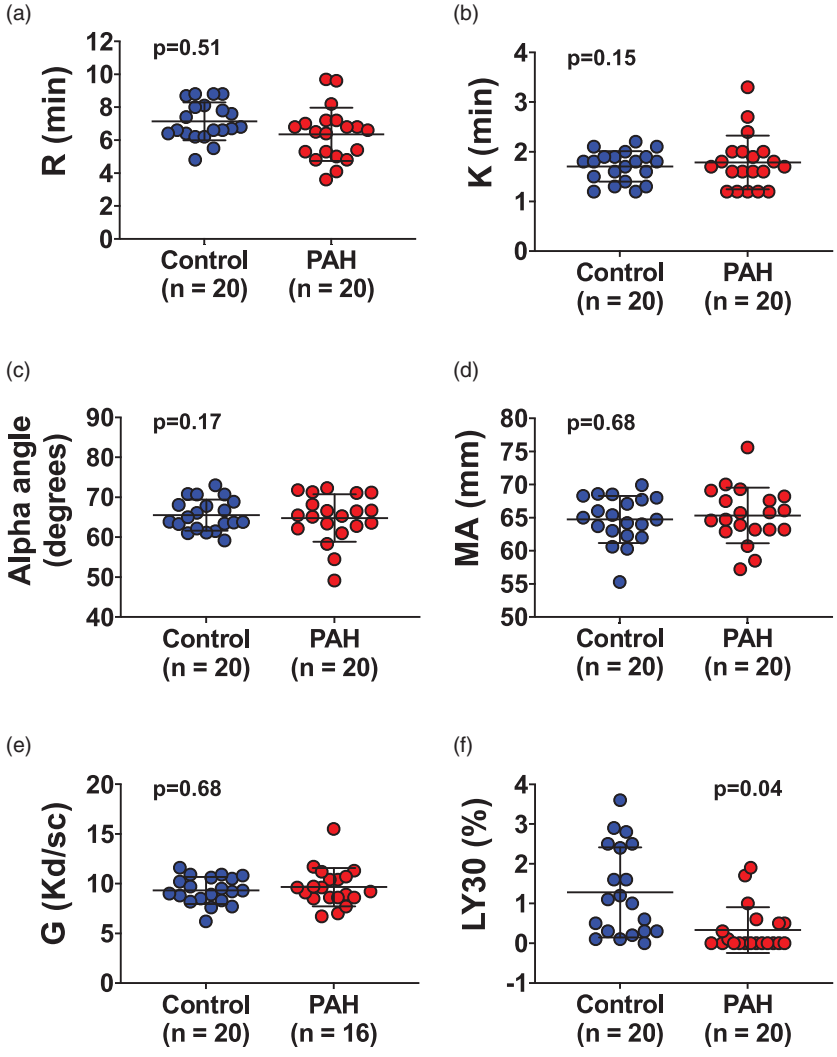

Fig. 2. Individual TEG parameters in PAH patients and healthy controls. Plots show the distribution for each parameter: (a) R, (b) K, (c) alpha angle, (d) MA, (e) G, and (f) LY30. Individual data points and mean ± SD are shown. *p*-Values were adjusted to account for rater effects. PAH: pulmonary arterial hypertension; R: reaction time; K: kinetic time; MA: maximum amplitude; G: shear elastic modulus strength; LY30: degree of fibrinolysis at 30 min.

Table 3. TEG parameters (mean ± SD).

Reference	R (min) 5–10	K (min) 1–3	Alpha (deg) 53–72	MA (mm) 50–70	G (Kd/sc) 4.5–11	LY30 (%) 0–8
Control ($n = 20$)	7.1 ± 1.2	1.7 ± 0.3	65.6 ± 3.9	64.7 ± 3.6	9.3 ± 1.4	1.3 ± 1.1
PAH ($n = 20$)	6.4 ± 1.6	1.8 ± 0.5	64.8 ± 5.9	65.4 ± 4.2	9.7 ± 1.9	0.3 ± 0.6

PAH: pulmonary arterial hypertension; R: reaction time; K: kinetic time; alpha: alpha angle; MA: maximum amplitude; G: shear elastic modulus strength; LY30: degree of fibrinolysis at 30 min.

Discussion

To our knowledge, this study represents the first evaluation of TEG in patients with PAH on current pulmonary vasodilator therapy. Degree of fibrinolysis (LY30) and alpha2-antiplasmin activity were significantly different between PAH patients and healthy volunteers, yet the results for each PAH patient fell within the normal reference range of the two assays. We did not detect significant alterations in any of the other individual TEG parameters, the calculated coagulation index, or the remaining hematologic markers in PAH patients compared to healthy volunteers. Furthermore, we did not detect any association of these coagulation studies with prostacyclin pathway-directed therapy or disease severity.

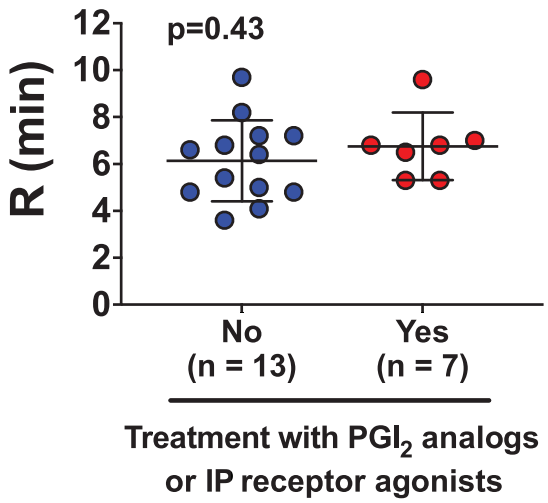

Fig. 3. Reaction time (R) is not significantly different between PAH patients that were treated with prostacyclin (PGI_2) analogs or prostacyclin receptor (IP receptor) agonists compared to patients who were not. Reaction time, the time to initiate clot formation, and therefore the parameter most relevant to anticoagulation with warfarin, is shown for simplicity while comparisons across the other TEG parameters are included in the Supplement (e-Figure 2). Individual data points and mean ± SD are shown.

A recent study evaluated coagulation and fibrinolysis in newly diagnosed PAH patients prior to treatment initiation with rotational thromboelastometry (ROTEM), a viscoelastic assay similar to TEG.[14] In contrast to our findings, these authors found abnormalities in the clotting time and clot formation time using a non-activated thromboelastometry (NATEM) assay, a ROTEM assay that uses whole blood without an agonist. The authors concluded that their constellation of findings were consistent with diminished initiation of clot formation and propagation in untreated PAH patients. Unfortunately, NATEM is not a widely used ROTEM modality, given substantial readout variability owing to differences in sample storage and processing. Without independently confirming these results using better validated and more reliable ROTEM assays, it is difficult to draw any definitive conclusions. There are a few important differences between our study and the NATEM study. The PAH patients in the NATEM assay study had lower platelet counts compared to the healthy volunteers, which might prolong clot initiation.[24] In addition, our study assessed PAH patients on pulmonary vasodilator therapy, which may mitigate coagulation abnormalities present in newly diagnosed, untreated patients. This possibility is consistent with a study conducted prior to the availability of modern pulmonary vasodilator therapy, which demonstrated a survival benefit of warfarin primarily in patients not responding to calcium channel blocker therapy.[4] Finally, the present study used a kaolin contact activator. In clinical and research contexts, native ROTEM and native TEG are rarely performed due to their high variability and limited interpretation. We did assess native TEG (non-activated whole blood) in a subset of subjects in our cohort; however, as expected, the results were highly variable. Furthermore, a number of healthy controls had abnormal native TEG results (e-Table 1), consistent with previous descriptions regarding the lack of reliability for non-activated viscoelastic assays.[25]

A number of studies from the 1990s reported abnormal levels of hematology markers in PAH patients, including increased plasma fibrinogen, factor VIII, and vWF antigen, some of which were found to normalize after patients received prostacyclin therapy.[23,26–28] While endothelial dysfunction is a central feature to PAH pathobiology that may contribute to both in situ[29] and systemic thromboembolism,[30] the PAH patients included in this study did not have a history of venous or arterial thromboembolism. Interestingly, while all patients in the current study were treated, 90% were on combination PAH therapy which may mitigate an underlying pro-thrombotic tendency due to endothelial dysfunction. In our cohort of PAH patients, only alpha2-antiplasmin levels were significantly different compared to controls. Though fibrinogen, vWF activity, and vWF antigen trended toward higher levels in PAH patients, the majority of patients had levels of these markers that were within the normal reference range, including alpha2-antiplasmin where all patient results fell within the normal range. Therefore, our data support that appropriate medical therapy may have a corrective effect on coagulation abnormalities in PAH patients. Nonetheless, higher alpha2-antiplasmin levels in PAH patients is notable given its role in vascular injury and remodeling.[31] Alpha2-antiplasmin expression is regulated by stress kinase pathways, and in particular ERK1/2 activation, which we have demonstrated as an important downstream, noncanonical effect of BMPR2 loss-of-function in pulmonary artery endothelial cells.[32] Furthermore, a recent study observed a significant association between apolipoprotein A-2-rich HDL-4 (Apo A-2 HDL-4) levels in PAH patients and survival.[33] In that study, alpha2-antiplasmin was among the nine proteins significantly associated with plasma Apo A-2 HDL-4 concentrations in PAH patients out of over 1100 circulating proteins analyzed, suggesting that alpha2-antiplasmin may play a role in PAH pathobiology.

One limitation of the present study is the small sample size. Additionally, TEG is less widely used clinically with applications generally limited to the trauma, surgical, and critical care settings. As such, there are no standardized thresholds for what would signify a clinically significant change for each parameter, especially in patients with PAH.[17] However, mean values for each individual TEG parameter in the PAH patients fell within normal manufacturer reference ranges, suggesting broad similarity between PAH patients and healthy volunteers. We are also limited in making conclusions for PAH patients at intermediate or high risk of death, since the majority of patients included had REVEAL 2.0 scores that placed them in a low-risk

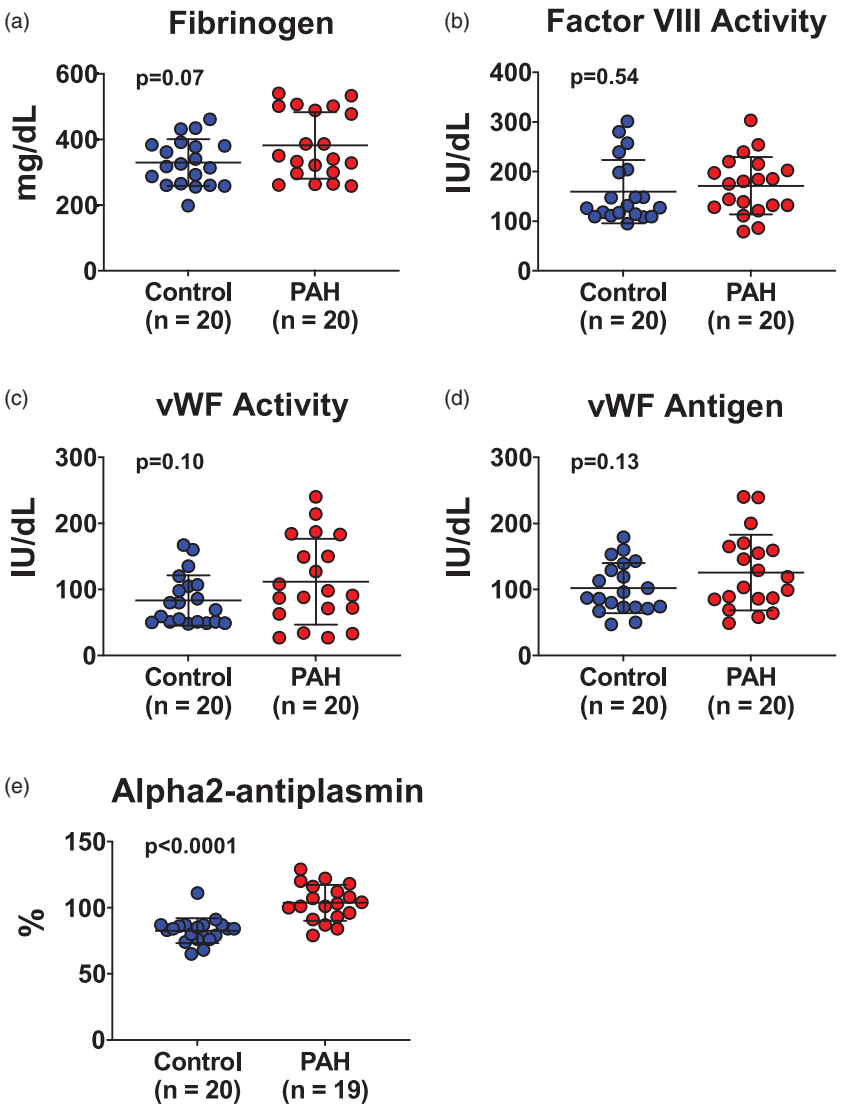

Fig. 4. Hematology markers in PAH patients and healthy controls. Plots show the distribution for each hematology marker: (a) fibrinogen, (b) factor VIII activity, (c) vWF activity, (d) vWF antigen, and (e) alpha2-antiplasmin. Data are presented as mean ± SD.
vWF: von Willebrand factor; PAH: pulmonary arterial hypertension.

Table 4. Hematology markers (mean ± SD).

Reference	Fibrinogen 177–466 mg/dL	Factor VIII activity 41–184 IU/dL	vWF activity 52–156 IU/dL	vWF antigen 50–197 IU/dL	Alpha2-antiplasmin 75–132%
Control ($n = 20$)	329.4 ± 71.3	159.4 ± 63.7	83.5 ± 38.1	102.1 ± 38.0	82.6 ± 9.5
PAH ($n = 20$)	381.6 ± 101.5	171.3 ± 57.9	111.7 ± 64.9	125.6 ± 57.2	103.7 ± 13.6[a]

[a]Results for alpha2-antiplasmin are from 19 subjects.
vWF: von Willebrand factor; PAH: pulmonary arterial hypertension.

Table 5. Pearson correlation between coagulation index and hematology markers.

	Fibrinogen	Factor VIII activity	vWF activity	vWF antigen	Alpha2-antiplasmin
R	0.22	0.42	0.40	0.51	–0.03
p-value	0.18	0.007	0.01	0.0008	0.85

R: correlation coefficient; vWF: von Willebrand factor.

category.[21] Likewise, our results may not apply to NYHA/WHO functional class IV patients since there were none in our cohort. Additionally, because recruitment took place over three years, multiple TEG machines were used for analysis. All machines used were the same model (TEG 5000 Hemostasis Analyzer), but in two different laboratory departments by more than one trained technician. These factors were taken into consideration during statistical analyses based on duplicate testing done concurrently in both locations. Finally, it is important to recognize the inherent limitations of TEG testing, which measures clot formation in a static environment, which can be especially useful in the surgical or trauma setting when a patient may experience similar disruptions in blood flow.[34] In an ambulatory setting, TEG may not fully represent the in vivo hemostatic clotting environment in subjects with dynamic blood flow. That being said, flow may be less dynamic in the narrowed pulmonary vessels of PAH patients. Despite these limitations, when we compare blood samples from PAH patients to healthy volunteers under the same static conditions, we are able to gain useful insights into multiple phases of the coagulation process, including the enzymatic, polymerization, and thrombolysis phases of clot formation. As none of the TEG parameters were significantly different from healthy volunteers, PAH patients do not appear to have altered thrombin generation, fibrin cross-linking, or platelet strength.

The use of empiric anticoagulation is an important and controversial consideration in the modern care of PAH patients. Anticoagulation comes with obvious risks, including adverse bleeding and drug–drug interactions, which complicate its widespread use. Thus, studies which aim to elucidate the biologic plausibility of its benefit in PAH can enable clinicians to better interpret the conflicting results of observational studies.[6,8] Our study demonstrates that PAH patients on stable medical therapy do not have abnormalities of clotting kinetics, fibrinolysis, or in key markers related to coagulation, supporting that anticoagulation may not be required. However, the true impact of anticoagulation on PAH mortality remains to be definitively defined by further prospective randomized controlled clinical trials.

PAH patients, particularly those who have achieved a low-risk status on stable medical therapy, do not demonstrate abnormal clotting kinetics or fibrinolysis by TEG. Additionally, these patients do not demonstrate abnormal levels of hematologic markers associated with thrombosis and fibrosis. These results support exercising caution when using anticoagulation in PAH patients in the absence of other standard indications such as venous thromboembolism or atrial fibrillation.

Author contributions

M.A.S. and J.M.E. conceived and designed research. G.M.G., B. H., K.T., and C.F.B. recruited and enrolled study subjects. M.L., K.P.B., A.C., C.H., and A.D.-F. performed thrombelastography testing and reviewed results. M.L., H.F.C., and J.K. collected and organized patient demographic information. M.L., J.S., J.M.E., and M.A.S. interpreted results. M.L., J.S., and J.M.E. prepared figures. M.L. drafted the manuscript. M.L., K.P.B., J.S., S.B.B., J. M.E., and M.A.S. edited and revised the manuscript. M.L., K.P. B., A.C., C.H., A.D.-F., J.S., H.F.C., G.M.G., B.H., K.T., J.K., C. F.B., S.B.B., J.M.E., and M.A.S. approved final version of manuscript.

Ethical approval

The NIH National Heart, Lung, and Blood Institute institutional review board (Intramural NHLBI IRB) approved these protocols (Natural History Study: 13-CC-0012. Human Sample Collection Protocol: 17-CC-0148) and all participants provided written informed consent.

Conflict of interest

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The NIH Clinical Center PAH Program received support from Aadi Bioscience through a Cooperative Research and Development Agreement. The authors have no personal financial relationship with Aadi Bioscience or any other entity.

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Guarantor statement

M.A.S. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Supplemental material

Supplemental material for this article is available online.

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