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Angiopoietin-2 and D-dimer add prognostic information to clinical risk in pulmonary arterial hypertension

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BACKGROUND: Thrombosis and endothelial injury are pathologic hallmarks of pulmonary arterial hypertension (PAH). We aimed to evaluate whether markers of endothelial dysfunction and coagulation in the blood would provide insight into disease activity, treatment response, and outcomes in PAH.
METHODS: We prospectively collected baseline and 3-month follow-up blood samples from treatment-naïve patients with PAH ($n = 22$) and those who had a clinical indication to intensify therapy ($n = 19$). In addition, we recruited 12 healthy people and clinically stable patients with PAH ($n = 45$) as controls who had 2 blood samples collected twice within 14 days. We generated platelet-free plasma and measured D-dimer, angiopoietin-2, thrombin time, soluble P-selectin, von Willebrand factor, and vascular endothelial growth factor. We assessed treatment response with Reveal Lite 2 scores (all patients had N-terminal-pro-brain natriuretic peptide, 6-minute walk, and functional class assessment at both visits) and followed clinical outcomes for 3 years.
RESULTS: Angiopoietin-2 levels were elevated and fell in response to effective therapy (drop in Reveal Lite 2 score). At follow-up, persistently elevated angiopoietin-2 levels predicted clinical events and even identified low-risk participants who subsequently had events. D-dimer levels were also elevated in patients with PAH but did not change in response to therapy. Several other abnormalities in endothelial and platelet activation were identified (including elevated soluble P-selectin, elevated von Willebrand factor, and elevated vascular endothelial growth factor) but these did not change with treatment or predict outcome.
CONCLUSIONS: Angiopoietin-2 and D-dimer are elevated in patients with PAH and may add prognostic information to routine clinical assessment.
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Background

Pulmonary arterial hypertension (PAH) is a rare, enigmatic lung vasculopathy that frequently progresses to right ventricular failure despite contemporary therapy. The pathogenesis is poorly understood but inflammation, endothelial damage, and thrombosis are characteristic vascular features at autopsy or transplant.[1,2] Currently, clinicians monitor treatment response using right ventricular imaging and clinical risk scores (which incorporate N-terminal-pro-brain natriuretic peptide [NT-proBNP], 6-minute walk distance, and functional class).[3] There is growing evidence that a "liquid biopsy" of disease activity could further characterize patients with PAH and help predict outcomes and treatment response.[4-6] Abnormal coagulation parameters have previously been noted in patients with PAH, including increased procoagulant tissue factor expression (circulating[7] and lung[8]), elevation of fibrinogen and fibrin degradation products,[9] endothelial activation markers,[7] and abnormal platelet function.[10] These biologic observations and clinical data[11] collectively led to guideline recommendations for warfarin anticoagulation as a treatment strategy; subsequent data highlighted a larger than expected bleeding risk, and anticoagulation is no longer routinely recommended.[12,13] It is unknown how coagulation and endothelial activation parameters compare in the era of combination therapy and how they change over time.

In this study, we measured markers of coagulation and endothelial activation in a contemporary cohort of group 1 patients with PAH. We hypothesized that a subgroup of patients with PAH would have ongoing evidence of increased coagulation, fibrinolysis, and/or endothelial activation despite approved therapy and abnormal parameters would be associated with worse clinical outcomes. We also correlated our measures with treatment response as assessed by Reveal Lite 2.

Materials and methods

Patient selection and consent

This was a single-center observational study with approval from the Institutional Review Board of University of Rochester Medical Center; all participants had provided written informed consent before the first blood draw. We recruited patients with PAH (by 2018 criteria)[14] between 2016 and 2019 from our PHA-accredited clinic. Demographics and clinical information were collected at each study visit which was paired with a 6-minute walk, New York Heart Association classification, NT-proBNP, and Reveal Lite 2.0 score. Patients were initially categorized as either (1) treatment-naïve, (2) treated and clinically stable (or maximally treated, including parenteral therapy), or (3) treated but requiring treatment intensification. Stable refers mainly to the clinician's assessment and plan for treatment change on the day of first blood draw, but none of the stable participants changed in the brief time between blood draws; 14 were on parenteral therapy. Healthy controls (HC) were also recruited during the same time period.

Sample collection

Peripheral blood samples were collected at enrollment (baseline) and then again within 14 days (for HC and those stable on therapy) or at 3 months (if treatment naïve or requiring treatment intensification). The purpose of collecting blood twice in HC and stable PAH was to establish reproducibility in the measures themselves before drawing conclusions about the magnitude of change after initiating or escalating treatment. We collected blood in ethylenediaminetetraacetic acid and sodium citrate tubes (to facilitate different assays that require 1 or the other), spun the blood according to International Society of Thrombosis and Hemostasis guidelines to obtain platelet-free plasma (PFP),[15] and stored PFP at −80°C. Processing occurred within 2 hours of collection for all samples.

Coagulation assays

D-dimer, von Willebrand factor (vWF) antigen, and thrombin time (TT) were batch measured in citrated PFP using the clinical grade Stago STA Compact Max ES analyzer and reagents. The proprietary reagents were used according to the manufacturer. We defined "normal" levels as those within the lower 90%-ile of the healthy group (for D-dimer, ≤0.4 mcg/ml fibrinogen equivalence units).

Endothelial injury and activation assays

Q-plex Human Array kits (4-plex) customized for the detection of angiopoietin-2 (Ang-2), soluble P-selectin (sP-selectin), vascular endothelial growth factor (VEGF), and soluble vascular cell adhesion molecule-1 (VCAM-1) (sVCAM-1) were purchased from Quansys Biosciences (Logan, UT) and used according to the manufacturer. To optimize assay performance (i.e., each analyte measured within linear portion of the standard curve), we first tested samples at dilutions of 1:2, 1:20, and 1:200 (Quansys recommendations). Plate results were reviewed with Quansys support to verify assay performance and determine subsequent dilutions. Following assay optimization, each sample was tested at 2 dilutions (1:2 and 1:4) in duplicate; each patient value (at each time point) is thus constructed from 4 measures. Plates were read using the Q-View Imager LS and standard curves were constructed using Q-View Software. We defined "normal" levels as those within the lower 90%-ile of the healthy group (for Ang-2, ≤865.6 pg/ml).

Outcomes

On follow-up blood draw, treatment response was defined as any decrease in Reveal 2.0 Lite score.[16] Participants with an initial Reveal 2.0 Lite score of ≤5 were excluded from treatment response analysis as it is not clear that one is "improved" below 5. Clinical worsening was defined as PAH hospitalization, lung transplantation, or death. Data were collected from the electronic medical record up to 3 years after enrollment; all participants remained active patients in our program and hospitalizations were known even when outside our

system. A PAH clinician expert (D.L., while blind to the biology data) reviewed the hospitalization data for each participant to determine if the hospitalization was attributable to PAH (i.e., heart failure, respiratory failure, etc.).

Statistics

Data were analyzed using Prism 9 GraphPad. Continuous variables are reported as median with interquartile range. Categorical data are reported as count with percent. Wilcoxon matched pairs signed rank test, Mann-Whitney test, and Kruskal-Wallis were used for testing between groups; we chose nonparametric testing given the heterogeneous data and relatively small sample sizes. When comparing PAH to HC, the initial sample was included for all participants. Some patients may not have had enough blood for a given assay (or the assay failed quality control); patients with missing data at one time point were excluded from the treatment response paired analysis (N should be 41 but may have been less because of missing samples). Bland-Altman plot was used to assess the variability of measures in stable participants and HC. Clinical event data were analyzed by Kaplan-Meier curves and log-rank test based on the results of the second blood draw (after starting or escalating treatment for those whose treatment changed). We used Cox regression to create univariate and multivariate analysis in a model including age, sex, follow-up Reveal Lite 2, and follow-up Ang-2 levels. We did not correct any of our reported statistics for the number of measures that we made.

Results

Characteristics of pulmonary arterial hypertension cohort

Baseline patient characteristics are shown in Table 1. Eighty-six patients with PAH had blood samples taken: 22 treatment naïve, 19 treatment intensification, and 45 stable patients. The population was representative of a PAH cohort with 71% female, 52% idiopathic or heritable PAH, and 32% connective-tissue associated disease PAH. The HC population was predominantly female but younger than our PAH population (30 vs 61 years). Platelet counts were within the normal range; 36% were on a platelet inhibitor (mostly 81 mg aspirin). There was a mix of Reveal Lite 2 strata at baseline for the entire group. For those intensifying therapy, Table 2 shows core clinical assessments before and after intensification as well as invasive hemodynamic data before therapy change.

Table 1 Baseline Patient Characteristics

	PAH (n = 86)	Healthy control (n = 12)
Age, years	61 (51, 68)	30 (30, 46)
Female (%)	61 (71)	8 (67)
BMI, kg/m^2	29 (25, 34)	
PAH type (%)		
Idiopathic	45 (52)	
Connective tissue disease	28 (33)	
Other[a]	13 (15)	
NT-proBNP, ng/liter	507 (164, 3,125)	
Functional class, I/II/III (%)	10 (12)/ 38 (44)/ 38 (44)	
6MWD, m	357 (219, 434)	
PAH therapies (%)		
None	22 (26)	
PDE5I/ERA	30 (35)	
Background + prostacyclin[b]	34 (40)	
Reveal Lite 2 Strata (%)		
Low risk	32 (37)	
Intermediate risk	16 (19)	
High risk	38 (44)	
Anticoagulation (%)	13 (15)	
Therapies (%)		
Adding therapy	41	
Stable	45	

Abbreviations: 6MWD, 6 minute walk distance; BMI, body mass index; ERA, endothelin receptor antagonist; NT-proBNP, N-terminal-pro-brain natriuretic peptide; PAH, pulmonary arterial hypertension; PDE5i, phosphodiesterase 5-inhibitor.

Data presented as median with interquartile range or count with percentage.

[a]Includes congenital heart disease, portopulmonary, HIV-related pulmonary hypertension.

[b]Usually triple therapy with oral or parenteral prostacyclin; rarely on prostacyclin monotherapy.

Angiopoietin-2 is elevated in patients with PAH, drops with treatment response, and correlates with survival

We observed that patients with PAH had elevated Ang-2 levels in plasma compared to controls (Figure 1A). The elevation was similar among patients with idiopathic PAH and connective tissue disease-pulmonary arterial hypertension (CTD-PAH) (Figure S1A). Participants who started or intensified therapy and had clinical improvement (drop in Reveal Lite 2) had a decrease in Ang-2 at follow-up (Figure 1B). Participants with elevated Ang-2 levels at the second blood draw (after therapy intensification, if therapy had changed) had significantly decreased event-free survival over 3 years (Figure 1C). We then investigated if Ang-2 would stratify patients deemed low risk by Reveal Lite 2 scoring; low-risk patients (Reveal Lite 2 ≤5) with high Ang-2 levels appeared to have worse event-free survival than low-risk patients with low Ang-2 levels (Figure 1D). Table 3 reinforces the heterogeneity in Ang-2 and D-dimer levels among patients with low- and intermediate-risk Reveal Lite 2 scores. While there were no high-risk patients with low D-dimer and low Ang-2, there were some low-risk patients who had elevation in one or both of these biomarkers. To strengthen the association between Ang-2 and outcomes, we conducted a multivariate Cox regression including age, sex, Reveal Lite 2, and Ang-2 levels dichotomized as normal or high ("high" > 90%-ile of healthy). Table S1 shows that the best model (highest concordance index, 0.78) was fit with follow-up Reveal Lite 2 score and the categorization of Ang-2 as high/low (also at

Table 2 Baseline and Follow-up Assessment in Treatment Naïve and Intensification Patients

	Baseline (n = 41)	3-Month follow-up (n = 41)
Right heart catheterization[a]		
Right atrium, mm Hg	8 (6, 13)	-
Mean pulmonary artery pressure, mm Hg	47 (42, 52)	-
Pulmonary capillary wedge pressure, mm Hg	10 (8, 12)	-
Cardiac index, liter/min/m^2	1.8 (1.6, 2.1)	-
Pulmonary vascular resistance, Woods Units	11 (8, 14)	-
NT-proBNP, ng/liter	2,451 (404, 5,571)	501 (216, 1,267)
6-minute walk distance, m	280 (145, 386)	344 (274, 443)
Reveal Lite 2	10 (7, 13)	7 (5, 12)

Abbreviation: NT-proBNP, N-terminal-pro-brain natriuretic peptide.
Data presented as median with interquartile range.
[a]Right heart catheterization was obtained as a standard of care procedure at baseline within 21 days of the blood sampling for n = 41 participants.

follow-up); heart rate was 7.7 [cardiac index, 1.8-32.9] for an elevated Ang-2, p = 0.005.

VEGF and vWF levels are elevated in patients with PAH

Two other markers of endothelial injury, VEGF and vWF antigen, were elevated in patients with PAH compared to HC (Figure 2A, Figure S2A). In patients who started new or additional therapy, lower baseline VEGF levels may have predicted a favorable treatment response (Figure 2B; participants with lower baseline VEGF were more likely to have a drop in Reveal score), but VEGF did not appear to change with treatment. vWF was not associated with treatment response and neither VEGF nor vWF predicted event-free survival at 3 years (Figure 2C, Figure S2C).

D-dimer is elevated in patients with PAH compared to healthy controls

We found that patients with PAH had elevated levels of D-dimer compared to HC (Figure 3A). For patients with PAH

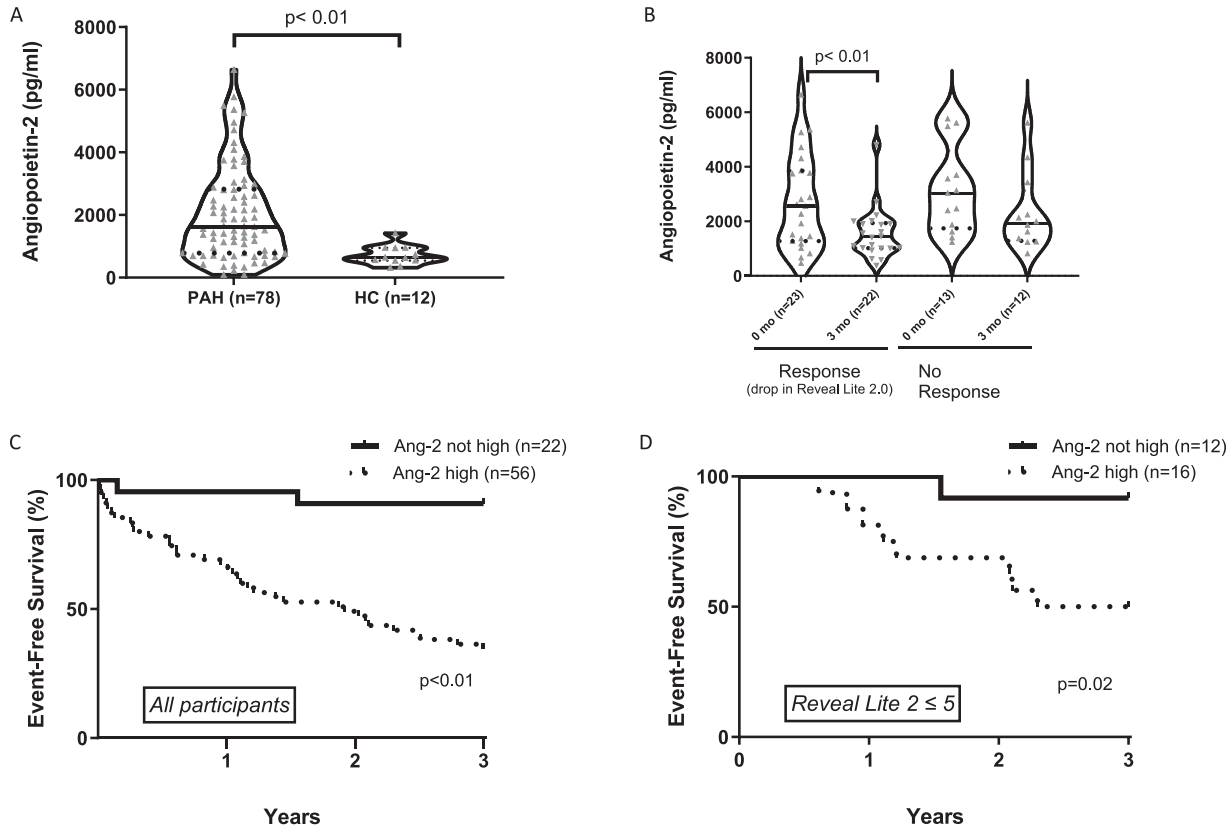

Figure 1 Ang-2 is elevated in patients with PAH, responds to treatment, and may predict outcomes. (A) Ang-2 levels in patients with PAH vs healthy controls. (B) Ang-2 drops after adding therapy for those who had improvement in risk score. (C) Event-free survival in patients with PAH (whether or not intensifying treatment) with high Ang-2 (> 90%-ile of healthy) vs normal Ang-2 levels. (D) In participants with follow-up Reveal Lite 2 ≤5, those with elevated Ang-2 levels had worse event-free survival. Ang-2, angiopoietin-2; PAH, pulmonary arterial hypertension.

Table 3 Elevated Ang-2 and D-dimer in Low and Intermediate-risk Patients

Reveal 2.0 Lite Score	Ang-2 high, D-dimer high	Ang-2 high, D-dimer nl	Ang-2 nl, D-dimer high	Ang-2 nl D-dimer nl
≤ 5	8	8	3	8
6	0	2	2	0
7	7	2	1	1
≥ 8	19	5	5	0

Abbreviation: Ang-2, angiopoietin-2.
Ang-2 and D-dimer values were dichotomized as "high" (> 90-%ile for healthy controls) or not high; cut-off values for "not high" were Ang-2 ≤865.6 pg/ml and D-dimer ≤0.4 mcg/ml fibrinogen equivalence units.
'Green' is low risk and shows the unexpectedly large number of abnormalities. The 'yellow' is intermediate risk.

who were initiating or intensifying treatment, a lower baseline D-dimer suggested an increased likelihood for favorable change (decrease) in Reveal 2.0 Lite score (Figure 3B). Participants with elevated D-dimer trended toward decreased event-free survival rates (Figure 3C). Bland-Altman analysis showed that elevated D-dimer was reproducible when repeated in HC and stable patients with PAH (Figure S3A). Considering Ang-2 and D-dimer together, we observed that patients with high Ang-2 and D-dimer (at the time of the second blood draw) had significantly lower event-free survival, and patients with lower Ang-2 had the highest event-free survival (Figure 3D). Considering the small numbers, it appeared that D-dimer added information only in those with elevated Ang-2.

Thrombin time

We found that thrombin time was highly variable in patients with PAH compared to controls (the median was skewed toward shorter times, but some had considerably longer thrombin times; Figure 4A). Thrombin time did not predict treatment response, did not change with treatment, and was not associated with event-free survival (data not shown).

sP-selectin and sVCAM-1 are elevated in patients with PAH but do not correlate with survival or treatment response

sP-selectin (released from endothelial cells and platelets) and sVCAM-1 levels were significantly elevated in patients with PAH (Figure 4B and C). Levels of these markers did not change significantly in patients after treatment initiation/intensification and did not predict event-free survival (data not shown).

Discussion

In this single-center prospective cohort, we found that Ang-2, and to a lesser extent, D-dimer, had prognostic value for hospitalization-free survival. Ang-2 may have prognostic value for significant clinical events even among those who had low risk for mortality as estimated by Reveal Lite 2; furthermore, Ang-2 levels tended to drop with effective treatment, further suggesting that they reflect something relevant about the disease process. Previous studies have demonstrated abnormal coagulation profiles in PAH,[9,17] and both classic and recent pathologic studies have shown

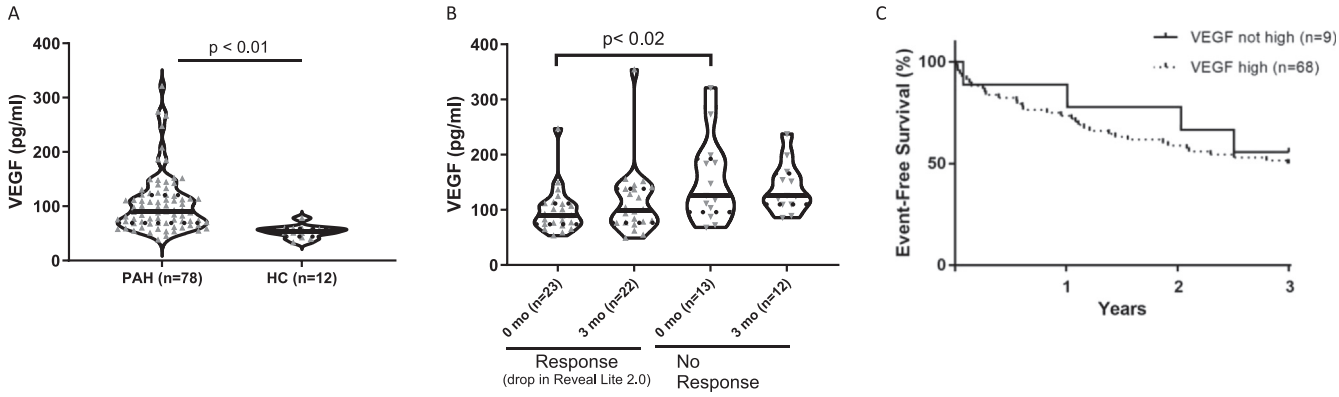

Figure 2 VEGF is elevated in patients with PAH. (A) VEGF levels in patients with PAH are elevated compared to healthy controls. (B) VEGF levels in treatment responders (indicated by drop in Reveal Lite 2.0 score) vs nonresponders among patients with PAH starting or intensifying treatment. Lower levels at baseline may predict treatment responders. (C) There was no difference in event-free survival based on VEGF level. PAH, pulmonary arterial hypertension; VEGF, vascular endothelial growth factor.

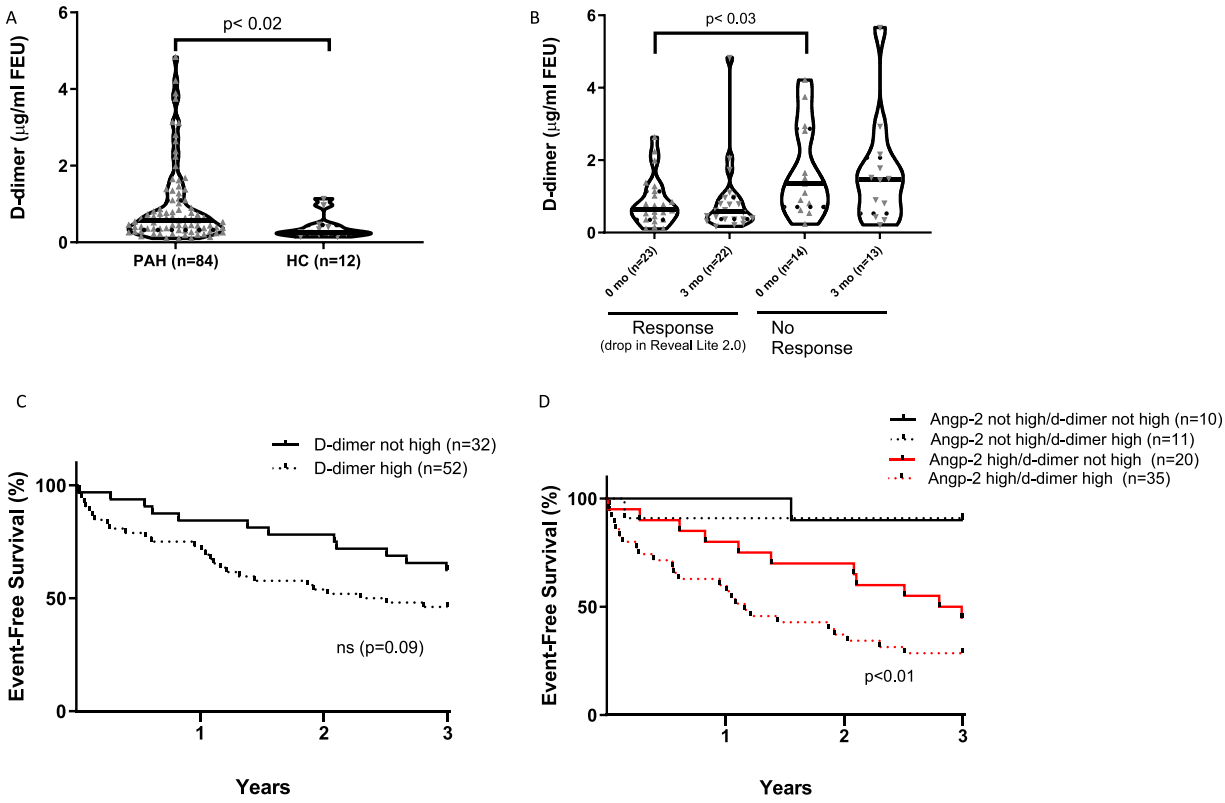

Figure 3 D-dimer is elevated in patients with PAH and predicts treatment response. (A) D-dimer level in patients with PAH compared to healthy controls. (B) D-dimer levels were lower at baseline in patients who had a drop in Reveal Lite 2 score after adding therapy; D-dimer did not fall with new therapy, but higher D-dimer levels were observed in those who did not improve with therapy. (C) Event-free survival in patients with PAH (whether or not intensifying treatment) with high D-dimer (> 90%-ile of healthy) vs normal D-dimer levels. (D) Event-free survival in patients with high (90%-ile of healthy) or normal Ang-2 and D-dimer levels; for those with high Ang-2 levels, a high D-dimer predicted even higher risk. D-dimer added nothing for those with more normal Ang-2 levels. Data are shown as median + IQR or Kaplan-Meier event curves. Ang-2, angiopoietin-2; IQR, inter-quartile range; PAH, pulmonary arterial hypertension.

thrombotic lesions (including recanalized thrombi) in patients with PAH at transplant or autopsy.[2,18] Here, we added to previous literature by measuring baseline and follow-up assays of coagulation, fibrinolysis, and endothelial dysfunction in patients with PAH intensely treated on contemporary 2- and 3-drug regimens. We included a cohort of treatment-naïve patients with PAH to see if initial combination therapy might impact fibrinolysis or markers of endothelial injury, and we incorporated clinical outcomes into our analyses.

Endothelial activation (or injury) converts the normally "anticoagulant" molecular expression on the endothelial surface to a procoagulant signature. Injury also activates endothelial cells to express intracellular adhesion molecules, including intracellular adhesion molecule -1 (ICAM-1) and VCAM-1, while releasing sP-selectin, which mediate further inflammatory cell and platelet adhesion, thrombosis, and downstream vascular injury.[19] We demonstrated increased expression of adhesion molecules sVCAM-1 and sP-selectin in the serum of patients with PAH, compared to HC (elevated sP-selectin in PAH is likely from both platelets and endothelial cells). However, these markers did not correlate with survival or treatment response.

A different marker of endothelial injury, Ang-2, was significantly elevated in patients with PAH; Ang-2 reductions correlated with a drop in clinical risk, while persistent elevations in Ang-2 predicted a significantly decreased event-free survival (and predicted events even among those with reassuring Reveal scores). Ang-2, stored in Weibel-Palade bodies, is released from activated or damaged endothelium and in turn binds Tie-2 tyrosine kinase on endothelial cells, causing upregulation of adhesion molecules and proinflammatory cytokine/chemokine expression. Ang-2 also results in destabilization of the endothelium and vascular bed remodeling.[20] Elevated Ang-2 has been demonstrated in patients with PAH previously.[21-23] Kumpers et al observed that a decrease in Ang-2 levels 3 months after PAH therapy intensification correlated with improvements in 6 minute walk distance, right atrial pressure, pulmonary vascular resistance, and mixed venous oxygen saturation (SvO_2). Our study replicates the prior work indicating that elevated Ang-2 is a strong predictor of future clinical events and adds to this work by demonstrating that elevated Ang-2 at follow-up may identify patients with low Reveal Lite 2 scores who are nonetheless still at increased risk for clinical events. This targeted "liquid biopsy" may help to identify a subset of patients who appear to be clinically low risk but might benefit from earlier treatment intensification. In chronic thromboembolic dyspnea, elevated Ang-2 distinguished chronic thromboembolic patients with and without pulmonary hypertension,[24] further confirming that this molecule has promise as a barometer of distal pulmonary vasculopathy.

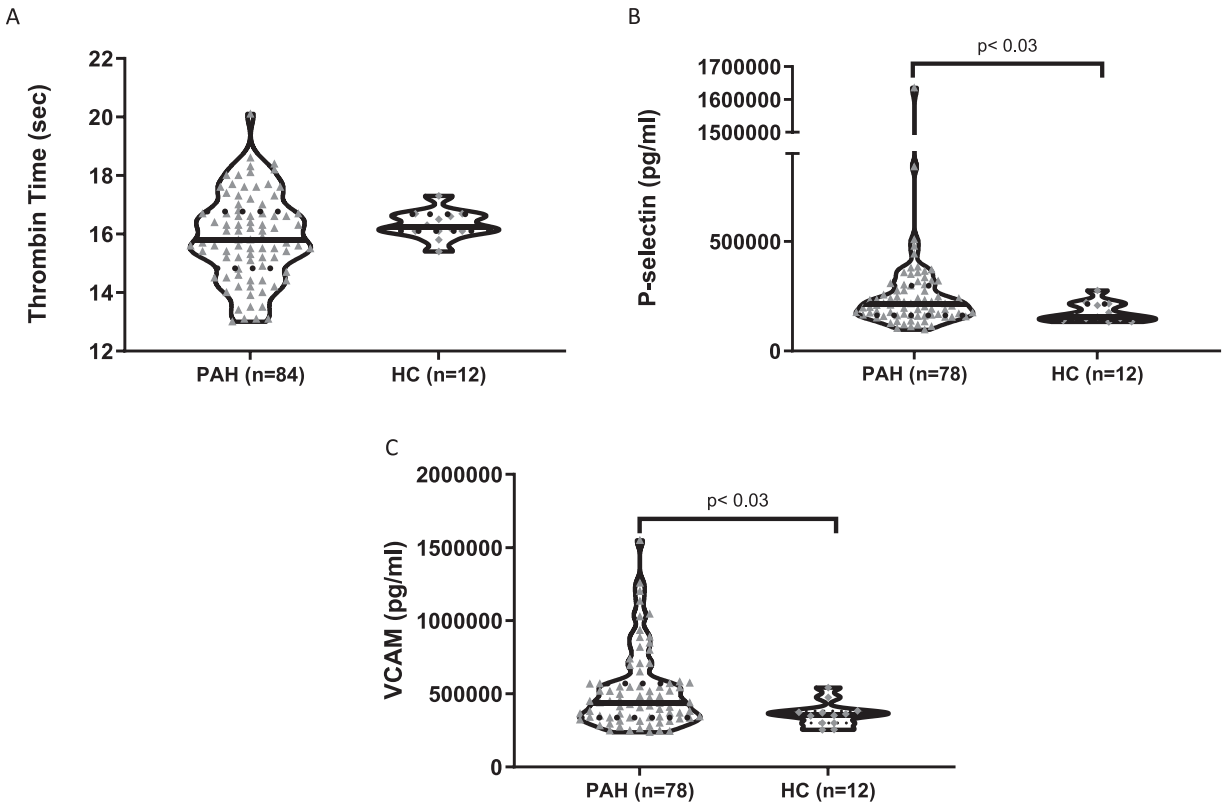

Figure 4 Thrombin time, P-selectin, and VCAM (A) thrombin time was generally shorter but also more variable in PAH compared to healthy controls, resulting in no net difference. (B) P-selectin levels are elevated in patients with PAH vs HC. (C) VCAM levels are elevated in patients with PAH vs HC. Data are shown as median + IQR. HC, healthy controls; IQR, inter-quartile range; PAH, pulmonary arterial hypertension.

vWF was also elevated in patients with PAH compared to HC, which has previously been noted.[25-27] In our study, we did not find that vWF correlated with event-free survival (different from Kawut et al). Reported vWF levels in patients with chronic thromboembolic pulmonary hypertension vs idiopathic vs patients with CTD-PAH have varied[25,26,28]; this may point to differing endothelial phenotypes in pulmonary hypertension (PH). Although both vWF and Ang-2 are released from Weibel-Palade bodies, they seem to report different features of endothelial injury.

Elevated levels of prothrombotic factors, including fibrinogen, vWF, and tissue factor, have been previously identified in patients with PAH, along with evidence of coagulation activation, including D-dimer and increased thrombin activity.[9,10,17,29] In 2007, Bakouboula et al specifically implicated the pulmonary circulation as the source of procoagulant activity and correlated markers of vascular injury with severity of hemodynamic profile.[7] Here, we found elevated D-dimer as an in vivo indicator for coagulation cascade activation and fibrinolysis. One strength of D-dimer is that it reflects in situ coagulation and subsequent fibrin clot degradation as opposed to other in vitro measures of thrombin generation, all of which require additional reagents and assumptions.

D-dimer elevation has been consistently observed in patients with PAH[29]; we add to these previous studies by correlating D-dimer with therapy response and clinical events. Patients with high baseline D-dimer levels were less likely to improve (by Reveal Lite 2) with treatment intensification, and those with persistently elevated D-dimer at follow-up trended to worse event-free survival (and the combination of high D-dimer and high Ang-2 was particularly noteworthy, Figure 3D). Because D-dimer is inexpensive, readily available in clinical labs, and consistently elevated in various PAH cohorts, our new data to suggest its utility as a prognostic indicator in PAH deserve further study. For now, it appears that D-dimer may add prognostic information (Figure 3C and D) which suggests higher risk even for some individuals with low Reveal Lite 2 score (Table 3).

Clinical scoring systems to predict prognosis and assess treatment response are robust.[30] Circulating proteins, such as Ang-2, which reflect some component of the underlying disease pathology are therefore most useful when they add to what are already useful clinical scores; the UK consortium recently demonstrated that a 6-protein "score" strongly predicted clinical outcomes among a group of prospectively enrolled French participants who had met at least 2 clinical "low-risk" criteria from the French non-invasive score.[6] In the UK cohort, Ang-2 tracked closely with BNP and did not independently predict survival (personal communication, Christopher Rhodes); further study in larger cohorts to include both hospitalizations and mortality seems appropriate based on our results suggesting that Ang-2 did add significant prognostic information to low-risk individuals (Figure 1D, Table 3). We will best treat our patients when we predict risk for hospitalization and the need for advanced therapies (not just mortality).[31]

Our study is limited by the single-center design, the modest sample size, and the age discrepancy between HC and PAH participants which may have influenced all of our comparisons. In particular, D-dimer is known to increase with age, and thus the "cut-off" (90%-ile of HC) we utilized to distinguish an abnormal D-dimer was relatively low for our older PAH participants. On the other hand, the cut-off is similar to the threshold which is generally used to identify those with venous thromboembolic disease; moreover, our data suggest that this threshold may have had clinical significance in terms of predicting outcomes, mitigating against the limitation. We did not correct any of our statistical testing for multiple comparisons; the purpose of our study was to interrogate previously described abnormalities in a cohort of patients with PAH treated with modern combinations of therapy. We did not cast a particularly broad net, but we did not correct any of the statistics for the number of measures we made.

In summary, we replicated previous reports that D-dimer is elevated in PAH, reflecting coagulation cascade activity; thrombin time was also reduced as a marker of increased procoagulant activity. Ang-2 levels as a marker of endothelial injury were treatment responsive and prognostically significant even in those with a "low-risk" Reveal Lite 2 score; this marker may represent an important risk stratification tool. Future work in biomarkers will need to determine which readily measurable proteins add value to the robust clinical scoring systems we are already using.

Author Contributions

D.L. and R.J.W. conceived and designed the study with input from A.N.L. and N.M.; D.H., D.L., and A.N.L. collected blood and performed assays; H.C., D.L., S.L., and R.J.W. analyzed data and wrote the article. All authors critically revised and approved the work for submission.

Disclosure statement

Authors have no conflicts of interest.

Participants and their families; Andrew Mintz, RN and Ali Theuer, RN helped recruit and manage the patients. Funding was provided by an Entelligence Career Development Award (now known as ATS Young Investigator) to D.L. and a Janssen Investigator Initiated Study award to R.J.W. The project described in this publication was supported in part by University of Rochester CTSA award No. KL2 TR001999 (to D.L.) from the National Center for Advancing Translational Sciences of the National Institutes of Health.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhlto.2024.100178.

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