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ORIGINAL ARTICLE

Effects of inflammation on thrombosis and outcomes in COVID-19: secondary analysis of the ATTACC/ACTIV-4a trial

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Abstract

Background: Patients hospitalized for COVID-19 are at high risk of thrombotic complications and organ failure, and often exhibit severe inflammation, which may contribute to hypercoagulability.

Objectives: To determine whether patients hospitalized for COVID-19 experience differing frequencies of thrombotic and organ failure complications and derive variable benefits from therapeutic-dose heparin dependent on the extent of systemic inflammation and whether observed benefit from therapeutic-dose anticoagulation varies depending on the degree of systemic inflammation.

Methods: We analyzed data from 1346 patients hospitalized for COVID-19 enrolled in the ATTACC and ACTIV-4a platforms who were randomized to therapeutic-dose heparin or usual care for whom levels of C-reactive protein (CRP) were reported at baseline.

Results: Increased CRP was associated with worse patient outcomes, including a >98% posterior probability of increased organ support requirement, hospital length of stay, risk of 28-day mortality, and incidence of major thrombotic events or death (patients with CRP 40-100 mg/L or ≥100 mg/L compared to patients with CRP <40 mg/L). Patients with CRP 40 to 100 mg/L experienced the greatest degree of benefit from treatment with therapeutic doses of unfractionated or low molecular weight heparin compared with usual-care prophylactic doses. This was most significant for an increase in organ support-free days (odds ratio: 1.63; 95% confidence interval, 1.09-2.40; 97.9% posterior probability of beneficial effect), with trends toward benefit for other evaluated outcomes.

Conclusion: Moderately ill patients hospitalized for COVID-19 with CRP between 40 mg/L and 100 mg/L derived the greatest benefit from treatment with therapeutic-dose heparin.

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KEYWORDS
anticoagulation, COVID-19, CRP, heparin, thrombosis

Essentials

- Patients with COVID-19 may be more prone to thrombosis based on the degree of inflammation.
- Data from 1346 COVID-19 patients were stratified based on levels of C-reactive protein (CRP).
- Increased CRP was associated with worse patient outcomes.
- Patients with CRP 40 to 100 mg/L derived the greatest benefit from therapeutic-dose heparin.

1 | INTRODUCTION

Throughout the progression of the COVID-19 pandemic, thrombosis and inflammation have gained recognition as significant contributors to the morbidity and mortality associated with this disease [1,2]. Rates of venous thromboembolism (VTE) as high as 25% were reported for patients hospitalized for COVID-19 early in the pandemic [3–6]. Numerous subsequent studies have reported rates of thrombotic complications as high as 20% to 30% in critically ill patients [7–14], significantly higher rates of thrombosis were observed in patients hospitalized with other viral respiratory illnesses [15,16]. In addition to macrovascular VTE events, including deep vein thrombosis and pulmonary embolism (PE), COVID-19 patients have also been shown to have increased rates of immunothrombosis [16] and elevated risk of microvascular [17–22] and arterial [21,23–25] thrombotic events. Widespread vaccination and the emergence of newer viral variants have contributed to a reduction in overall morbidity and mortality associated with COVID-19 and may be associated with decreased rates of thrombosis; however, thrombotic complications are still a concern among patients seeking medical care for COVID-19 infection [26,27]. The prevalence of these events as well as the associated morbidity and mortality has led to interest in the use of anticoagulation in patients hospitalized with COVID-19.

The recently published multiplatform randomized controlled trial (RCT) involving the Antithrombotic Therapy to Ameliorate Complications of COVID-19 (ATTACC; NCT04372589), Accelerating COVID-19 Therapeutic Interventions and Vaccines 4 ACUTE (ACTIV-4a; NCT04505774), and Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP; NCT02735707) platforms [28] enrolled 2,244 moderately ill (noncritically ill) patients and evaluated the efficacy of therapeutic heparin in patients hospitalized for COVID-19, without known thrombotic complications [29]. This open-label, adaptive, multiplatform RCT (mpRCT) compared therapeutic-dose anticoagulation with unfractionated heparin or low molecular weight heparin (LMWH) to usual-care thromboprophylaxis dosing and demonstrated an improvement in the composite of days alive without intensive care unit (ICU)-level organ support and hospital survival in noncritically ill patients treated with therapeutic-dose heparin.

Another hallmark of COVID-19 is inflammation. A widely available clinical laboratory test, C-reactive protein (CRP), is commonly used as a non-specific measure of inflammation. In patients with COVID-19, elevated CRP has been associated with mortality [30–34], disease severity [35,36], thromboembolism [36,37], and severity of pulmonary dysfunction [38].

Inflammation is known to contribute to the development of hypercoagulability [39] and may be of particular relevance in COVID-19. Anti-inflammatory therapies are of significant interest in COVID-19, including corticosteroids [40,41], and are widely used in patients requiring supplemental oxygen.

The aim of this secondary analysis was to examine the treatment interactions between patients' systemic inflammatory state and clinical outcomes and to evaluate whether treatment with therapeutic-dose heparin appeared more efficacious based on the degree of underlying systemic inflammation.

2 | METHODS

2.1 | Patients

This study was performed as a secondary analysis of data collected by 2 of the 3 participating trials in the mpRCT of therapeutic-dose anticoagulation with heparin in patients hospitalized with COVID-19, the ATTACC and ACTIV-4a platforms [28]. This secondary analysis was not a pre-specified subgroup analysis. Briefly, this multiplatform RCT enrolled patients hospitalized for COVID-19 at 121 study sites worldwide and was conducted with approval from the relevant ethics committees, in accordance with the Good Clinical Practice guidelines of the International Council for Harmonization, with informed consent obtained from all patients or surrogates. A data-sharing statement for this trial is provided at NEJM.org. Patients were randomized to therapeutic-dose anticoagulation with unfractionated heparin or LMWH or to usual care with prophylactic dose

anticoagulation administered according to local practice. Participants and primary medical teams were not blinded to group assignments. Patients were enrolled in the trial within 72 hours of admission for COVID-19 and were excluded if the anticipated length of hospital stay was <72 hours, patients had a clinical indication for therapeutic anticoagulation, an elevated risk of bleeding, were receiving dual antiplatelet therapy, or had a known allergy to heparin including a history of heparin-induced thrombocytopenia. In noncritically ill patients hospitalized for COVID-19, this study demonstrated improved probability of survival to hospital discharge and reduced requirement for ICU-level organ support in patients treated with therapeutic-dose heparin but did not demonstrate this same benefit for critically ill patients [28,42].

This secondary analysis includes data extracted from the noncritically ill participants (hospitalized but with no requirement for ICU-level organ support at time of trial enrollment) enrolled by the ATTACC and ACTIV-4a cohorts only; relevant data were not available for patients enrolled through the REMAP-CAP platform. This analysis includes all patients from the ATTACC and ACTIV-4a noncritically ill cohorts who did not withdraw from the trial and who had levels of CRP available at baseline or within 24 hours of screening.

2.2 | Outcomes

The primary outcome evaluated in this secondary analysis was organ support-free days, defined as days alive without requirement for oxygen delivery via high flow nasal cannula, non-invasive or invasive mechanical ventilation, vasopressors, or inotropes. As in the mpRCT, individuals who died were assigned the worst organ support-free days value of -1. The secondary outcomes were hospital length of stay, 28-day all-cause mortality, the composite of major thrombotic events (myocardial infarction, PE, ischemic stroke, systemic arterial embolism), and death.

Clinical outcomes were analyzed for association with baseline degree of inflammation. The interaction of the degree of inflammation with treatment was also evaluated using interaction effects. All statistical analyses were adjusted for gender and age, as fixed effects, and site and time period, as random effects. Organ support-free days were modeled as an ordinal outcome with a cumulative logistic model to calculate the posterior probability distribution for the proportional odds ratio (OR). We used flat non-informative priors for the fixed effects and minimally informative centered student t priors (degrees of freedom 3, scale 2.5) for the proportional odds intercepts and the standard deviations of the random effects. The posterior probability in Bayesian statistics represents the updated probability of an event (ie, reduction in requirement for organ support) occurring given the availability of new information (ie, stratification based on level of CRP).

Length of stay in hospital was measured in days with death considered a censoring event and modeled using a Bayesian Cox proportional hazard model using the same parameterization and minimally informative priors as were used for 28-day mortality.

Mortality at 28 days was modeled using a Bayesian Cox proportional hazards model fit using the Integrated Nested Laplace Approximation (INLA) algorithm [43]. INLA parameterizes the baseline hazard using a piecewise constant function with a 1-step random walk. Minimally informative priors were used with a log-gamma distribution (parameters 1, 0.00005) for the log-precision of the random walk and the random effects and a normal (mean 0, precision 0.001) for the fixed effects.

The composite of major thrombotic events and death (defined as myocardial infarction, PE, ischemic stroke, systemic arterial embolism, or in-hospital death) was modeled as a binary outcome using Bayesian logistic regression. The model was fit using INLA using minimally informative priors of a log-gamma distribution (parameters 1, 0.00005) for the log-precision of the random effects and a normal (mean 0, precision 0.001) for the fixed effects.

2.3 | Stratification by CRP level

Cutoff values for CRP were determined on the basis of a literature review of measurements of CRP in COVID-19. CRP can increase more than 1000-fold from baseline in severe inflammation, and CRP levels reported in COVID-19 patients vary significantly based on patient cohort characteristics and outcomes of interest. The normal value for CRP is typically reported as <3 to 5 mg/L; however, levels below 10 mg/L may be considered as low grade inflammation [44]. A CRP level of 10 mg/L was selected as the cutoff between normal/low and moderate inflammation. Systematic reviews and meta-analyses have proposed levels of CRP >10 mg/L as a risk factor for disease progression [45] and as a predictor of composite poor outcomes including mortality, ICU admission, and severe disease [35]. A CRP value of 40 mg/L was selected as the cutoff between moderate and high inflammation. Levels of >40 mg/L have been shown to be predictive of mortality [30,46] or disease severity [47] in moderately ill patients. Furthermore, CRP >100 mg/L was selected as the cutoff for severe inflammation. Values in the 40 to 100 mg/L range are more commonly reported in studies of patients ultimately developing severe or critical illness [48,49].

3 | RESULTS

3.1 | Baseline characteristics

Noncritically ill patients hospitalized for COVID-19 were stratified on the basis of the degree of inflammation determined by CRP plasma level at the time of trial enrollment. Measurement of CRP was not required for enrollment in the trial and was performed at the discretion of the primary medical team. Of the 1922 patients for whom data was available, 1346 patients (67.6%) had baseline CRP levels. Baseline characteristics were compared between patients with and without baseline CRP levels to evaluate for bias in the collection of this laboratory test, as shown in Table 1. Demographic information

TABLE 1 Baseline characteristics of patients with CRP measurements.

	ATTACC and ACTIV-4A trial population	CRP not reported within 24 h of enrollment	CRP reported within 24 h of enrollment	Posterior Probability of Increased Disease Prevalence in Patients with CRP Level
No. of patients	1,922	576	1346	NA
Proportion female	0.41	0.44	0.40	4.8%
Age (y, mean ± SD)	58.90 ± 14.1	58.45 ± 14.3	59.07 ± 14	79.5%
Hypertension (%)	53.4%	52.1%	53.9%	76.2%
Diabetes (%)	30.1%	33.8%	29.8%	4.8%
Cardiovascular disease (%)	56.8%	55.8%	57.2%	71.7%
Chronic kidney disease (%)	7.5%	7.5%	7.5%	49.9%
Immune disease (%)	8.7%	8.4%	8.8%	62.3%
Respiratory disease (%)	18.8%	18.3%	19%	64.8%

Baseline characteristics of patients enrolled in the ATTACC and ACTIV-4a trial with and without CRP values reported within 24 hours of trial enrollment. Percent with comorbidities represents the percent of patients for with a given comorbidity was reported among patients for whom data was available for a given comorbidity. An equivalent Bayesian analysis was performed for each baseline characteristic. The results of this analysis are reported as posterior probability, representing the probability that the prevalence of a given comorbidity is higher among patients with a reported CRP level. CRP, C-reactive protein.

and the prevalence of comorbidities were comparable between patients with and without baseline CRP values. Information on patient race or ethnicity was not available. The proportion of patients with measured CRP levels was also consistent throughout the trial enrollment period. All further analyses were performed on the 1346 patients with reported CRP values.

3.2 | Stratification by degree of inflammation

Patients were subdivided into 4 groups on the basis of CRP levels reported within 24 hours of trial enrollment (low, moderate, high, and severe inflammation). As shown in Table 2, only 70 patients were found to have low (<10 mg/L) CRP. Moreover, 291 patients were classified as having moderate inflammation (10-40 mg/L), 496 as high inflammation (40-100 mg/L), and 489 as severe inflammation (>100 mg/L).

3.3 | Association of CRP with laboratory evidence of coagulopathy

The association of degree of inflammation with laboratory evidence of coagulopathy as assessed using D-dimer, platelet count, international normalized ratio, and activated partial thromboplastin time is summarized in Table 3.

High and severe inflammation was associated with increased D-dimer. Consistent with the ATTACC and ACTIV-4a studies, a relative D-dimer, defined relative to the reference normal at each trial site, was used; absolute D-dimer levels were not available. Compared with patients with a low inflammatory state, relative D-dimer levels were 1.056 (credible interval 0.86-1.30), 1.21 (credible interval, 0.99-1.47), and 1.53 (credible interval, 1.25-1.87) higher for patients with moderate, high, and severe inflammatory states, respectively. The posterior probability of a true increase in D-dimer compared with patients with low inflammation was relatively modest at 69.8% for patients with moderate inflammation but demonstrated significant association with elevated D-dimer at higher levels, with posterior probabilities of 97% for high inflammation and 100% for severe inflammation.

Platelet count exhibited minimal variability based on inflammatory state. The 95% confidence interval (CI) crossed the threshold for no effect for the moderate and high inflammatory categories, with posterior probabilities of 33% and 69%, respectively. A small increase in platelet count (246 ± 93 vs 230 ± 117K/μL) was seen in severe inflammation with a posterior probability of 99.6%.

Standard laboratory coagulation tests international normalized ratio and activated partial thromboplastin time were measured in a small number of patients, precluding formal statistical analysis. Descriptive analyses are shown in Table 3.

This analysis demonstrates an association between laboratory evidence of coagulopathy and degree of inflammation in COVID-19 patients. Formal evaluation for disseminated intravascular coagulation was not performed as part of this study; however, the increase, rather than decrease, in platelet count associated with severe inflammation argues against a disseminated intravascular coagulation-like consumptive coagulopathy in these patients.

3.4 | Association of inflammation with patient outcomes

The small number of patients with low levels of CRP precluded statistical analysis of patient outcomes for this category. Accordingly, the low and moderate inflammation categories were combined, resulting

TABLE 2 Distribution of patients by CRP level.

Category	CRP range (mg/L)	No. of patients	CRP (mg/L, mean ± SD)	Usual care (*N*)	Therapeutic heparin (*N*)
Low	<10	70	5.91 ± 2.44	28	42
Moderate	10-39.9	291	24.98 ± 8.32	138	153
High	40-99.9	496	69.59 ± 16.89	237	259
Severe	≥100	489	181 ± 100	224	265

Patients were divided into inflammatory category on the basis of CRP level as measured within 24 hours of trial enrollment with cutoff values as shown above. Patients were randomized to usual care with prophylactic dose anticoagulation administered according to local practice or to therapeutic-dose heparin. CRP, C-reactive protein.

in a low-moderate inflammation category composed of 333 patients with 166 receiving usual-care prophylactic dose anticoagulation and 195 receiving therapeutic-dose heparin.

Increased inflammation was associated with worse patient outcomes. This included a reduction in organ support-free days, and increased hospital length of stay, mortality, and composite major thrombotic events and death. Results are summarized in Table 4. For each outcome, models were constructed as described above, with the combined low-moderate inflammation group used as the reference category in all analyses. The posterior probability that high and severe inflammation respectively are associated with decreased organ support-free days was 99.4% (median adjusted OR, 0.46; 95% CI, 0.28-0.75) and 99.9% (median adjusted OR, 0.40; 95% CI, 0.24-0.66). Patients with high and severe inflammatory states also experienced increased hospital length of stay, represented by hazard ratios below one, with median hazard ratios of 0.74 (95% CI, 0.60-0.92; 99.7% probability) for patients with high inflammation and 0.68 (95% CI, 0.55-0.85; 100% probability) for patients with severe inflammation compared to patients with low-moderate inflammation.

For 28-day mortality and composite thrombotic event and death, ORs above one signify increased risk of mortality and major thrombotic event and death, respectively. Patients in the high and severe inflammation categories had an increased risk of death compared to patients with normal-moderate inflammation, with hazard ratio of 2.64 (95% CI, 1.20-6.47; posterior probability, 99.3%) and 2.35 (95% CI, 1.11-5.62; posterior probability, 98.7%) respectively. Patients in the high inflammation category had a median hazard ratio for combined major thrombotic event and mortality of 1.80 (95% CI, 0.83-4.24; posterior probability, 93.2%) while patients with severe inflammation had a median hazard ratio of 2.79 (95% CI, 1.34-6.42; posterior probability, 99.8%).

Patients enrolled in this study were randomized with respect to anticoagulation with no restriction on the utilization of other treatments for COVID-19. Treatment with the corticosteroid dexamethasone has become standard of care for patients with COVID-19 and hypoxia, with implications for inflammatory state. Of patients included in this analysis, 906 (67%) received treatment with steroids. The incidence of steroid treatment was unequally distributed across inflammation groups, with 58% of normal inflammation patients, 65% of moderate inflammation patients, 68% of high inflammation patients, and 70% of severe inflammation patients receiving steroid treatment. Further analysis was not performed regarding the

TABLE 3 Laboratory markers of coagulopathy by patient inflammatory status.

	Relative D-dimer			Platelet count			International normalized ratio		aPTT	
Inflammation category (Total *N*)	*N*	Mean ± SD	Posterior probability	*N*	Mean ± SD (K/μL)	Posterior probability	*N*	Mean ± SD	*N*	aPTT (s, mean ± SD)
Normal CRP <10 mg/L (70)	63	2.175 ± 3.282	–	66	230 ± 117	–	31	1.06 ± 0.09	23	30.38 ± 4.57
Moderate CRP 10-39.9 mg/L (291)	253	2.041 ± 3.296	69.8%	287	216 ± 89	33%	126	1.07 ± 0.11	92	30.29 ± 6.35
High CRP 40-99.9 mg/L (496)	413	2.393 ± 5.170	97%	492	222 ± 85	69%	189	1.05 ± 0.11	148	30.58 ± 6.29
Severe CRP >100 mg/L (498)	413	2.990 ± 3.840	100%	487	246 ± 93	99.6%	181	1.09 ± 0.13	126	31.26 ± 6.30

Laboratory markers of coagulopathy by patient inflammatory category. *N* for each marker represents number of patients in whom baseline values were reported. D-dimer was reported as relative D-dimer relative to the local normal at each trial site. Posterior probability reported for D-dimer and platelets represents the probability of a true increase in marker for a given inflammatory category compared to patients with normal inflammatory state. aPTT, activated partial thromboplastin time; CRP, C-reactive protein.

TABLE 4 Association of inflammatory state with patient outcome.

Outcome	Normal-moderate inflammation						High inflammation						Severe inflammation					
	No. of occurrences		Median (IQR)		Odds ratio (95% CI)	Probability of effect (%)	No. of occurrences		Median (IQR)		Odds ratio (95% CI)	Probability of effect (%)	No. of occurrences		Median (IQR)		Odds ratio (95% CI)	Probability of effect (%)
	UC	TH	UC	TH			UC	TH	UC	TH			UC	TH	UC	TH		
28-d mortality	8	4					21	4			2.64 (1.20-6.47)	99.3%	24	5			2.35 (1.11-5.36)	98.7%
Major thrombotic event or death	9	161					24	19			1.80 (0.84-4.24)	93.2%	33	41			2.79 (1.34-6.42)	99.8%
Hospital length of stay			4 (3-7)	5 (3-8)					5 (3-10)	4 (3-8)	0.74 (0.60-0.92)	99.7%			6 (4-10)	5 (4-10)	0.68 (0.55-0.85)	100%
Organ support-free days			22 (22-22)	22 (22-22)					22 (22-22)	22 (22-22)	0.46 (0.28-0.74)	99.4%			22 (20-22)	22 (20-22)	0.40 (0.24-0.66)	99.9%

Association between inflammatory state and patient outcome. Probability of effect calculated relative to the normal-moderate group. For organ support-free days, odds ratio <1 indicates an increased reduced risk of requirement for organ support. For hospital length of stay, an odds ratio <1 indicates a longer length of hospitalization. For all outcomes, probability of effect is reported as probability of clinically negative effect in patients with high or severe inflammation compared to normal-moderate inflammation. For 28-day mortality and major thrombotic event or death, an odds ratio of greater than one indicates an increased risk of the indicated detrimental event. TH, therapeutic heparin; UC, usual care.

interaction of steroid use and coagulopathy and response to anticoagulation due to the potential confounding effects of unequal steroid treatment across patient groups.

3.5 | Interaction between inflammation and treatment with therapeutic-dose heparin

For all outcomes, the treatment effect of therapeutic-dose heparin was calculated separately for the combined low-moderate, high, and severe inflammatory groups. Results are reported as ORs or hazard ratios for patients in the therapeutic-dose heparin vs usual-care arms for each inflammatory category as well as the posterior probability of a true protective treatment effect. For all outcomes, the greatest treatment benefit was seen in patients in the high inflammation category, with the probability of a protective treatment effect greater than 95% for hospital length of stay and greater than 90% for 28-day mortality and major thrombotic event or death. Patients in the severe inflammatory category showed minimal treatment effect for all outcomes. Results are summarized in Table 5.

Treatment with therapeutic-dose heparin resulted in a statistically significant increase in organ support-free days in patients with high inflammation with minimal effect in patients with low-moderate or severe inflammation. For the high inflammation group, the proportional odds of increased organ support-free days was 1.63 (95% CI, 1.09-2.40; posterior probability, 98%), indicating that treatment with therapeutic-dose heparin increases organ support-free days for this group. Limited benefit was seen in the severe and normal-moderate inflammation groups, with a treatment effect of 1.02 (95% CI, 0.71-1.43; posterior probability, 53%) for the severe group and 0.76 (95% CI, 0.44-1.31; posterior probability, 18.9%) for the normal-moderate group.

For hospital length of stay, similar results were seen. A hazard ratio of greater than one indicates decreased length of hospitalization. Patients with high inflammation trended toward a decrease in hospital length of stay with treatment, with a hazard ratio 1.20 (95% CI, 0.99-1.45; 96.9% posterior probability of decreased length of hospital stay). Again, limited treatment effect was seen in length of hospitalization in the severe inflammation group with median hazard ratio 1.02 (0.84-1.23, 56.4% posterior probability) and in the normal-moderate inflammation group with hazard ratio of 0.85 (95% CI, 0.70-1.08; 90.5% posterior probability of increase in hospital length of stay).

For 28-day mortality, the 95% credible interval for treatment effect in all 3 inflammation groups crossed one, indicating insufficient evidence to conclude the treatment effect for each group. However, a similar trend was seen as for organ support-free days, with a trend toward benefit from treatment in patients with high inflammation with increased risk of death or no effect seen in patients with normal-moderate or severe inflammation. Similar results were seen for the major thrombotic event and death composite outcome, with a treatment effect approaching one for both the

TABLE 5 Interaction effects between treatment with therapeutic-dose heparin and inflammation.

	Normal-moderate inflammation		High inflammation		Severe inflammation	
Outcome	Odds ratio (95% CI)	Probability of effect	Odds ratio (95% CI)	Probability of effect	Odds ratio (95% CI)	Probability of effect
Organ support-free days	0.75 (0.44-1.31)	18.9%	1.63 (1.09-2.40)	97.9%	1.01 (0.71-1.43)	53.7%
Hospital length of stay	0.86 (0.70-1.08)	9.5%	1.20 (0.99-1.45)	97%	1.02 (0.84-1.23)	56%
28-d mortality	1.09 (0.47-2.67)	42.2%	0.63 (0.33-1.20)	91.9%	1.16 (0.67-2.02)	29.9%
Major thrombotic events and death	1.38 (0.61-3.37)	23%	0.64 (0.33-1.22)	91.4%	0.97 (0.58-1.61)	56%

Odds ratio and probability of effect calculated for treatment with therapeutic-dose heparin vs treatment with usual-care prophylactic dose for patients at each level of inflammation. For organ support-free days and hospital length of stay odds ratio >1 indicates an increase in days free of organ support or alive without requirement for hospitalization. For 28-day mortality and major thrombotic events or death, an odds ratio >1 indicates a reduced risk of negative outcome. Probability of effect indicates posterior probability of clinically beneficial effect from treatment with therapeutic heparin in a given inflammatory category.

normal-moderate and severe inflammation categories and a protective effect of treatment within the high inflammation category, with a probability of reduced risk of 91.4% (median OR, 0.64; 95% CI, 0.33-1.21).

4 | DISCUSSION

This secondary analysis of data from the ATTACC and ACTIV-4a trials sought to assess the interaction between inflammation, as measured by CRP level and treatment with therapeutic-dose heparin in noncritically ill patients hospitalized with COVID-19. The data presented in this study suggest that the patients deriving greatest benefit from therapeutic-dose heparin are those with high but not extreme degrees of inflammation, defined in this study as CRP levels in the range of 40 to 100 mg/L, with minimal treatment effect observed in patients with both low-moderate and severe degrees of inflammation. As therapeutically dosed heparin carries definitive, although low, risk of harm, as well as cost, this information may ultimately prove useful in personalizing the risk-benefit discussion surrounding therapeutic heparin in COVID-19 patients without a prior indication for anticoagulation. Patient baseline level of inflammation as measured by CRP should be accounted for in future studies of anticoagulation in COVID-19 patients and considered in the development of clinical guidelines for anticoagulation in this patient population.

Although CRP is not currently used to guide decision-making regarding anticoagulation in patients with COVID-19, the ubiquitous availability and non-invasive nature of this test make it a favorable candidate as a marker to guide anticoagulation management in COVID-19 patients. Furthermore, in a large analysis of the association of biomarkers with thrombosis in patients with COVID-19, CRP was found to be one of the most useful markers for the assessment of prognosis and thrombotic risk in this patient population [36]. We identified a wide variation in the degree of inflammation measured among noncritically ill patients, with >10-fold variation in CRP levels measured at baseline. Patients hospitalized with COVID-19 are highly heterogeneous in terms of symptoms, timing of disease course relative to hospital presentation, disease severity, and outcome as well as underlying comorbidities. However, CRP levels may represent a tool to identify patients with the potential to benefit from therapeutic-dose heparin treatment.

The use of markers such as CRP to identify patients who are likely to benefit from treatment with therapeutic heparin is particularly relevant in light of the fact that current research demonstrates that critically ill patients do not experience the benefit from anticoagulation that noncritically ill patients do, as seen in the results of the ATTACC, ACTIV-4a, and REMAP-CAP mpRCT critically ill cohort study [42]. Here, patients requiring ICU-level care did not benefit from therapeutic-dose heparin, and the probability that therapeutic-dose heparin was inferior to usual-care prophylactic dose anticoagulation with respect to organ support-free days or probability of survival was high. The increased bleeding events seen with therapeutic-dose heparin in the critically ill cohort (3.8% vs 2.3%) may account for some of this effect, as bleeding rates were lower in the noncritically ill cohort analyzed in this secondary analysis (1.9% vs 0.9%). In conjunction with the results of this analysis, this suggests that there is an inflammatory stage in COVID-19 infection at which treatment with therapeutic-dose anticoagulation offers benefit.

Further studies are also warranted to evaluate whether the evaluation of other markers to define inflammation, including interleukin (IL)-6, ferritin, and fibrinogen, leads to the same differential benefit and to elucidate the mechanisms behind this differential benefit. Additionally, analysis of additional inflammatory markers may provide greater insight into the mechanism of disease. Further study is also warranted into the interaction of anti-inflammatory therapies such as dexamethasone with therapeutic-dose heparin.

It has been hypothesized that the development of thrombotic microangiopathy in the lungs may contribute to the development of acute respiratory distress syndrome (ARDS) and subsequent respiratory failure in COVID-19 patients [20]. In the last decade, microvascular thrombosis has become recognized as a complication of ARDS regardless of the underlying cause [50], and this phenomenon has been observed more extensively in COVID-19 than in other types of viral pneumonia [16]. It is conceivable that appropriately timed

intervention with anticoagulation may reduce this progression. It is plausible that patients who do not exhibit a significant inflammatory response are less likely to develop thromboembolic complications and therefore derive less benefit from anticoagulant therapy, with risk outweighing benefit for these patients. In this study, patients with the most severe degree of inflammatory response did not benefit from anticoagulant therapy. One hypothesis to explain this phenomenon is that these patients have already experienced organ damage due to hypercoagulability or thromboinflammation prior to the initiation of heparin, and that treatment in these patients was initiated too late to provide significant benefit. It is also plausible that these patients experience disease progression and poor outcomes due to non-thrombotic mechanisms of disease, such as ARDS. In accordance with other studies of patients with COVID-19, we demonstrated worse clinical outcomes associated with a higher degree of inflammation across all evaluated outcome measures.

Inflammatory mediators released at high levels in COVID-19, including CRP, IL-6, IL-8, and tumor necrosis factor α (TNFα) can contribute to the development of a hypercoagulable state *in vitro* and *in vivo* through mechanisms including upregulation of tissue factor and dysregulation of endogenous anticoagulants. Neutrophil extracellular traps (NETs) are mediators of thromboinflammation of particular interest in COVID-19 [51,52]. NETs have been shown to be strongly pro-thrombotic [53–55], cause damage to the endothelium, increase global inflammation, and contribute to the development of ARDS-like pathology [56,57]. NETs have been identified within microvascular thrombi found in the lungs of patients with COVID-19 at autopsy [51,54,58,59], and at higher levels in the serum from patients with COVID-19 who developed thrombosis than in those without [60]. Endothelial damage and dysregulation, either as a primary viral mechanism or as a result of high levels of inflammation, may also contribute to the development of thrombosis in COVID-19, with markers of endothelial damage associated with increased levels of D-dimer at autopsy [61]. Furthermore, structural and functional differences have been demonstrated between thrombi from patients with COVID-19 and patients with other ARDS pathologies, including inhibited fibrinolysis and more thrombus contraction, which may also contribute to the degree of clinical thrombosis seen in these patients [62].

Further study is also required into the mechanisms by which anticoagulation with heparin exerts benefit in patients with COVID-19, particularly in light of the fact that the benefits of anticoagulation were not replicated in a trial using rivaroxaban in patients hospitalized with COVID-19 [63]. Heparin has been proposed to have multiple beneficial mechanisms in addition to antithrombin-mediated inactivation of the coagulation cascade, including endothelial protection, inhibition of heparinases, and interactions with pro-coagulant factors such as extracellular histones [64,53]. Heparin has also been proposed to have pleiotropic effects that may impact the development of disease or thrombosis in ways other than antithrombin-mediated anticoagulation [65].

This study was performed as a secondary analysis of a larger clinical trial and was not a pre-specified subgroup analysis. Measurement of CRP was not obligatory for participation in the trial, and the trial was not designed to stratify patients based on CRP levels. Insufficient data was available regarding other markers of inflammation to allow for analysis of these markers in combination with CRP. While this study assesses for major thromboembolic events detected in hospitalized patients, it is limited by the lack of systematic screening for subclinical thrombotic events and the reliance on individual clinician's suspicion and ability to acquire evidence of VTE in highly heterogeneous practice environments. A recent meta-analysis demonstrated significant variability in the reported incidence of VTE based on study methodology, with an aggregate incidence of VTE of 33% in studies utilizing systematic screening and an incidence of 9.8% in studies relying on clinician diagnosis [66]. This suggests that VTE may go undiagnosed in many COVID-19 patients; however, the clinical significance of this is unclear.

The patient population included in this study was enrolled early in the pandemic and consequently is likely to differ from current cohorts of patients hospitalized with COVID-19 in terms of additional treatments received, vaccination status, and viral variant. Dexamethasone has become the standard of care for the treatment of patients hospitalized with COVID-19 and hypoxemia with implications for inflammation and thrombosis. The impact of steroid treatment was not analyzed in this patient cohort due to a lack of randomization and the potential for confounding factors.

Patients included in this analysis were recruited prior to the availability of vaccines against COVID-19. Vaccination against COVID-19 reduces disease severity and accordingly may reduce the risk of thromboembolic complications as well as the level of inflammation as measured by CRP. Studies comparing CRP levels in vaccinated vs unvaccinated patients have shown reduced CRP among vaccinated patients [67,68]. However, CRP levels in excess of 40 mg/L and 100 mg/L have been reported in fully vaccinated, hospitalized patients, infected with more recent viral variants [68,69]. A reduction in median CRP level among infected patients from 49.5 mg/L in 2020 to 33 mg/L in 2022 has been reported [68]. This may reduce the number of patients to whom the data presented in this study is applicable, as fewer patients infected with newer COVID-19 variants may experience high or severe degrees of inflammation. However, this does not indicate that evaluation of inflammation as a means to guide treatment is irrelevant in these patients.

5 | CONCLUSIONS

This secondary analysis of the ATTACC and ACTIV-4a trials evaluated the relationship between degree of inflammation based on CRP level and efficacy of treatment with therapeutic doses of unfractionated or LMWH for patients hospitalized with COVID-19. Patients with high levels of inflammation (CRP, 40-99.9 mg/L) derived the greatest benefit from treatment with therapeutic-dose heparin. This study underscores the connection between inflammation and the development of coagulopathy in patients with COVID-19 and demonstrates the need for further research into the mechanisms of

thromboinflammation in COVID-19 in order to provide optimally safe and effective treatment for this disease.

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AUTHOR CONTRIBUTIONS

A. Walborn and J. Paul designed the secondary analysis and prepared the manuscript. A. Heath provided guidance for the statistical design of the study, performed all statistical analysis, and contributed to the writing of the manuscript. P. Lawler, M. Neal, and R. Zarychanski assisted with the study design and the editing of the manuscript. L. Kornblith, B. Hunt, L. Castellucci, and J. Hochman contributed to the editing of the manuscript.

RELATIONSHIP DISCLOSURE

A. Walborn, A. Heath, and B. Hunt report no conflicts of interest. P. Lawler received grant support for conduct of the ATTACC trial (NCT04372589) from the Canadian Institutes for Health Research, the Peter Munk Cardiac Centre, the LifeArc Foundation, the Thistledown Foundation, and the Province of Ontario, as well as support for the ACTIV-4a trial (NCT04505774) from the National Institutes of Health. He is supported by a Heart and Stroke Foundation of Canada National New Investigator award and reports unrelated consulting fees from Novartis, CorEvitas, and Brigham and Women's Hospital, and unrelated royalties from McGraw-Hill Publishing. M. Neal has received grants from the NIH and the United States Department of Defense. He has received research support from Haemonetics, Janssen, and Instrumentation Laboratories. He has received honoraria from Haemonetics, Janssen, and CSL Behring. He serves on the Scientific Advisory board of Haima Therapeutics. R. Zarychanski receives operating grants from Canadian Institutes of Health Research (CIHR), Research Manitoba, LifeArc, Thistledown Foundation, CancerCare Manitoba Foundation, Peter Munk Cardiac Centre, Victoria General Hospital Foundation, and the Manitoba Medical Services Foundation. L. Kornblith serves as a member of the Scientific Advisory Board for Cerus., L. Castellucci's institution received funding from the Academy, Bayer, Amag Pharmaceuticals, LEO Pharma, Sanofa, and Servier and BMS/Pfizer Alliance. J. Hochman reports grants from NHLBI-University of Pittsburgh during the conduct of the study. J. Paul has received research support and consulting fees from Inari Medical, AIDOC, and Boston Scientific.

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