Journal of Thrombosis and Thrombolysis
https://doi.org/10.1007/s11239-020-02145-0

Heparin resistance in COVID-19 patients in the intensive care unit

D. White[1] · S. MacDonald[1] · T. Bull[1] · M. Hayman[1] · R. de Monteverde-Robb[2] · D. Sapsford[2] · A. Lavinio[3] · J. Varley[3] · A. Johnston[3] · M. Besser[1] · W. Thomas[1]

Abstract
Patients with COVID-19 have a coagulopathy and high thrombotic risk. In a cohort of 69 intensive care unit (ICU) patients we investigated for evidence of heparin resistance in those that have received therapeutic anticoagulation. 15 of the patients have received therapeutic anticoagulation with either unfractionated heparin (UFH) or low molecular weight heparin (LMWH), of which full information was available on 14 patients. Heparin resistance to UFH was documented in 8/10 (80%) patients and sub-optimal peak anti-Xa following therapeutic LMWH in 5/5 (100%) patients where this was measured (some patients received both anticoagulants sequentially). Spiking plasma from 12 COVID-19 ICU patient samples demonstrated decreased in-vitro recovery of anti-Xa compared to normal pooled plasma. In conclusion, we have found evidence of heparin resistance in critically unwell COVID-19 patients. Further studies investigating this are required to determine the optimal thromboprophylaxis in COVID-19 and management of thrombotic episodes.

Keywords Thrombosis · Intensive care · COVID-19 · Heparin

Highlights

- Heparin resistance with unfractionated heparin or sub-optimal anti-Xa peak with low molecular weight heparin appeared common in COVID-19 intensive care unit patients that received therapeutic anticoagulation.
- In-vitro spiking of COVID-19 samples from patients in intensive care unit with low molecular weight heparin failed to recover the anti-Xa level as would have been predicted.
- COVID-19 patients have high factor VIII and fibrinogen with low antithrombin which could contribute to the picture seen.
- Further studies are needed to confirm our findings and also describe the mechanism of heparin resistance in these patients as well as optimal management of thrombosis in COVID-19.

Introduction

COVID-19 has been associated with hyper-inflammation, coagulopathy and also a high thrombotic risk in critically unwell patients [1, 2]. Previously we have found a cumulative incidence of 30% for arterial and venous thrombosis on the intensive care unit (ICU) at our hospital for COVID-19 patients [3]. This figure is comparable to other published series of critically unwell patients [4–7]. In an autopsy series pulmonary embolism and microvascular thrombosis was described in patients that had died from COVID-19 [8]. There is a report from an intensive care unit (ICU) cohort of COVID-19 patients that has demonstrated that patients on a therapeutic dose of LMWH, as primary thromboprophylaxis, may develop thrombosis despite this escalated dose; the authors found a cumulative incidence for venous thromboembolism (VTE) of 56% in those patients [9]. There is therefore concern that thrombosis is a significant pathological mechanism of COVID-19. In light of this we aimed to review the clinical and laboratory evidence for heparin

✉ W. Thomas
william.thomas@addenbrookes.nhs.uk

[1] Department of Haematology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

[2] Pharmacy Department, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

[3] Department of Intensive Care Medicine, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

Published online: 22 May 2020

resistance in patients with COVID-19 at our intensive care unit.

Methods

This was a retrospective study of patients with COVID-19 admitted to the ICU at Addenbrooke's Hospital from 1st March 2020 until 21st April 2020 with COVID-19 confirmed by polymerase chain reaction swab of the respiratory tract. This study had full institutional approval from the Trust research and development department. The medical notes (Epic, WI, USA) were interrogated and statistical analysis was performed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). A p-value of 0.05 was considered significant. Clinical information and laboratory information was collected from the medical notes. Patient records were identified that had therapeutic anticoagulation with either subcutaneous LMWH (dalteparin, Pfizer, UK, was the only LMWH used) or intravenous unfractionated heparin (UFH) (Wockhardt UK, UK). Indications for anticoagulation included either radiologically proven venous thromboembolism or prophylaxis of filter thrombosis for patients receiving continuous haemodiafiltration. Heparin resistance was defined as requiring > 35,000 units of heparin per day for those on UFH [10]. For patients on LMWH expected anti-Xa was defined as a 2–4 h peak anti-Xa activity of 0.6–1.0 IU/mL for those on twice daily dosing and > 1.0 IU/mL for those on once daily dosing [11]. At our hospital an activated partial thromboplastin ratio (APTR, defined as the activated partial thromboplastin time (APTT) divided by the mean of the reference range) of 1.5–2.5 is used as a target for therapeutic UFH. Concordance of the APTR/anti-Xa (performed on the same sample) was defined as: (i) APTR 1.5–2.5 and paired anti-Xa 0.3–0.7 IU/mL (ii) APTR < 1.5 and anti-Xa < 0.3 IU/mL or (iii) APTR > 2.5 and anti-Xa > 0.7 IU/mL.

An in-vitro LMWH spiking study was conducted on a convenience sample of ICU COVID-19 confirmed patients, sampled from the patients on ICU that 1 day, by spiking citrated plasma with 0.70 IU/mL dalteparin. Baseline antithrombin (Stago, France), One-stage factor VIII (FVIII:C1), Clauss fibrinogen and anti-Xa (Werfen, UK) were assessed using the ACL TOP 750 (Werfen, UK) prior to spiking. Exogenous antithrombin is not added to this anti-Xa assay. Once spiked, the anti-Xa was reassessed immediately post spiking then at hourly intervals for 3 h. In-vitro recovery of the anti-Xa was determined by spiking a commercial pool of normal pooled plasma (Cryocheck, Precision Biologic, USA) to a target concentration of 0.70 IU/mL with dalteparin. The normal pooled plasma anti-Xa after spiking, with the deduction of the baseline anti-Xa, was defined as a 100% recovery of anti-Xa activity. The process was repeated with 12 ICU COVID-19 positive patient samples. Patient recovery was reported relative to that of the reference recovery from the normal pooled plasma. In-vitro recovery = (observed increase in anti-Xa activity of patient sample from baseline/observed increased in anti-Xa activity of normal pooled plasma from baseline) × 100.

Results

Clinical evaluation of ICU patients

In total 69 patients have been admitted with COVID-19 to the ICU in the study period and 15 patients were identified that had received therapeutic anticoagulation with either LMWH or UFH. The characteristics of these 15 patients are summarised in Table 1; one patient in the group received LMWH (and no UFH) and did not have peak anti-Xa levels performed. Of the 10 infusions of UFH, 8 (80%) patients had heparin resistance (> 35,000 units/day) and of these 8 patients, 3 required > 50,000 units heparin per day to maintain the target APTR (not including the 5000 unit loading dose). In total 17 anti-Xa assays were performed as a pair with an APTR and the results from this are demonstrated in Fig. 1. All patients on UFH had the APTR prolonged to > 1.5 in the first 24 h of starting the infusion. The APTR was concordant with the anti-Xa in 11/17 (73%) results. In total 7 patients received therapeutic LMWH (dalteparin) for management of venous thromboembolism, of which 5 patients had peak anti-Xa performed (after at least 3 doses and whilst still on ICU). For the 3 patients on twice daily therapeutic LMWH regimens the peak was < 0.5 IU/mL in all cases and for the 2 patients on once daily LMWH regimens the peak levels were 0.82 and 0.46 IU/mL. There was no association of heparin resistance with smoking (p = 1.00, Fisher exact test) and no patients had COPD; both previously identified risk factors for heparin resistance [12]. We are not aware of any of these patients having had a further thrombosis on therapeutic anticoagulation however systematic investigation for this has not occurred.

Laboratory testing for heparin resistance

In-vitro spiking, of 12 plasma ICU samples (3 of which came from patients in the cohort that had received therapeutic anticoagulation described above) from 1 day, showed a statistically significant decreased recovery of anti-Xa within 12/12 of the COVID19 patients assessed, p < 0.05 (t test), with the maximum recovery being 82% and the minimum recovery being 58% when compared with a calculated expected recovery. The results are displayed in Table 2. The anti-Xa remained stable in these patients post spiking for

Table 1 Patient characteristics in the study

Number of patients (%)	
Male	11 (73)
Female	4 (27)
Total	15 (100)
Age (%)	
40–49	1 (7)
50–59	5 (33)
60–69	4 (28)
70–79	5 (33)
Weight (%)	
50–99 kg	10 (66)
100–139 kg	3 (20)
> 140–179 kg	2 (13)
Co-morbidities	
Arterial disease[a]	3 (20)
Diabetes mellitus	3 (20)
Chronic respiratory disease	2 (13)
Number of patients receiving haemofiltration (%)	11 (73)
Indications for anticoagulation (%)[b]	
UFH for recurrent haemofiltration circuit clotting	9
UFH for pulmonary embolism	1
LMWH for pulmonary embolism	6
LMWH for line associated thrombosis	1
Number with risk factors for heparin resistance (%)	
Chronic obstructive pulmonary disease	0 (0)
Current or ex-smoker	5 (33)
Median maximum UFH dose/24 h in units (range)	38,400 (22,800–57,600)

LMWH low molecular weight heparin, *UFH* unfractionated heparin

[a]Known atherosclerosis including cerebrovascular disease, peripheral arterial disease or cardiac atheroma

[b]2 patients received therapeutic LMWH for thrombosis followed by UFH for haemofiltration circuit clotting

at least 3 h (data not shown). No correlation was observed between baseline factor VIII, antithrombin or Clauss fibrinogen with the anti-Xa recovery (data not shown). It is notable that these patients have grossly raised fibrinogen and factor VIII levels with mild decreases in the antithrombin (Table 2).

Discussion

The rates of heparin resistance with intravenous UFH are high (80%) and are likely due to the effects of increased fibrinogen and factor VIII on the APTT, which has been described previously and acts to lower the APTT and as a risk factor for heparin resistance [12, 13]. Previously it has been recommended that the anti-Xa should be used for UFH monitoring and dosing however this was not proved to be beneficial over APTT monitoring when examined in a randomised control trial, though potentially lower doses of heparin would be needed [10, 14]. In this study monitoring the heparin effect with anti-Xa for patients on UFH did not appear to add benefit to monitoring via the APTR, which is not unexpected as both measure the effects of heparin in plasma. Given the reasonable performance of the APTR against the anti-Xa assay and concordance in 73% of the paired samples then this could also suggest that higher heparin doses are required to ensure the anti-Xa is 0.3–0.7 IU/mL. In the small cohort of critically ill patients on LMWH with dose based on weight the anti-Xa levels indicate subtherapeutic levels per Garcia et al., 2012 [11]. We have demonstrated that ICU patients with COVID-19 have a decreased in-vitro recovery of anti-Xa activity compared to normal control plasma when spiked to therapeutic concentrations of LMWH; this did not correlate to either factor VIII, fibrinogen or antithrombin levels (data not shown). Previously it has been shown that after 2500 units of dalteparin the anti-Xa activity is in ICU patients is approximately half of the value of that in healthy volunteers, and our findings are in accordance with that data [15].

In-vitro spiking of COVID patients with LMWH demonstrate a decreased recovery of anti-Xa; this further demonstrates heparin resistance within this subset of patients. The difference in recovery by patients suggests there is not a linear response to dosing of LMWH in these patients, when LMWH is known to have a linear dose-dependent response [16]. No correlation was found between the anti-Xa in-vitro recovery and antithrombin, factor VIII and fibrinogen levels however a combination of these factors may contribute to heparin resistance [17].

Spiking of plasma was used to assess recovery of anti-Xa within COVID19 patients in comparison to a normal pool, to ensure commutability of the in-vitro spiking assessment. Dalteparin was used as it has been shown to be commutable to patient samples on comparison to UFH [18]. The ICU patients that had plasma samples spiked with heparin had elevated baseline anti-Xa as many are on UFH in haemodiafiltration circuits and because we are using escalated doses of prophylactic LMWH [3].

This study has limitations. It is a single centre study on a relatively small collection of heterogeneous patients and clinical end points were not examined and laboratory results were not available for all patients. In addition, we have not systematically collected data for every day of a patient's admission and there is a relatively limited number of laboratory data available. We also have not been able to as of yet define an exact mechanism of heparin resistance however as we did not observe a fall in anti-Xa activity over time this suggests there is not time dependant degradation of heparin. Some patients may also be on UFH

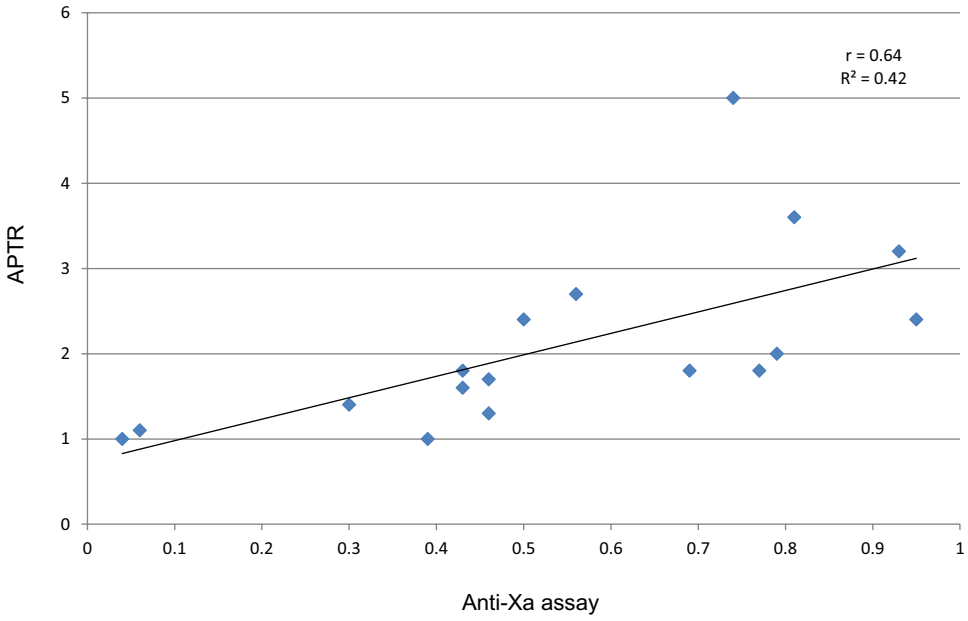

Fig. 1 Relationship between the anti-Xa assay and APTR where paired testing was performed

Table 2 Laboratory analysis of the recovery of anti-Xa levels in 12 patients from ICU with COVID-19. In-vitro recovery of 100% was defined as the increase in anti-Xa activity from baseline in the normal pooled plasma after the addition of the low-molecular weight heparin

Patient number	Antithrombin activity (U/dL; reference range ≥ 79U/dL)	Factor VIII:C1 (IU/mL; reference range 0.52–1.43 IU/mL)	Clauss fibrinogen (g/L; reference range 1.46–3.33 g/L)	Baseline anti-Xa (IU/ML)	Anti-X after addition of LMWH (IU/mL)	In-vitro percentage recovery of anti-Xa
Normal pooled plasma	120.0	0.88	2.85	0.05	0.76	100
1	60.8	1.19	3.63	0.00	0.52	73
2	71.2	3.34	7.39	0.56	0.99	61
3	69.6	4.17	8.20	0.11	0.61	70
4	78.3	2.71	5.52	0.36	0.77	58
5	108.0	2.04	6.52	0.11	0.69	82
6	48.6	2.84	4.67	0.19	0.70	72
7	57.9	2.59	4.69	0.09	0.58	69
8	71.5	3.34	5.52	0.16	0.64	68
9	62.2	4.80	6.54	0.21	0.80	83
10	73.7	2.59	6.54	0.35	0.93	82
11	59.5	3.01	8.50	0.42	0.93	72
12	62.9	2.24	4.31	0.05	0.61	79

however we performed experiments on LMWH which may have differing properties in plasma. In addition the definition of UFH resistance as > 35,000 units per day is arbitrary though is widely quoted in the medical literature, and the evidence for the use of the anti-Xa to guide LMWH dosing limited. Whilst we cannot exclude an effect of the haemodiafiltration on removing heparin, this has not been previously described as a significant effect with negligible amounts of heparin found in ultrafiltrate [19].

Conclusion

Our data demonstrates that patients with COVID-19 in the ICU are heparin resistant that receive therapeutic anticoagulation with UFH and may have decreased peak anti-Xa levels with therapeutic LMWH. The clinical significance of this requires further study, as do the biological mechanisms. Given the resistance to heparin seen then this could

offer some insights into why high rates of thromboprophylaxis failure have been seen in COVID-19 when standard thromboprophylactic LMWH doses are used [3, 4, 6–8]. On the basis of our work we will measure anti-Xa levels for patients on therapeutic LMWH in the ICU to ensure adequate dosing and continue with careful monitoring for those on UFH. In the acute phase UFH may be desirable for thrombosis in the ICU in COVID-19 to ensure therapeutic anticoagulation, which can be closely monitored in this setting, however this could be inconvenient and resource intensive. Further clinical and laboratory studies are required to determine the optimal thromboprophylaxis and management of venous/arterial thrombosis in patients with COVID-19 as well as mechanism of heparin resistance.

Author contributions WT and DW—designed the study, performed the data collection and wrote the manuscript. DW performed the laboratory work. MH—performed data collection. TB, SM, MB, DdM-R, AJ, AL, JV, DS—provided intellectual input.

Funding None received.

Availability of data and material The dataset will not be published.

Compliance with ethical standards

Conflicts of interest MB—speakers fee STAGO, Advisory board Novartis, Cosmopharma, Werfen. WT—speakers fees from Pfizer, Bayer, Takeda and advisory boards for Daiichi-Sankyo, Sanofi and Ablynx. No other authors declared relevant conflicts of interest.

Ethical approval This study had institutional research and ethics approval. As this was a retrospective study patient consent was not deemed necessary.

References

1. Tang N, Li D, Wang X, Sun Z (2020) Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 18:844–847
2. Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, Pesenti A, Peyvandi F, Tripodi A (2020) Hypercoagulability of COVID-19 patients in intensive care unit. A report of thromboelastography findings and other parameters of hemostasis. J Thromb Haemost. https://doi.org/10.1111/jth.14850
3. Thomas W, Varley J, Johnston A, Lavinio A, Symington E, Robinson M et al (2020) Thrombotic complications of patients admitted to intensive care with COVID-19 at a teaching hospital in the United Kingdom. Thromb Res. In-press
4. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM et al (2020) Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. ISSN 0049-3848
5. Cui S, Chen S, Li X et al (2020) Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. J Thromb Haemost. https://doi.org/10.1111/jth.14830
6. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Muller MC, Bouman CC, Beenen LF, Kootte RS, Heijmans J, Smits LP, Bonta PI, van Es N (2020) Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. https://doi.org/10.20944/preprints202004.0345.v1
7. Helms J, Tacquard C, Severac F et al (2020) High risk of thrombosis in patients in severe SARS-CoV-2 infection. Intensive care medicine. https://www.esicm.org/wpcontent/uploads/2020/04/863_author_proof.pdf
8. Llitjos J-F, Leclerc M, Chochois C, Monsallier J-M, Ramakers M, Auvray M, Merouani K (2020) High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. J Thromb Haemost. https://doi.org/10.1111/jth.14869
9. Fox SE, Akmatbekov A, Harbert JL, Li G, Quincy Brown J, Vander Heide RS (2020) Pulmonary and Cardiac Pathology in Covid-19: The first autopsy series from New Orleans. MedRxiv. https://doi.org/10.1101/2020.04.06.20050575
10. Levine MN, Hirsh J, Gent M, Turpie AG, Cruickshank M, Weitz J, Anderson D, Johnson M (1994) A randomized trial comparing activated thromboplastin time with heparin assay in patients with acute venous thromboembolism requiring large daily doses of heparin. Arch Intern Med 154:49–56
11. Garcia DA, Baglin TP, Weitz JI, Samama MM (2012) Parenteral anticoagulants: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 141(2 Suppl):e24S–e43S
12. Kawatsu S et al (2018) Predictors of heparin resistance before cardiovascular operations in adults. Ann Thorac Surg 105(5):1316–1321
13. Uprichard J, Manning RA, Laffan MA (2010) Monitoring heparin anticoagulation in the acute phase response. Br J Haematol 149:613–619
14. Arachillage DRJ, Kamani F, Deplano S, Banya W, Laffan M (2017) Should we abandon the APTT for monitoring unfractionated heparin? Thromb Res 157:157–161
15. Rommers MK, Van der Lely N, Egberts TC, van den Bemt PM (2006) Anti-Xa activity after subcutaneous administration of dalteparin in ICU patients with and without subcutaneous oedema: a pilot study. Crit Care 10(3):R93
16. Burton E (2006) Applied pharmacokinetics & pharmacodynamics: principles of therapeutic drug monitoring. Lippincott Williams & Wilkins. ISBN 0781744318
17. Finley A, Greenberg C (2013) Heparin sensitivity and resistance: management during cardiopulmonary bypass. Anaesth Analg 116:1210–1222
18. Jennings I, Kitchen D, Kitchen S, Woods T, Walker I (2018) The importance of commutability in material used for quality control purposes. IJLH. https://doi.org/10.1111/ijlh.12918
19. Singer M, McNally T, Screaton G, Mackie I, Machin S, Cohen SL (1994) Heparin clearance during continuous veno-venous haemofiltration. Intensive Care Med 20(3):212–215