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COVID-19 patients often show high-titer non-platelet-activating anti-PF4/heparin IgG antibodies

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Running head: Risk for overestimating HIT in COVID 19

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Abstract

Background: Heparin-induced thrombocytopenia (HIT) is a severe adverse reaction to heparin caused by heparin-dependent, platelet activating anti-platelet factor 4 (PF4)/heparin antibodies. Heparin is a cornerstone of treatment in critically ill COVID-19 patients. HIT antibodies can be detected by antigen tests and functional tests. Often strong reactivity in the antigen test is used as surrogate marker for the presence of clinically relevant, platelet activating antibodies. We observed an unexpectedly high percentage of COVID-19 patients, clinically suspected to have HIT, with high titer anti-PF4/heparin antibodies, but a negative functional test.

Objective: We investigated whether in COVID-19 patients a serum-derived factor inhibits the heparin-induced platelet activation test (HIPA).

Methods and Results: 12 COVID-19 patients with suspected HIT were tested. Three samples tested negative in all assays; nine samples tested positive by antigen tests, among which only three tested also positive by HIPA. When we spiked COVID-19 serum or control serum with the human HIT antibody like mAb 5B9, reactivity of 5B9 remained the same. Also the purified IgG fractions of COVID-19 sera testing strongly positive in the PF4/heparin antigen test but negative in the functional test did not show increased reactivity in the functional test in comparison to the original serum. Both results make a functionally inhibitory factor in the serum/plasma of COVID-19 patients highly unlikely.

Conclusion: COVID-19 patients often present with strong reactivity in PF4/heparin antigen tests without the presence of platelet-activating antibodies. Diagnosis of HIT requires confirmation of heparin-dependent, platelets activating antibodies to avoid overdiagnosis and overtreatment with non-heparin anticoagulants.

Keywords: COVID-19, Heparin, Platelet Factor 4, Thrombocytopenia, Thrombosis;

Essentials:

- COVID-19 patients often present with thrombocytopenia and new thrombosis while receiving heparin resulting in suspicion of HIT.

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- High titer of anti-PF4/heparin antibodies are often used as surrogate marker to predict clinically relevant HIT antibodies.
- In COVID-19-patients, a high titer of anti-PF4/heparin antibody test does not strongly predict clinically relevant HIT antibodies.
- Confirmation of HIT in COVID-19 patients requires demonstration of platelet-activating antibodies, regardless of the anti-PF4/heparin antibody titer.

1. Introduction

Heparin-induced thrombocytopenia (HIT) is a severe adverse reaction to heparin. Heparin forms complexes with platelet factor 4 (PF4), which induces anti-PF4/heparin IgG antibodies. When these antibodies activate platelets, this causes a prothrombotic syndrome. HIT typically occurs between day five and 14 of heparin treatment, is characterized by a decrease in platelet counts by more than 50%, and an increased risk for new thrombotic complications.[1] Diagnosis of HIT is based on clinical criteria and laboratory tests. However, diagnosis of HIT in critically ill patients is challenging. As thrombocytopenia and thrombotic complications may occur for many reasons other than HIT, [2] diagnosis strongly relies on laboratory tests for anti-PF4/heparin antibodies. Antigen tests detecting anti-PF4/heparin antibodies are widely available [3] and reliable for ruling out HIT but have limitations in confirming HIT. At best, 50% of patients with a positive anti-PF4/heparin antibody test result will also test positive in sensitive platelet activation assays, such as the serotonin-release assay (SRA) or the heparin-induced platelet activation test (HIPA). These functional tests are restricted to specialized laboratories and turn-around times of results are often longer than one day. Therefore, a high titer of anti-PF4/heparin antibodies, together with clinical symptoms suggestive for HIT, are often used for the decision to switch heparin to an alternative anticoagulant.

COVID-19 is a severe complication of coronavirus SARS-CoV-2 infection, leading to respiratory failure and the need of ventilation or even extracorporeal oxygenation (ECMO) in some patients. COVID-19 is associated with a prothrombotic state, at least in critically ill patients, [4, 5] and heparin is a cornerstone of treatment in this setting.[6, 7] Thrombocytopenia is frequent in critically ill and in intensive care unit patients, especially when extracorporeal circuits, like ECMO are required. Timing of the worsening of COVID-19, usually a week after onset of disease, overlaps with the typical time window of HIT occurrence, i.e. between day five and 14 after initiation of heparin treatment.

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In patients without COVID-19, high titer anti-PF4/heparin antibodies, usually predict reasonably well a positive platelet activation test. [4, 5] We observed several COVID-19 patients with clinical symptoms suggestive of HIT and high titer anti-PF4/heparin antibodies, but a negative HIPA test. We therefore investigated whether a serum-derived factor interfering with the functional HIPA test in COVID-19 patients might be present, similar to the situation faced in patients treated with ticagrelor.[8]

2. Material and methods

2.1 Antigen assays

The presence of anti-PF4/heparin antibodies in serum samples was assessed by one in-house and two commercially available tests. The in-house PF4/heparin EIA was performed as described.[9] The HemosIL AcuStar-HIT IgG CLIA (Instrumentation Laboratory GmbH, Munich, Germany) and the GTI-PF4 ELISA (GTI Diagnostics, Waukesha, WI) were performed according to manufacturer's instructions.

2.2 Functional assays, IgG-fraction and monoclonal antibody 5B9

The heparin-induced platelet activation test was performed as described.[10] Briefly, washed platelets of healthy donors were incubated with patient serum in the presence of buffer, low molecular weight heparin, reviparin 0.2 aFXaU; and unfractionated heparin 100 units. Platelet aggregation was optically assessed every five minutes.

The IgG fractions of patient and control sera were prepared using a protein G column according to standard methods. The IgG fraction was adjusted to a concentration that gave a similar OD result in the EIA as the original serum and then assessed in the HIPA test.

Monoclonal antibody 5B9 with a human Fc part recognizes PF4/heparin complexes and activates platelets in presence of low concentrations of heparin/reviparin in functional HIT tests [11] and mimics a typical human HIT antibody. Serum samples of COVID-19 patients and of a healthy control were spiked with 5B9 in concentrations from 10 to 400 µg/mL and then assessed in the HIPA test.

3. Results and Discussion

3.1 High titer of anti-PF4/Heparin antibodies

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During the observation period from March 2020 - April 2020; 12 COVID-19 patients with suspected HIT were tested. Three samples tested negative in all assays; nine samples tested positive by antigen test among which only three tested also positive by the functional test. The usual frequency of a positive functional test in antigen test positive patients in samples referred to reference laboratories is considerably higher with about 45-50%.[12, 13] We therefore assumed that a factor in the patients' sera might inhibit the functional test. To test for the presence of an inhibitory factor in the serum of COVID-19 patients, potentially associated with false negative results in the HIPA test, we followed two approaches. The first was to use serum samples of COVID-19 patients who tested negative or only very weakly positive (OD value <0.6; or <0.5 U/L) in the antigen assays and negative in the HIPA test and to spike them with the monoclonal antibody 5B9 in increasing concentrations. The reactivity of 5B9 remained the same, whether it was diluted in COVID-19 or control serum (table 1).

3.2 Purification of IgG fraction

We next purified the IgG fraction of COVID-19 serum samples strongly positive in the antigen tests but negative in the HIPA test. The concentration of the purified IgG fractions was adjusted to react with a similar OD in the PF4/heparin EIA as the original sample. When tested with the HIPA, we observed no or a very weak reactivity of the IgG fraction after 20 to 30 minutes in some of the four test cells per sample (table 1). We did not consider these reactions as reflecting the presence of typical HIT antibodies as purified IgG fractions may contain some aggregated IgG, which can alter the HIPA test.

These findings suggest that COVID-19 patients exhibit a different reactivity pattern in HIT tests compared to other patient groups. In non-COVID-19 patients, the likelihood for a positive functional test increases along OD values of the PF4/heparin EIA and the U/ml of the AcuStar HIT IgG test, [12] especially if clinical symptoms suggestive for HIT are present.[14] If the result of the AcuStar HIT-IgG is >4.00 U/mL, the likelihood ratio for the presence of platelet-activating antibodies is 47.53, and at a result of >8.00 U/mL it is 103.4.[12] In contrast, COVID-19 patients may have strongly positive antigen tests, without platelet-activating anti-PF4/heparin antibodies. It is unlikely that this is caused by an inhibitory factor present in the serum of COVID-19 patients. An alternative explanation might be that sera which test only positive in the functional test after addition of PF4 [14-16] are prevalent in COVID-19 patients at an unusually high rate. Although we have not tested this, we regard this explanation as very unlikely. In addition, we have also excluded anti-phospholipid antibodies in these sera.

Some current reports on the incidence of HIT in COVID-19 patients base their diagnosis on clinical criteria only, [17, 18] or use only anti-PF4/heparin antibody tests.[17-19] Because no functional test was used to

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confirm the presence of heparin-dependent platelet-activating antibodies, these reports most likely overestimate the incidence of HIT in COVID-19. This is underscored by the findings of others. Most reports on case series of COVID 19 patients suspected to have HIT found a positive functional HIT test only in less than 35% of anti-PF4/heparin antibody positive samples (Table 2). Although Patell et al report a positive SRA in 3 of 4 patients who had been tested by SRA with suspected HIT and a positive antigen test, [20] two sera gave a borderline positive result only in the SRA, which is rather untypical for real positive HIT sera, which typically give a strong positive (all or nothing) reaction. Daviet et al reported that 7/7 COVID 19 patients with HIT tested positive by the anti-PF4/heparin antibody test and also positive by a functional test (HIPA).[19] However, this report does focus on HIT in COVID 19 patients and patients testing positive by the antigen test only, may not have been reported. At the same time it might very well be that the prevalence of HIT is higher in severely ill COVID 19 patients receiving UFH compared to other ICU patients and HIT should always be considered as an important differential diagnosis in patients with a rapid decrease in platelet count associated with new thrombotic complications.

In conclusion, COVID-19 patients often present with strong reactivity in PF4/heparin antigen tests without the presence of platelet-activating antibodies. In COVID-19 patients, suspicion of HIT requires confirmation of heparin-dependent, platelet-activating antibodies in spite of strong reactivity in PF4/heparin antigen tests to avoid overtreatment with non-heparin anticoagulants.

Addendum

J. Brodard, J.A. Kremer Hovinga and J-D. Studt made the first observation that a strongly positive antigen test may not predict for a positive functional test in COVID-19 patients suspected for HIT and performed the initial laboratory tests for HIT.

P. Fontana, J-D. Studt managed COVID-19 patients suspected to have HIT and provided patient sera.

Y. Gruel provided the 5B9 antibody.

A. Greinacher designed the study interpreted the experiments and wrote the manuscript.

All authors contributed to writing the manuscript and reviewed the final version of the manuscript.

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Conflicts of interest:

AG performed consultant work for Instrumentation Laboratories.

JB, JKH, PF, J-DS, YG have no conflicts of interest to declare

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Table 1. COVID-19 patients evaluated for heparin-induced thrombocytopenia

Pat.	Sex	Age	4Ts score	Antigen assays			HIPA		Purified IgG fraction	
				HIT-IgG Acustar (U/L)	GTI-PF4 IgG ELISA (OD)	PF4/heparin EIA (OD)	Standard	in presence of mAb 5B6	PF4/heparin EIA (OD)	HIPA
1	M	54	5	0.13	0.262	0.26	negative	positive	n.d	n.d
2	M	73	6	0.3	0.5	0.5	negative	positive	n.d	n.d
3	M	59	4	0.4	0.558	0.56	negative	positive	n.d	n.d
4	M	56	4	0.0	0.127	0.13	negative	positive	n.d	n.d
5	M	58	4	2.6	n.a	1.71	negative	n.d	1.2	negative
6	F	54	4	41.3	2.63	2.43	negative	n.d	1.91	1/4 test cells with very weak reactivity after 30’
7	M	81	5	11.3	2.9	2.18	negative	n.d	2.11	2/4 test cells with very weak reactivity after 20’ and 25’, respectively

Abbreviations: EIA, enzyme-linked immunosorbent assay; HIPA, heparin-induced platelet activation test; HIT, heparin-induced thrombocytopenia; mAb 5B9, monoclonal antibody 5B9; n.d, not done; OD, optical density.

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Table 2: Published references in COVID-19 patients with suspected heparin-induced thrombocytopenia

Published references	Patients population	Antigen assay positive	Functional test positive
1. Parzy G et al., (2020)[21]	14 COVID-19 related ARDS on ECMO	No information given how many patients tested by PF4/heparin antigen test only	3
2. May JE et al., (2020)[22]	7 hospitalized COVID 19 patients	7	1
3. Riker RR et al., (2020)[23]	3 COVID 19 patients with thrombocytopenia	3	1
4. Lingamaneni P et al., (2020)[24]	5 COVID 19 patients admitted to ICU with clinically suspected HIT	3	1
5. Patell R et al., (2020)[20]	8 COVID-19 patients receiving UFH with clinically suspected HIT	5	1 of 4 tested borderline pos 2 of 4 tested cells
6. Daviet F et al., (2020)[19]	7 COVID 19 patients with ARDS and HIT	7	7

Abbreviations: ECMO, Extracorporeal Membrane Oxygenation; ARDS, Acute Respiratory Distress Syndrome ARDS; ICU, Intensive Center Unit.

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