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Contrast between Prevalence of HIT Antibodies and Confirmed HIT in Hospitalized COVID-19 Patients: A Prospective Study with Clinical Implications

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In hospitalized severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)-infected patients, elevated prevalence of thromboembolic events (TE) has been reported with subsequent recommendations to reinforce prophylactic anticoagulation using low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH).[1] Heparin-induced thrombocytopenia (HIT) is an immune-mediated prothrombotic disorder resulting from immunoglobulin G (IgG) platelet-activating antibodies against platelet chemokines, mainly platelet factor-4 (PF4), bound to heparin (HIT-associated antibodies).[2] Due to SARS-CoV-2-mediated exacerbated inflammatory and procoagulant response, patients may be subject to HIT. To date, HIT data in SARS-CoV-2-infected patients are scarce, limited to the report of a few HIT cases confirmed using platelet activation assay, and HIT-associated antibody prevalence remains poorly investigated.[3–13] Our prospective cohort study aimed to characterize patients with clinical suspicion of HIT and determine HIT-associated antibody prevalence in hospitalized SARS-CoV-2-infected patients. This study was part of the ICU-COVID and French-COVID cohort registries approved by our institutional ethics committee (IDRCB, 2020-A00256–33; CPP, 11–202020.02.04.68737).

All consecutive SARS-CoV-2-infected adults admitted from March 2, 2020 to May 7, 2020 to the intensive care unit (ICU) and medical wards were included. Lower limb deep vein thrombosis (DVT) was diagnosed using duplex ultrasound performed weekly for critically ill patients and based on clinical suspicion for noncritically ill patients. Suspected pulmonary embolism was confirmed using chest computed-tomography/angiography.

For each patient referred by the attending physician for HIT suspicion, we systematically performed (1) 4Ts-score[2]; (2) qualitative particle gel immunoassay (PaGIA) (ID-PaGIA Heparin/PF4-Antibody Test, Bio-Rad, United States); (3) enzyme-linked immunosorbent assay (EIA; ZYMUTEST-HIA-IgG, HYPHEN BioMed, France) with 0.500 optical density (OD) as a positivity threshold; and (4) heparin-induced platelet activation-assay (HIPLA) with a positivity threshold of 13%

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Table 1 Characteristics of SARS-CoV-2-infected patients with heparin-induced thrombocytopenia suspicion

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Age/gender	77/M	63/M	60/M	63/M	71/M	64/M	66/M	50/M	67/M	65/M
Heparin in the 100 days prior to hospitalization (number of days)	No	No	No	No	No	No	No	No	No	Enoxaparin 8,000 IU bid (5 d)
Recent surgery (<72 h)	No	No	No	No	No	No	No	No	No	No
Heparin exposure daily dose (number of days)	Enoxaparin 4,000 IU od (2) UFH 20,000 IU (6)	Enoxaparin 4,000 IU od (3) UFH 16,000 IU (6)	Enoxaparin 3,000 IU bid (5) UFH 50,000 IU (16)	Enoxaparin 4,000 IU bid (2) UFH 40,000 IU (9)	Enoxaparin 7,000 IU bid (4) UFH 46,000 IU (17)	Enoxaparin 3,000 IU bid (5) UFH 20,000 IU (13)	UFH 35,000 IU (2)	Enoxaparin 4,000 IU bid (3)	UFH 30,000 IU (23)	Enoxaparin 4,000 IU bid (2) UFH 30,000 IU (22)
Indication for anticoagulation	Prophylaxis	Prophylaxis	DVT (day 6)	AF[a]	AF[a]	Prophylaxis	PE (day 2)	Prophylaxis	AF[a]	PE suspicion (day 3)
Platelet count at start of heparin (G/L)/in-hospital day	136/3	250/5	153/2	177/1	240/1	223/1	121/2	227/1	363/1	317/1
Nadir platelet count (G/L)/in-hospital day	59/8	11/11	36/23	38/12	77/21	67/19	59/9	136/12	138/24	138/36
Number of days of heparin when tested for HIT	11	14	21	12	21	18	2	12	23	24
Confirmed thrombosis/ in-hospital day	None	None	DVT/6	None	None	DVT/24	PE/2 and DVT/7	Stroke/7	DVT/2	PE/4 and DVT/17
4Ts-score	5	4	4	4	4	6	4	6	6	6
PaGIA rapid test (Bio-Rad)	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
EIA HIT-associated IgG antibody(OD) (Zymutest)[b]	0.10/negative	0.28/negative	0.33/negative	0.12/negative	NP	2.40/positive	0.28/negative	0.34/negative	0.10/negative	0.12/negative
Emo-test (HEPLA %) functional test[c]	0.1	11	16	0	12	74	7	11	NP	7
Serotonin release assay functional test[d]	NP	NP	NP	NP	NP	Positive[a]	NP	NP	NP	NP
Alternative nonheparin treatment	None	None	None	None	None	Argatroban then danaparoïd	None	None	None	argatroban
Outcome/in-hospital day	Death/25	Alive	Death/31	Death/33	Death/21	Alive	Alive	Death/7	Death/24	Death/36

Abbreviations: AF, atrial fibrillation; bid, twice daily; DVT, deep venous thrombosis; HEPLA, heparin-induced platelet activation index; NP, not performed; od, once daily; OD, optical density units; PE, pulmonary embolism; SRA, serotonin release assay; UFH, unfractionated heparin.

Note: Only patient 3 required extracorporeal membrane oxygenation. Seven patients died from multiorgan failure (patients 4, 8, 9, and 10), cardiorespiratory arrest (patients 3 and 5), and bacterial superinfection-related multiorgan failure.

[a]SRA was 94% at 0.1 IU/mL, 103% at 0.5 IU/mL, and 3% at 1 IU/mL heparin.

[b]Optical density, with 0.500 as positivity threshold.

[c]Heparin-induced platelet activation index based on P-selectin expression measured using anti-CD62 in flow cytometry, with 13% as positivity threshold.

[d]Serotonin release assay (0.1 IU/mL) with 30% as positivity threshold.

(Emo-test HIT-CONFIRM, EMOSIS-SAS, France).[14] Serotonin release assay (SRA; positivity threshold of 30%; 0.1, 0.5, and 10 IU/mL heparin), considered as the gold standard to confirm HIT diagnosis, was performed if HIT was highly likely.[2]

Additionally, in an exploratory study, we screened HIT-associated antibodies using EIA with both ZYMUTEST-HIA-IgG and ZYMUTEST-HIA-IgGAM (HYPHEN BioMed, France) in consecutive patients admitted from March 17, 2020 to April 21, 2020.

Quantitative variables are expressed as medians [25th–75th percentiles] and categorical variables as percentages. Mann–Whitney and Fisher's exact tests were used for comparisons as appropriate. Pearson correlation coefficients were determined. $p < 0.05$ was considered as significant.

From March 2, 2020 to May 7, 2020, 626 SARS-CoV-2-infected patients were admitted, 184 patients to the ICU and 442 to the medical ward. HIT was clinically suspected in 10 patients and confirmed in one (►**Table 1**). Considering that all patients were exposed to heparin, HIT prevalence was 1.6/1,000 patients. All patients with suspected HIT were in the ICU and had received UFH (except for one patient). Patient 6 (body mass index: 30 kg/m^2) developed cholecystitis complicated by renal failure, requiring cholecystectomy. On HIT suspicion associated with confirmed DVT, he received argatroban during 13 days (initial dose: 0.5 µg kg^{-1} min^{-1}). Meanwhile, the platelet count increased on day 3 (89 G/L) and normalized on day 7 (264 G/L). Danaparoid (1,250 IU/12 h) was further administered for 19 days, once the renal function was normalized. HIT diagnosis was confirmed with positive SRA. He was discharged with 5 mg bid apixaban. In the nine other patients with HIT suspicion, platelet counts declined within 6 to 36 days of heparin exposure; however, HIT-associated antibodies were undetectable with PaGIA, anti-IgG EIA (OD: 0.100–0.340), and functional HIPLA (<13%, except patient 3).

Using EIA in 172 consecutive SARS-CoV-2-infected patients including 64 ICU and 108 noncritically ill patients admitted from March 17, 2020 to April 21, 2020 (►**Table 2**), we observed an overall 33% prevalence of anti-IgG/A/M and 11% anti-IgG HIT-associated antibodies (all positive for anti-IgG/A/M), without significant differences in relation to the hospitalization site. These patients have received LMWH (enoxaparin) in 87% of cases. TE compared with non-TE patients exhibited no difference between HIT-associated IgG/A/M antibody titers (OD: 0.401 [0.211–0.672] vs. 0.328 [0.217–0.534], $p = 0.11$) or between HIT-associated IgG/A/M antibody proportions with OD >1.0 (17 vs. 9%, $p = 0.4$). Moreover, HIT-associated IgG/A/M antibodies (OD: 0.344 [0.218–0.550] vs. 0.318 [0.206–0.686], $p = 0.69$) and the proportion of HIT-associated IgG/A/M antibodies with OD >1.0 (20 vs. 10%, $p = 0.09$) did not significantly differ between survivors and nonsurvivors. In 11 samples with the highest HIT-associated IgG/A/M antibodies (OD range: 1.020–4.500; five also positive for IgG), HIPLA was weakly positive in one patient only (14%; OD: 4.500) and negative in all others. None of the patients received HIT diagnosis. HIT-associated IgG/A/M antibody titers were correlated neither

Table 2 Clinical characteristics and laboratory data in the 172 consecutive COVID-19[a] patients screened for HIT-associated antibodies (EIA)

Demographics and past medical history	
Female/male gender	53/119 (31%/69%)
Age, y	68 [58–77]
Body-mass index, kg/m^2	27 [24–31]
Diabetes	76 (45%)
Ischemic heart disease	44 (26%)
Autoimmune disease	8 (5%)
Severity of disease and outcomes during hospitalization stay	
Critically ill patients	64 (37%)
PaO_2/FiO_2, mmHg ($N = 64$)[b]	144 [95–142]
Maximal oxygen flow, L/min ($N = 108$)[c]	3 [1–9]
Thrombotic events	41 (25%)
• Isolated deep venous thrombosis	24
• Isolated pulmonary embolism	11
• Deep venous thrombosis and pulmonary embolism	5
• Stroke	1
• Death	25 (15%)
Anticoagulant treatment at the time of sampling	
Heparin exposure ($N = 172$)	
Unfractionated heparin	23 (13%)
• Prophylactic dose	9
• Therapeutic dose	14
Low-molecular-weight heparin (enoxaparin)	149 (87%)
• Prophylactic dose (standard or reinforced)	105
• Therapeutic dose	44
Median time of blood sampling postadmission ($N = 172$)	4 [3–8]
Laboratory parameters, units (reference interval)	
Leukocytes, G/L (4.0–10.0)	7.8 [5.6–10.4]
Hemoglobin, g/dL (*N*, 13.0–17.0 in males; 12.0–16.0 in females)	11.7 [10.3–12.8]
Platelets, G/L (150–450)	285 [190–364]
Prothrombin time, ratio (0.8–1.20)	1.08 [1.03–1.16]
Fibrinogen, G/L (2.0–4.0)	6.69 [5.15–8.03]
D-dimers, ng/mL (< 500)	1,900 [805–3,275]
Serum creatinine, µmol/L (64–104) ($N = 167$)	80 [62–121]
C-reactive protein, mg/L (≤5) ($N = 130$)	91 [39–175]
Antithrombin, IU/dL (80–120)	93 [82–103]
Anti-Xa activity, IU/mL	
• Unfractionated heparin ($N = 23$)	0.25 [0.19–0.46]
• Low-molecular-weight heparin ($N = 147$)	0.19 [0.10–0.35]

(*Continued*)

Table 2 (*Continued*)

HIT-associated antibodies (EIA)	
IgG/A/M antibodies, OD ($N = 172$)	0.343 [0.217–0.570]
• Critically ill ($N = 64$)	0.330 [0.210–0.600]
• Noncritically ill ($N = 108$)	0.346 [0.219–0.557]
IgG antibodies, OD ($N = 145$)	0.104 [0.057–0.202]
• Critically ill ($N = 50$)	0.098 [0.053–0.185]
• Noncritically ill ($N = 95$)	0.120 [0.070–0.230]
Positive HIT-associated IgG/A/M antibodies (>0.500 OD)	57 (33%)
• Critically ill patients	21
• Noncritically ill patients	36
Positive HIT-associated IgG antibodies (>0.500 OD)	16 (11%)
• Critically ill patients	6
• Noncritically ill patients	10
HIT-associated IgG/A/M antibodies with OD > 1.0	19 (11%)
In relation to thromboembolic events (TE)	
• Patients with TE ($N = 41$)	7 (17%)
• Patients without TE ($N = 131$)	12 (9%)
In relation to outcome	
• Nonsurvivors ($N = 25$)	5 (20%)
• Survivors ($N = 147$)	14 (10%)

Abbreviations: HIT, heparin-induced thrombocytopenia; IgG, immunoglobulin G; OD, optical density.
Note: data are expressed as median [25th–75th percentiles] or numbers (percentages) as appropriate. Five COVID-19 patients required extracorporeal membrane oxygenation, with the following IgG/A/M and IgG HIT-antibody titers: (0.812 and 1.125), (1.703 and 0.097), (0.297 and 0.092), (0.400 and 0.056), and (0.484 and 0.143), respectively.
[a]SARS-CoV-2 infection was diagnosed on admission using RT-PCR (Cobas-SARS-CoV-2 kits, Roche, France).
[b]In the ICU patients.
[c]In the medical ward patients.

with fibrinogen ($p = 0.09$), nor with C-reactive protein ($p = 0.11$).

In addition to the HIT prevalence, we provided new data on the overall HIT-associated antibody prevalence in hospitalized SARS-CoV-2-infected patients, predominantly receiving LMWH. The 1.6/1,000 patient prevalence of HIT is closer to values previously reported in critically ill patients (0.20–0.45%) than to values in cardiac surgery patients (~1–3%)[5] and lower than the estimated overall HIT incidence (0.76%).[6] Patell et al reported a cumulative 12% incidence of positive antibodies in hospitalized COVID-19 patients using an immunoassay, with one confirmed HIT case.[6] Daviet et al confirmed HIT diagnosis in seven out of 86 ICU COVID-19 patients.[9] The lower frequency of confirmed HIT in our ICU patients could be partly explained by the preferential use of LWMH (in ~70% of our ICU patients) rather than UFH. Upon clinical HIT suspicion, we calculated the 4Ts-score and whatever the score probability was, we performed HIT-associated antibody immunoassays since this score had not been validated in SARS-CoV-2-infected patients. We were able to exclude HIT diagnosis in nine patients. In addition, we systematically performed a supplemental functional assay (HIPLA), which turned out to be negative, but for one patient (weakly positive). Noteworthy, in our series of patients referred for HIT suspicion, non-HIT-related thrombocytopenia/thrombosis was associated with a high risk for fatal outcome, mostly in the setting of multiorgan failure. Conversely, we found an elevated frequency of HIT-associated IgG/A/M antibodies in hospitalized SARS-CoV-2-infected patients, consistent with the highest seroconversion rates observed in cardiac surgery patients assisted with extracorporeal membrane oxygenation or ventricular assist device (~25–75%). These antibodies seem to be nonfunctional since the platelet activation assay (HIPLA) was essentially negative in the patients with the highest EIA OD ($N = 11$). However, HIT-associated IgG antibody frequency was as expected lower, similar to seroconversion rates observed among medical and surgical patients (~4–17%).

Limitations of the current study include the single-center setting and the short study period.

To conclude, our data suggest that COVID-19 patients receiving LMWH do not appear to be especially susceptible to HIT. Prevalence of HIT-associated antibodies is comparable to other critical illness settings and those antibodies do not seem to be associated with increased risk of TE and death.

Conflict of Interest
None declared.

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