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

Maxime Delrue1,2 Virginie Siguret1,3 Marie Neuwirth1,3 Caren Brumpt1 Sebastian Voicu4,5 Ruxandra Burlacu6 Damien Sène6 Benjamin G. Chousterman7,8 Nassim Mohamedi9 Thomas Lecompte10,11 Bruno Mégarbane4,5 Alain Stépanian1,2

1 Hematology Laboratory, AP-HP Lariboisière Hospital, Paris University, Paris, France
2 EA 3518, Paris University, Paris, France
3 INSERM UMRS 1140, Paris University, Paris, France
4 Department of Medical and Toxicological Critical Care, AP-HP Lariboisière Hospital, Paris University, Paris, France
5 INSERM UMRS 1144, Paris University, Paris, France
6 Department of Internal Medicine, AP-HP Lariboisière Hospital, Paris University, Paris, France
7 Department of Anesthesiology and Critical Care, AP-HP Lariboisière Hospital, Paris University, Paris, France
8 INSERM UMRS 942, Université de Paris, Paris, France
9 Department of Clinical Physiology, AP-HP Lariboisière Hospital, Paris University, Paris, France
10 Département de Médecine, Service d’Angiologie et d’Hémostase, Hôpitaux Universitaires de Genève, Genève, Switzerland
11 Faculté de Médecine Geneva Platelet Group (GpG), Université de Genève, Geneva, Switzerland

Address for correspondence Alain Stépanian, PharmD, PhD, AP-HP Hôpital Lariboisière, Service d’Hématologie biologique, 2 rue Ambroise Paré, 75010 Paris, France (e-mail: alain.stepanian@aphp.fr).

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-score2; (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%
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/gender</th>
<th>Heparin in the 100 days prior to hospitalization (number of days)</th>
<th>Recent surgery (&lt;72 h)</th>
<th>Heparin exposure daily dose (number of days)</th>
<th>Indication for anticoagulation</th>
<th>Platelet count at start of heparin (G/L)/in-hospital day</th>
<th>Nadir platelet count (G/L)/in-hospital day</th>
<th>Number of days of heparin when tested for HIT</th>
<th>4T5-score</th>
<th>PaGIA rapid test (Bio-Rad)</th>
<th>EIA HIT-associated IgG antibody (OD) (Zymutest)</th>
<th>Emo-test (HEPLA %) functional test</th>
<th>Serotonin release assay functional test</th>
<th>Alternative nonheparin treatment</th>
<th>Outcome/in-hospital day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>77/M</td>
<td>No</td>
<td>No</td>
<td>Enoxaparin 4,000 IU od (2) UFH 20,000 IU (6)</td>
<td>Prophylaxis</td>
<td>136/3</td>
<td>59/8</td>
<td>11</td>
<td>5</td>
<td>Negative</td>
<td>0.10/negative</td>
<td>0.1/negative</td>
<td>NP</td>
<td>None</td>
<td>Death/25</td>
</tr>
<tr>
<td>Patient 2</td>
<td>63/M</td>
<td>No</td>
<td>No</td>
<td>Enoxaparin 4,000 IU od (3) UFH 16,000 IU (6)</td>
<td>Prophylaxis</td>
<td>250/5</td>
<td>111/11</td>
<td>14</td>
<td>4</td>
<td>Negative</td>
<td>0.28/negative</td>
<td>0.28/negative</td>
<td>NP</td>
<td>None</td>
<td>Alive</td>
</tr>
<tr>
<td>Patient 3</td>
<td>60/M</td>
<td>No</td>
<td>No</td>
<td>Enoxaparin 3,000 IU bid (5) UFH 50,000 IU (16)</td>
<td>DVT/6</td>
<td>153/2</td>
<td>36/23</td>
<td>21</td>
<td>4</td>
<td>Negative</td>
<td>0.33/negative</td>
<td>0.33/negative</td>
<td>NP</td>
<td>None</td>
<td>Death/31</td>
</tr>
<tr>
<td>Patient 4</td>
<td>63/M</td>
<td>No</td>
<td>No</td>
<td>Enoxaparin 4,000 IU bid (2) UFH 40,000 IU (9)</td>
<td>DVT/6</td>
<td>177/1</td>
<td>38/23</td>
<td>12</td>
<td>4</td>
<td>Positive</td>
<td>0.12/negative</td>
<td>0.12/negative</td>
<td>NP</td>
<td>None</td>
<td>Death/33</td>
</tr>
<tr>
<td>Patient 5</td>
<td>71/M</td>
<td>No</td>
<td>No</td>
<td>Enoxaparin 7,000 IU bid (4) UFH 46,000 IU (17)</td>
<td>DVT/24</td>
<td>240/1</td>
<td>77/21</td>
<td>21</td>
<td>6</td>
<td>Positive</td>
<td>2.40/positive</td>
<td>0.28/negative</td>
<td>NP</td>
<td>None</td>
<td>Death/21</td>
</tr>
<tr>
<td>Patient 6</td>
<td>64/M</td>
<td>No</td>
<td>No</td>
<td>Enoxaparin 3,000 IU bid (5) UFH 20,000 IU (13)</td>
<td>PE (day 2)</td>
<td>67/19</td>
<td>67/19</td>
<td>18</td>
<td>4</td>
<td>Positive</td>
<td>0.28/negative</td>
<td>0.28/negative</td>
<td>Positive</td>
<td>None</td>
<td>Alive</td>
</tr>
<tr>
<td>Patient 7</td>
<td>66/M</td>
<td>No</td>
<td>No</td>
<td>UFH 35,000 IU (2)</td>
<td>Prophylaxis</td>
<td>223/1</td>
<td>67/9</td>
<td>21</td>
<td>6</td>
<td>Positive</td>
<td>0.12/negative</td>
<td>0.12/negative</td>
<td>NP</td>
<td>None</td>
<td>Alive</td>
</tr>
<tr>
<td>Patient 8</td>
<td>50/M</td>
<td>No</td>
<td>No</td>
<td>Enoxaparin 4,000 IU bid (3) UFH 30,000 IU (23)</td>
<td>PE (day 2 and DVT/7)</td>
<td>121/2</td>
<td>59/9</td>
<td>12</td>
<td>6</td>
<td>Positive</td>
<td>0.1/negative</td>
<td>0.1/negative</td>
<td>NP</td>
<td>None</td>
<td>Death/7</td>
</tr>
<tr>
<td>Patient 9</td>
<td>67/M</td>
<td>No</td>
<td>No</td>
<td>Enoxaparin 4,000 IU bid (2) UFH 30,000 IU (22)</td>
<td>Stroke/7</td>
<td>227/1</td>
<td>136/12</td>
<td>23</td>
<td>6</td>
<td>Positive</td>
<td>0.1/negative</td>
<td>0.1/negative</td>
<td>NP</td>
<td>None</td>
<td>Death/24</td>
</tr>
<tr>
<td>Patient 10</td>
<td>65/M</td>
<td>Enoxaparin 8,000 IU bid (5 d)</td>
<td>No</td>
<td>UFH 30,000 IU (2)</td>
<td>PE suspicion (day 3)</td>
<td>36/3</td>
<td>138/24</td>
<td>24</td>
<td>6</td>
<td>Positive</td>
<td>0.28/negative</td>
<td>0.34/negative</td>
<td>NP</td>
<td>None</td>
<td>Death/36</td>
</tr>
</tbody>
</table>

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.

aSRA was 94% at 0.1 IU/mL, 103% at 0.5 IU/mL, and 3% at 1 IU/mL heparin.
bOptical density, with 0.500 as positivity threshold.
cHeparin-induced platelet activation index based on P-selectin expression measured using anti-CD62 in flow cytometry, with 13% as positivity threshold.
dSerotonin release assay (0.1 IU/mL) with 30% as positivity threshold.
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 (\(\text{Table 1}\)). 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/C0 1 min) 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 11 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), anti-IgG HIT-associated antibodies were undetectable with PaGIA, declining within 6 to 36 days of heparin exposure; however, positive SRA. He was discharged with 5 mg bid apixaban. In 11 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 (\(\text{<13\%}, \text{except patient 3}\)).

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 (\(\text{Table 2}\)), 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)

<table>
<thead>
<tr>
<th>Demographics and past medical history</th>
</tr>
</thead>
</table>
| **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

| 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)

<table>
<thead>
<tr>
<th>Laboratory parameters, units (reference interval)</th>
</tr>
</thead>
</table>
| **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]  
| **Creatine protein, mg/L (<5) (N = 130)** | 91 [39–175]  
| **Antithrombin, IU/dL (80–120)** | 93 [82–103]  
| **Anti-Xa activity, IU/mL** | 0.25 [0.19–0.46]  
| **Unfractionated heparin (N = 23)** |  
| **Low-molecular-weight heparin (N = 147)** | 0.19 [0.10–0.35]  

(Continued)
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%) and lower than the estimated overall HIT incidence (0.76%).

Patel et al reported a cumulative 12% incidence of positive antibodies in hospitalized COVID-19 patients using an immunoassay, with one confirmed HIT case. Daviet et al confirmed HIT diagnosis in seven out of 86 ICU COVID-19 patients. 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.

Acknowledgments
The case investigations, analysis, and manuscript preparation were completed as part of the official duties at the university hospital. We would like to thank Pr. Dominique Helley (Hematology Laboratory, Hôpital Européen Georges Pompidou, AP-HP, Paris, France) for serotonin release assay. We would like to acknowledge the APHP Lariboisière COVID Group for the management of COVID-19 patients admitted in our hospital.

References


