Letter to the Editor

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The high frequency of anti-PF4/heparin antibodies in patients with COVID-19 is neither related to heparin treatment or to an increased incidence of thrombosis

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To the Editor,

Heparin-induced thrombocytopenia (HIT) is a severe, life threatening immune complication of heparin therapy associated with thrombocytopenia and high risk of thrombosis. HIT is caused by IgG-specific antibodies directed to complexes containing heparin (hep) and platelet factor 4 (PF4). The frequent use of anticoagulation during COVID-19 has raised the question of HIT diagnosis. On one hand, some cases of HIT confirmed by serotonin release assay have been reported in patients with COVID-19 [1–3], but on the other hand, one prospective study reported an overall 33% prevalence of anti-IgG/A/M and 11% anti-IgG HIT-associated antibodies after a 4 days

hospitalization without being related to the diagnosis of HIT [4]. In this latter study, the HIT prevalence remained extremely low (1.6/1,000 patients) [4]. We hypothesized that the presence of anti-PF4/hep antibodies may be related to the pathophysiology of the disease and not to the heparin therapy. Thus, we performed anti-PF4/hep antibodies detection in 119 hospitalized patients with COVID-19 pneumonia (confirmed by a positive RT-PCR) at the admission, before any heparin treatment, and during the hospitalization, in the absence of any suspicion of HIT. This prospective study was conducted in Foch Hospital (Suresnes, France) between July 6, 2020 and March 23, 2021. The study was approved by the local ethics committee of Foch hospital. Demographic, clinical, biological and radiological characteristics were recorded. Routine blood examination was performed including platelet count (DxH 800, Beckman Coulter, Inc., Brea, CA), fibrinogen and d-dimer measurement (ACL Top analyzer, Werfen, Le Pré-Saint Gervais, France). The presence of anti-PF4/hep antibodies was assessed using the polyspecific Asserachrom® HPIA assay (Stago, Asnières, France), according to the manufacturer's instructions. When positive, IgG-specific anti-PF4/hep antibodies were screened using the Asserachrom® HPIA-IgG (Stago) assay. Categorical variables were expressed as percentages and continuous variables as medians and interquartile range (IQR). In univariate analysis, we determined the differences in medians using unpaired t-test (Mann–Whitney U test) or paired t-test (Wilcoxon) as appropriate, for continuous variable, and differences in proportions were determined using the Fischer exact test.

We detected anti-PF4/hep IgG/A/M in 28 patients (overall 23.5% prevalence) and among them, 9 (32.1%) displayed IgG-specific anti-PF4/hep antibodies. In patients positive for anti-PF4/hep IgG/A/M antibodies, the median value of optical density (OD) of anti-PF4/hep IgG/A/M antibodies was 0.752 [IQR: 0.4823–1.315] with a maximum

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of 1.934. OD values were lower than one in 18/28 (64%) patients. Surprisingly, seven patients had anti-PF4/hep IgG/A/M upon admission, prior to initiation of heparin therapy (IgG-specific antibodies were detected in three of them). All were then treated by heparin during their hospitalization, without any HIT complication, and anti-PF4/hep IgG/A/M antibodies became negative in one of them after 12 days of hospitalization (Figure 1A). None of these seven patients were admitted to the ICU department. Anti-PF4/hep IgG/A/M antibodies also appeared in 21 patients during their hospitalization (IgG-specific antibodies were detected in six of them) with a median time of antibodies testing of 10 days [IQR: 7–14] after the admission (Figure 1B). Three positive patients were tested twice during their hospitalization and one of them showed a marked decrease in OD of anti-PF4/hep IgG/A/M antibodies 6 days after the positive sample (Figure 1B). Interestingly, OD values also increased significantly during the hospitalization in negative patients (Wilcoxon $p < 0.0001$) and three patients became even borderline positive (Figure 1C). Despite the presence of anti-PF4/hep IgG/A/M antibodies, platelet count was significantly higher during the hospitalization when compared to platelet count at admission ($242 \times 10^9/L$ [186–342] vs. $182 \times 10^9/L$ [137–240], $p = 0.031$), and the median platelet nadir was not significantly different between patients with and without anti-PF4/hep IgG/A/M antibodies (Table 1). Considering only patients with IgG-specific anti-PF4/hep antibodies, the presence of

IgG-specific anti-PF4/hep was associated with a mild thrombocytopenia in 4/9 (44%) patients ($91, 101, 104$ and $128 \times 10^9/L$) while the HIT pre-test probability was high in only one of them (4 Ts score: 6). Among these patients with IgG-specific anti-PF4/hep antibodies, two thrombotic events were recorded. The first one developed an abdominal aortic thrombosis occurring 17 days following initiation of unfractionated heparin (UFH) therapy. He also had an active bronchial adenocarcinoma. The second patient was admitted to the emergency department for respiratory distress which revealed a pulmonary embolism prior to initiation of low-molecular-weight heparin (LMWH). In the follow-up platelet count increased despite LMWH therapy in these two patients, which did not support HIT diagnosis.

In order to identify risk factors for development of anti-PF4/hep IgG/A/M antibodies, patients were divided into negative and positive groups, and a specific group including patients who became positive during the hospitalization. The overall median time from symptom onset to anti-PF4/hep testing was 16 days [IQR: 11–21] and was similar between patients with and without anti-PF4/hep antibodies (Table 1). In the positive group, UFH therapy was used in 25% whereas 61% of patients were treated with LMWH and 11% of patients on long-term anticoagulation did not receive heparin during their hospitalization. Surprisingly, two patients who did not receive heparin therapy developed anti-PF4/hep IgG/A/M antibodies during their hospitalization. Moreover, frequencies of UFH or LMWH

Figure 1: Optical density of IgG/A/M anti-PF4/heparin antibodies in patients with COVID-19.

Optical density of IgG/A/M anti-PF4/heparin antibodies in seven patients positive at admission (A), in 21 patients during their hospitalization (B) and in negative patients (C). Stars indicate patients with IgG-specific anti-PF4/heparin antibodies. Dotted lines indicate patients who had three measurements. Comparisons of OD between admission and the hospitalization were performed using Wilcoxon matched-pair signed rank test. **** $p < 0.0001$. ns: non-significant.

Table 1: Main characteristics of patients.

	PF4/hep Ab negative (n=91)	PF4/hep Ab positive All patients (n=28)	PF4/hep Ab positive during hospitalization (n=21)	Negative vs. positive (all patients)	Negative vs. positive during hospitalization
Demographical data and comorbidities					
Male sex	61 (67%)	19 (68%)	15 (71%)	> 0.999	0.799
Age, years	70 [61–79]	67 [60–77]	67 [61–78]	0.457	0.669
Diabetes	25 (27%)	13 (46%)	10 (48%)	0.068	0.115
Hypertension	49 (54%)	12 (57%)	9 (43%)	0.388	0.469
Body-mass index, kg/m ²	(n=57) 27.4 [24.9–33.15]	(n=18) 27.0 [23.1–31.7]	(n=16) 27.0 [23.3–30.8]	0.496	0.433
Timing of anti-PF4/heparin antibodies testing					
– From onset of symptoms, days	(n=84) 15 [11–23]	(n=23) 16 [14–19]	(n=19) 17 [14–19]	0.500	0.440
– From admission, days	8 [6–12]	9 [5–13]	10 [7–14]	0.726	0.369
Anticoagulant treatment					
Heparin treatment	80 (88%)	24 (86%)	19 (90%)	0.750	>0.999
– Unfractionated heparin	23 (25%)	7 (25%)	7 (33%)	>0.999	0.585
– Low-molecular-weight heparin	57 (63%)	17 (61%)	12 (57%)	>0.999	0.630
Others (VKA, DOAC)	10 (11%)	3 (11%)	2 (10%)	>0.999	>0.999
Biological testing					
Platelets, ×10 ⁹ /L					
– Platelets on admission	200 [150–253]	182 [137–240]	180 [136–228]	0.534	0.334
– Platelets concomitant with the anti-PF4/hep antibodies testing	252 [185–364]	242 [186–342]	261 [196–353]	0.502	0.855
– Platelet nadir	182 [138–230]	176 [136–214]	141 [129–213]	0.305	0.173
D-dimers, mg/L	(n=57) 0.992 [0.626–2.063]	(n=15) 2.120 [0.783–5.548]	(n=12) 2.211 [0.904–10.210]	0.078	0.028
Fibrinogen, g/L	(n=45) 4.13 [3.36–4.87]	(n=16) 5.10 [3.47–7.08]	(n=12) 5.10 [3.47–7.25]	0.055	0.106
Severity of disease					
Thrombotic event	14 (15%)	4 (14%)	2 (10%)	>0.999	0.732
Venous	9 (10%)	3 (11%)	2 (10%)		
Arterial	5 (5%)	1 (4%)	0 (0%)		
ECMO	0 (0%)	3 (11%)	3 (14%)	0.012	0.006
Lung involvement >75%	(n=77) 7 (9%)	(n=24) 6 (25%)	(n=18) 6 (33%)	0.074	0.015
ICU admission	39 (43%)	15 (54%)	15 (71%)	0.387	0.028
Mortality	18 (20%)	5 (18%)	5 (24%)	>0.999	0.765

Qualitative variables are expressed as number (percentage) and quantitative variables are expressed as median [interquartile range]. When data is not available for all patients, the number of patients is indicated between brackets. p-Value <0.05 are indicated in bold. Ab, antibodies; PF4, Platelet factor-4; DOAC, direct oral anticoagulant; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; VKA, vitamin K antagonist.

treatment were similar between negative and positive groups (Table 1). Thrombosis is the most severe complications of HIT. In our series, we recorded 18 (15%) thrombotic events (12 venous and six arterial) but the frequencies of thrombosis did not differ significantly between patients with anti-PF4/hep antibodies and those without ($p>0.999$). In contrast, the occurrence of anti-PF4/heparin antibodies appeared to be related to the severity of the disease. In this series, three patients required extracorporeal membrane oxygenation (ECMO) and all were tested positive for anti-PF4/hep antibodies during their hospitalization (IgG-

specific antibodies were detected in one of them). The frequencies of ECMO use were significantly different between negative and positive during the hospitalization groups ($p=0.012$). D-dimer, previously reported as a potential biomarker for severity of COVID-19 [5], were higher in patients positive during the hospitalization than in the negative group ($p=0.028$). While no correlations with fibrinogen or C-reactive protein were observed, anti-PF4/hep antibodies were positively correlated with D-dimer (Spearman's $r=0.244$, $p=0.002$). The occurrence of anti-PF4/hep antibodies during the hospitalization was also

related to a lung involvement >75% as well as ICU admission ($p=0.015$ and $p=0.028$ respectively).

As previously reported, we observed an increased prevalence of anti-PF4/heparin antibodies in hospitalized patients with COVID-19. A high prevalence (30.1%) was previously described during ECMO [6] or after a cardiopulmonary bypass where anti-PF4/hep antibodies positivity can reach 52% [7]; but the pathological HIT prevalence is extremely low in these clinical situations. Anti-PF4/hep antibodies previously reported in COVID-19 are neither all related to platelet activation nor to heparin treatment, suggesting that they do not necessarily reflect pathological HIT [8]. In our series, 25% of patients were treated with UFH, well known to more likely cause HIT than LMWH. However, the presence of anti-PF4/hep antibodies was not related to heparin therapy. Unexpectedly, we also observed that patients may have antibodies upon the admission prior to initiation of heparin therapy, or develop anti-PF4/hep IgG/A/M antibodies during their hospitalization, in the absence of heparin treatment. The presence of anti-PF4/hep antibodies is transient as they became negative in two patients on the 12th and 16th day of hospitalization. Finally, our results suggest that the positivity of anti-PF4/hep antibodies does not confer an increased risk of thrombotic complications or death. In conclusion, the occurrence of anti-PF4/hep antibodies in COVID-19 patients seems to be a marker of the severity of COVID-19 rather than a marker of thrombotic risk.

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Ethical approval: The study was performed in agreement with the French ethical laws (the patients and their family are informed that their biological data used for routine care may be used in an anonymous manner unless they express their opposition) and was approved by the local Ethics Committee of Foch hospital (approval number IRB00012437).

References

1. Riker RR, May TL, Fraser GL, Gagnon DJ, Bandara M, Zemrak WR, et al. Heparin-induced thrombocytopenia with thrombosis in COVID-19 adult respiratory distress syndrome. *Res Pract Thromb Haemostasis* 2020;4:936–41.
2. Patell R, Khan A, Bogue T, Merrill M, Koshy A, Bindal P, et al. Heparin induced thrombocytopenia antibodies in COVID-19. *Am J Hematol* 2020;95:E295–6.
3. Daviet F, Guervilly C, Baldesi O, Bernard-Guervilly F, Pilarczyk E, Genin A, et al. Heparin-induced thrombocytopenia in severe COVID-19. *Circulation* 2020;142:1875–7.
4. Delrue M, Siguret V, Neuwirth M, Brumpt C, Voicu S, Burlacu R, et al. Contrast between prevalence of HIT antibodies and confirmed HIT in hospitalized COVID-19 patients: a prospective study with clinical implications. *Thromb Haemostasis* 2020. <https://doi.org/10.1055/a-1333-4688>.
5. He X, Yao F, Chen J, Wang Y, Fang X, Lin X, et al. The poor prognosis and influencing factors of high D-dimer levels for COVID-19 patients. *Sci Rep* 2021;11:1830.
6. Laverdure F, Louvain-Quintard V, Kortchinsky T, Rezaiguia-Delclaux S, Imbert A, Stéphan F. PF4-heparin antibodies during ECMO: incidence, course, and outcomes. *Intensive Care Med* 2016;42:1082–3.
7. Gluckman TJ, Segal JB, Schulman SP, Shapiro EP, Kickler TS, Prechel MM, et al. Effect of anti-platelet factor-4/heparin antibody induction on early saphenous vein graft occlusion after coronary artery bypass surgery. *J Thromb Haemostasis* 2009;7:1457–64.
8. Favaloro EJ, Henry BM, Lippi G. The complicated relationships of heparin-induced thrombocytopenia and platelet factor 4 antibodies with COVID-19. *Int J Lab Hematol* 2021;43:1–12.