High prevalence of Lupus Anticoagulant in Ambulatory COVID-19 patients: interest of Hydroxychloroquine ?

Laurence Camoin-Jau[1,3,4] , Philippe Gautret[1,2], Philippe Colson[1,3], Jean Christophe Lagier[1,3], Hervé Tissot-Dupont[1,3], Matthieu Million[1,3], Audrey Giraud-Gatineau[1,3], Sophia Boudjema[1,3], Hervé Chaudet[1,2], Didier Raoult[1,3,*]

1 IHU Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005 Marseille, France;

2 Aix Marseille Univ, Institut de Recherche pour le Développement (IRD), Assistance Publique - Hôpitaux de Marseille (AP-HM), Service de Santé des Armées (SSA), Vecteurs - Infections Tropicales et Méditerranéennes (VITROME), Marseille, France;

3 Aix-Marseille Univ., Institut de Recherche pour le Développement (IRD), Assistance Publique - Hôpitaux de Marseille (AP-HM), Microbes Evolution Phylogeny and Infections (MEPHI), 27 boulevard Jean Moulin, 13005 Marseille, France;

4 Laboratoire d'Hématologie, Hôpital de la Timone, APHM, boulevard Jean- Moulin, 13005 Marseille, France

* Corresponding author: Didier Raoult, IHU Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005 Marseille, France. Tel.: +33 413 732 401, Fax: +33 413 732 402; email: didier.raoult@gmail.com

Running title: Lupus anticoagulant in COVID -19 patients

Abstract

Detection of lupus anticoagulant (LAC) was performed from June 15 to August 2020 for patients suspected of having COVID-19 and in people who had been in contact with confirmed cases in the Institut Hospitalo-Universitaire Mediterranee Infection in France. A high prevalence of LAC was detected among ambulatory and hospitalized patients.

Key words

Lupus anticoagulant, COVID-19, hydroxychloroquine

Introduction

Evidence of abnormal coagulation parameters associated with COVID-19 appeared in early reports from China (1). The most prevalent hemostatic abnormalities observed are mild thrombocytopenia and increased fibrinogen and D-Dimers. Elevated D-Dimers on admission is associated with increased mortality (2). A high prevalence of lupus anticoagulant (LAC) was reported in COVID-19 patients, notably in critically ill COVID-19 patients (3). An association between thrombosis and the presence of LAC in critically ill COVID-19 was suggested but has not yet been demonstrated (3).

In Marseilles, our institute has adopted a strategy of early and massive screening for SARS-CoV-2 infection. Detection of LAC was performed in patients with suspected or having COVID-19. The aim of this study was to evaluate the prevalence of LAC in COVID-19 patients between 15 June and 30 August 2020.

Methods

Patients and study design

The study was conducted in the Institut Hospitalo-Universitaire (IHU) Mediterranée Infection (https://www.mediterranee-infection. com/), Assistance Publique-Hôpitaux de Marseille (AP-HM), Southern France, from June 15 to August 2020. As previously described, we performed early massive PCR screening, both for patients suspected of having COVID-19 and for those who were in contact with confirmed cases. Viral load was analysed by qPCR from naso-pharyngeal swabs (Million) on admission prior to treatment. Demographic parameters (age, gender) and outcomes (hospitalization or ambulatory) were collected. Hospitalization was decided on the basis of clinical severity (2). The biological data collected were platelets, lymphocytes and eosinophils counts, prothrombin time, fibrinogen and D-Dimer.

Laboratory tests

Lupus anticoagulant (LA) was determined as recommended by International Society of Thrombosis and Haemostasis (ISTH) (4) using diluted Russel Viper Venom Time (dRVVT)

by Hyphen BioMed® (Neuville-sur-Oise, France). The Rosner Index (RI) was calculated and was considered positive when RI > 15. Results of dRVVT were expressed with a Normalized Ratio (NR) (positive value : > 1.2).

Statistical methods

Data were obtained retrospectively from patient files. All statistical analyses were carried out using R [R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2020. URL: https://www.R-project.org/]. Fisher's exact test was used to compare the differences between proportions (unilateral test used when indicated). Quantitative data means were compared using Student's t-test or Wilcoxon's rank test. The multifactorial analysis was performed using Factor Analysis for Mixed Data (FAMD) (5). A p-value < 0.05 was considered as statistically significant.

Ethics statement

Data presented herein were collected retrospectively from the routine care setting using the electronic health recording system of the hospital. This non-interventional retrospective study was approved by our institutional review board committee (Mediterranée Infection N: 2020–021). The analysis of the collected data followed the reference methodology MR-004 registered on NMR 5010010520 in the AP-HM register.

Results

Over the period from June 15 to August 30, 2020, 1194 patients were detected positive for COVID-19. Only a minority of patients was hospitalized (3.8 %) (Table 1). Compared to ambulatory patients, inpatients were older, had significantly lower platelet and eosinophil levels, and significantly higher fibrinogen and D-dimer levels. Considering ambulatory patients, prevalence of positive DRVVT test was 25.5 %. Interestingly, 62 % of hospitalized patients had a positive DRVVT ($p = 1.05\ 10^{-6}$) compared to ambulatory patients.

,

Discussion

To the best of our knowledge, we report here for the first time a high prevalence of LAC in ambulatory COVID-19 patients.

Between June 15 and August 30, a minority of patients were hospitalized. Although an increase in fibrinogen and D-Dimer was observed, their levels were lower than those observed in late winter and spring (2). However, 62% of them have DRVVT test positive. More interestingly, 25% of ambulatory patients were also positive for DRVVT test. DRVTT test is the more specific test for detecting LAC in patients with high risk of thrombosis (ISTH). This test was performed at low and high phospholipid concentrations, as recommended by ISTH. None of the patients were treated by anticoagulant at the time of blood puncture and patients presented a moderate inflammatory state.

The occurrence of LAC during viral infections is described in many viral infections, notably during HIV, hepatitis C, CMV, Varicella zoster virus and EBV infections. Prevalence of LAC was variable depending on the virus and the severity of the disease (6).

With regard to COVID-19, a prevalence of positive DRVVT tests, ranging from 45% to 82%, has been previously reported critically ill patients with COVID-19 (3, 7).

Actually, the role of LAC in hypercoagulabilty state observed in critically COVID patients is uncertain. We report here for the first time these abnormalities in ambulatory patients. Regarding their prothrombotic action, these coagulation abnormalities might justify HCQ treatment. Indeed, *in vitro* and animal models have demonstrated that HCQ had several antithrombotic effects (8). Several clinical studies have highlighted the value of HCQ for the prevention of thrombosis in antiphospholipid syndrome (9,10).

Our study presents limitations. The detection of LAC was performed at time of COVID-19 diagnosis and a second LAC detection needs to be performed within twelve weeks. Indeed, Siguret *et al*, underlined in a small cohort that LAC appeared labile (7). Moreover, we need to correlate our results with the occurrence of thrombotic events.

References

1. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395: 1054-62
2. Lagier JC, Million M, Gautret P, Colson P, Cortaredona S, Giraud-Gatineau A, Honoré S, Gaubert JY, Fournier PE, Tissot-Dupont H, Chabrière E, Stein A, Deharo JC, Fenollar F, Rolain JM, Obadia Y, Jacquier A, La Scola B, Brouqui P, Drancourt M, Parola P, Raoult D. Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France: A retrospective analysis.; IHU COVID-19 Task force.Travel Med Infect Dis. 2020 25; 36:101791. doi: 10.1016/j.tmaid.2020.101791
3. Helms J, Taquard C, Severac F, et al; CRICSTRIGGERSEP Group(Clinical Research in Intensive Care and Sepsis Trial Group for Global Evaluation and Research in Sepsis). High risk of thrombosis in patients with severe SARS- CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med. 2020; 46:1089-1098. doi:10. 1007/s00134-020-06062-x
4. Pengo V, Tripodi A, Reber G, Rand JH, Ortel TL, Galli M, et al. Update of the guidelines for lupus anticoagulant detection. J Thromb Haemost. 2009; 7:1737–40.
5. Pages J. Multiple factor analysis by example using R, Chapman & Hall Edn. Londont: CRC Press, 2014.
6. Martirosyan A, Amino R and Manukya G. Environmental Triggers of Autoreactive Responses: Induction of Antiphospholipid Antibody Formation. Frontiers in Immunology 2019, 10 : 1609
7. Siguret V, Voicu S, Neuwirth M, Delrue M, Gayat E, Stépanian A, Mégarbane B.Are antiphospholipid antibodies associated with thrombotic complications in critically ill COVID-19 patients? Thromb Res. 2020; 195:74-76
8. Edwards MH, Pierangeli S, Liu X, Barker JH, Anderson G, Nigel HE. Hydroxychloroquine reverses thrombogenic properties of antiphospholipid antibodies in mice. Circulation 1997; 96 : 4380-4 https://doi.org/10.1161/01.CIR.96.12.4380.
9. Schmidt-Tanguy A, Voswinkel J, Henrion D, Subra JF, Loufrani L, Rohmer V, et al. Antithrombotic effects of hydroxychloroquine in primary antiphospholipid syndrome patients. J Thromb Haemost 2013;11: 1927–1929. 10.1111/jth.12363

10. Kravvariti E, Koutsogianni A, Samoli, E, Sfikakis PP, Tektonidou MG. The effect of hydroxychloroquine on thrombosis prevention and antiphospholipid antibody levels in primary antiphospholipid syndrome: A pilot open label randomized prospective study. Autoimmunity Reviews 2020; 19: 102491

Competing interest

The authors declare that they have no competing interests.

Acknowledgments:

The authors

Table 1: Demographic and biological parameters

	Positive COVID -19 (n=1194)		
	Ambulatory patients	Hospitalized patients	p
Number of patients	1149 (96.2 %)	45 (3.8 %)	
Male (n, %)	565 (50)	26 (57.8)	0.289
Age (mean± SD)	38.7 ± 16.4	58.9 ± 17.7	$2.7\ 10^{-7}$
< 65 years (n, %)	1071 (93 %)	27 (60 %)	
> 65 years (n, %)	77 (7%)	18 (40 %)	
Haematological parameters			
Platelets (G/L) (mean ±SD)	226.2 ± 62.3	197.7 ± 77.9	0.022
Lymphocytes (G/L) (mean ± SD)	1.60 ± 0.61	1.39 ± 0.65	0.047
Eosinophils (G/L) (mean ± SD)	0.07 ± 0.08	0.05 ± 0.07	0.0013
Coagulation parameters			
Prothrombin time (%) (mean ± SD)	104.4 ± 11.0	96.6 ± 11.0	2.15^{10-5}
Fibrinogen (g/L) (mean ± SD)	3.63 ± 0.82	4.88 ± 1.35	$1.9\ 10^{-7}$
D-Dimer (µg/mL) (mean ± SD)	0.59 ± 1.65	1.20 ± 1.44	$1.32\ 10^{-13}$
LAC detection			
Positive DRVVT (Normalized ratio> 1.2), n (%)	293 (25.5 %)	28 (62%)	$1.05\ 10^{-6}$