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ORIGINAL RESEARCH—CLINICAL

# Validation of the Performance of A1HPV6, a Triage Blood Test for the Early Diagnosis and Prognosis of SARS-CoV-2 Infection

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**BACKGROUND AND AIMS:** Apolipoprotein A1 (A1) and haptoglobin (HP) serum levels are associated with the spread and severity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. We have constructed and validated a multivariable risk calculator (A1HPV6) integrating A1, HP, alpha2-macroglobulin, and gamma glutamyl transferase to improve the performances of virological biomarkers. **METHODS:** In a prospective observational study of hospitalized patients with nonsevere SARS-CoV-2 infection, A1HPV6 was constructed in 127 patients and validated in 116. The specificity was assessed in 7482 controls representing the general population. The primary diagnostic endpoint was the area under the receiver operating characteristic curve in patients with positive SARS-CoV-2 PCR. The primary prognostic endpoint was the age-and-sex adjusted risk of A1HPV6 to predict patients with WHO-stage > 4 (W > 4) severity. We assessed the kinetics of the A1HPV6 components in a nonhuman primate model (NHP), from baseline to 7 days (D7) after SARS-CoV-2 infection. **RESULTS:** The area under the receiver operating characteristic curve for A1HPV6 was 0.99 (95% CI 0.97–0.99) in the validation subset, which was not significantly different from that in the construction subset, 0.99 (0.99–0.99; $P = .80$), like for sensitivity 92% (85–96) vs 94% (88–97; $P = .29$). A1HPV6 was associated with W > 4, with a significant odds ratio of 1.3 (1.1–1.5; 0.002). In NHP, A1 levels decreased ($P <$ .01) at D2 and normalized at D4; HP levels increased at D2 and peaked at D4. In patients, A1 concentration was very low at D2 vs controls ($P < .01$) and increased at D14 ($P < .01$) but was still lower than controls; HP increased at D2 and remained elevated at D14. **CONCLUSION:** These results validate the diagnostic and prognostic performances of A1HPV6. Similar kinetics of apolipoprotein A1, HP, and alpha-2-macroglobulin were observed in the NHP model. ClinicalTrials.gov number, NCT01927133.

*Keywords:* COVID-19; Nonhuman Primate Model; Apolipoprotein A1; Haptoglobin

## Introduction

The pandemic of the respiratory disease (COVID-19) associated with the novel coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) has highlighted the need for biomarkers that detect different risks, that is, the risk of infection before the exposure, named "predisposing biomarkers", the prognostic factors during the exposure, named "acute phase biomarker", and the risk of sequelae after exposure, named "sequelae bio-

*These authors contributed equally to this work.

***Abbreviations used in this paper:*** **A1, Apolipoprotein A1; A2M, alpha2-macroglobulin; ALT, alanine aminotransferase; Dpi, days after infection; GGT, gamma glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; HP, haptoglobin; ICU, intensive care unit; NHP, non-human primate model; SAA, serum amyloid A.**

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marker".[1–3] Among simple available blood biomarkers, 2 proteins associated with cell repair, apolipoprotein-A1 (A1) and haptoglobin (HP), could be accurate components of such multianalyte risk markers.

Several prospective population-based studies have shown that a low level of A1 as well as an associated low level of high-density lipoprotein cholesterol were associated with a significant risk of hospitalization 10 years later, confirming their importance as a predisposing biomarker.[4–7] Several prospective studies in COVID-19 hospitalized patients have shown that A1 alone or associated with HP also had significant diagnostic and short-term prognostic value for the acute phase of infection.[8–12] Biologically, A1 interacts with lipid rafts on cellular membranes that are enriched in immune cell receptors such as toll-like receptors on macrophages, T-cell receptors, and B-cell receptors, which may all modulate immune responses.[9–13] HDL also has immunomodulatory, antithrombotic, and antioxidant effects that could provide important clarity why genetically determined levels of high-density lipoprotein cholesterol, but not low-density lipoprotein cholesterol, provide a protective effect against infectious diseases.[4,12]

The increase in serum HP levels associated with SARS-CoV-2 infection was expected based on its well-known acute-phase protein profile.[14–16] HP is the most abundant protein among those modulated in SARS-CoV-2 infection.[17] Unlike A1, there is no association between serum HP levels before SARS-CoV-2 exposure or genetic polymorphisms.[3,14] However, A1 and HP interact during the acute phase response to infection.[14–16] A1 collaborates with HP to downregulate hemoglobin-redox activity.[15,16] It also facilitates the uptake of hemoglobin by interacting with the HDL-scavenger receptor B type 1 (SR-B1) displayed on macrophages and hepatocytes.[16]

The interaction of the SARS-CoV-2 S1 spike protein with HDL cholesterol could also participate in the transient decrease of A1 by its main transporter during infection. The SARS-CoV-2 S protein binds to cholesterol, followed by an enhanced attachment via SR-B1, which facilitates the SARS-CoV-2 entry. SR-B1 and host cell entry are initiated through interactions with the angiotensin-converting enzyme 2.[18]

In a previous study, we described the temporal association between A1 and HP in patients with nonalcoholic fatty liver disease with the incidence of SARS-CoV-2-infected cases in the United States. The significant association between serum A1 levels and SARS-CoV-2 infection persisted after stratification of the main confounding factors.[3] The value of combining these 2 proteins was based on the high sensitivity and specificity of the combination in the general population. The kinetics of a transient low A1 and high HP were not observed in 4 million FibroTest (FibroSure in USA) results since 2001, which combine these 2 proteins to assess the stage of liver fibrosis.[3]

The primary endpoint of this study was to assess the diagnostic value of A1HPV6, a multianalyte SARS-CoV-2 risk marker integrating A1 and HP; alpha-2-macroglobulin (A2M), a marker of liver fibrosis; and the prognostic value of gamma glutamyl transpeptidase (GGT), a sensitive marker of liver injury.[3] We report the prospective construction of A1HPV6 during the first wave of COVID-19, the "construction subset", and the internal validation in the "validation-subset" during the following 2 waves.

The second aim was to assess the prognostic value of A1HPV6 to predict WHO stage $> 4$ (W $> 4$) severity COVID-19 disease in patients hospitalized in an internal medicine department (Table 1), who did not require direct admission to the intensive care unit (ICU). To confirm the value of A1HPV6 components, we compared the kinetics observed in the patients during hospitalization to those observed in a nonhuman primate (NHP) mild-disease model.[19,20] This is a unique model to assess the impact of SARS-CoV-2 on biomarkers in the first 14 days postinfection (Dpi), which is not feasible in humans.

Finally, to identify the risk of false positive or false negative A1HPV6 values and its components,[21,22] we assessed the A1HPV6 components in 3 extremely severe ICU patients with persistence of SARS-CoV-2 infection and multiple repeated samples.

## Patients and Methods

### Patients

Five subsets were analyzed, a "construction-subset," a "validation-subset," a "prognostic-subset," a "controls-subset," and a "kinetic-subset" combining all the positive SARS-CoV-2 PCR cases admitted in the internal medicine department, plus 3 severe cases with persistent plasma infection followed in the ICU, and controls from the general population (Figure 1).

### Ethics

The prospective observational study in COVID-19 patients was approved by CER-Sorbonne University IRB, CER-2020-14, with signed informed consent. All the previously published patient analyses from retrospective databases were non-interventional studies, without supplementary blood samples, and were exempt from IRB review (NCT01927133). This study was performed according to the principles of the Declaration of Helsinki. All authors had access to the study data and reviewed and approved the final manuscript.

### Construction, Validation, and Prognostic Subsets

The construction-subset was performed between January and June 2020 and the validation-subset between October 2020 and May 2021. The methods and characteristics of patients included in the construction-subset have already been described elsewhere, as well as the univariate performance of A1 and HP.[3] The inclusion criteria were the same in both subsets. Patients aged 18 years or older and hospitalized in the internal medicine department were eligible for the study if they had confirmed SARS-CoV-2 infection (positive on RT-PCR or typical chest CT scan). Very severe patients directly admitted in ICU were not eligible, as well as patients with serum assessments not contemporaneous, defined as more than 14 days before or after admission.

**Table 1.** Characteristics of COVID-19 Patients of the Construction and Validation Subsets to Assess Sensitivity of A1HPV6

| Characteristics | Construction subset | Validation subset | *P* value | All |
|---|---|---|---|---|
| Number n (%) | 127 | 116 | | 243 |
| Male sex | 83 (65.4) | 74 (63.4) | .90 | 157 (65.0) |
| Median age (IQR) year | 71 (57–81) | 67 (53–77) | .03 | 69 (56–78) |
| Age category | | | .22 | |
| <50 y | 16 (12.6) | 21 (18.1) | | 37 (15.2) |
| 50 to <70 y | 45 (35.4) | 47 (40.5) | | 92 (37.9) |
| ≥70 y | 66 (52.0) | 48 (41.4) | | 114 (46.9) |
| Geographic origin | | | .03 | |
| Caucasian | 75 (59.1) | 61 (52.6) | | 136 (56.0) |
| North African, Middle East | 36 (28.4) | 25 (21.6) | | 61 (25.1) |
| Other (Subsaharan, Asian) | 16 (12.6) | 30 (25.9) | | 46 (18.9) |
| Severity WHO stages | | | | |
| <5 | 40 (31.5) | 62 (53.5) | <.001[a] | 102 (42.0) |
| 0–2 not hospitalized | 2 (1.8) | 0 (0) | | 2 (0.8) |
| 3 hospitalized without oxygen | 3 (2.4) | 25 (21.6) | | 28 (11.5) |
| 4 oxygen support mask | 35 (27.6) | 37 (31.9) | | 72 (29.6) |
| 5–8 | 87 (68.5) | 54 (46.6) | | 141 (58.0) |
| 5 high flow or ventilation | 64 (50.4) | 29 (25.0) | | 93 (38.3) |
| 6–7 Invasive oxygen support | 8 (6.3) | 3 (2.3) | | 11 (4.5) |
| 8 death | 15 (11.8) | 22 (19.0) | | 37 (15.2) |
| Coexisting conditions | | | | |
| Obesity (BMI ≥ 30) | 27 (21.3) | 35 (30.2) | .14 | 62 (25.5) |
| Hypertension | 71 (55.9) | 59 (50.9) | .44 | 130 (53.5) |
| Diabetes type 2 | 33 (26.0) | 29 (25.0) | .88 | 62 (25.5) |
| Dyslipidaemia | 44 (34.7) | 37 (32.0) | .68 | 81 (33.3) |
| Stage liver fibrosis (FibroTest) | | | .15 | |
| F0F1F2 | 117 (92.1) | 100 (89.3) | | 217 (89.3) |
| F3F4 (cirrhosis) | 10 (7.9) | 16 (13.8) | | 26 (10.7) |
| Initial presentation | | | | |
| Anosmia | 15 (11.8) | 13 (11.2) | 1.00 | 28 (11.5) |
| Diarrhea | 28 (22.1) | 18 (15.5) | .25 | 46 (18.9) |
| Apolipoprotein ≤ 1.25g/L | 115 (90.6) | 105 (90.5) | 1.00 | 220 (90.5) |
| Median laboratory (IQR) | | | | |
| Apolipoprotein-A1 g/L | 0.86 (0.71–1.03) | 0.88 (0.67–1.07) | .96 | 0.87 (0.69–1.05) |
| Haptoglobin g/L | 3.16 (2.22–4.08) | 3.17 (1.91–4.11) | .78 | 3.17 (2.15–4.09) |
| Alpha-2 macroglobulin g/L | 1.41 (1.18–1.97) | 1.56 (1.20–2.00) | .46 | 1.50 (1.19–1.98) |
| GGT IU per liter | 50 (30–117) | 79 (40–134) | .02 | 63 (33–125) |
| ALT IU per liter | 32 (23–63) | 43.5 (24–83.3) | .02 | 36 (24–67) |
| Total bilirubin micromol/L | 7 (5–11) | 7 (5–10) | .96 | 7 (5–10) |
| Time clinic-PCR (d) | 6 (2–11) | 6 (0–11) | .82 | 6 (1–11) |
| Time clinic-serum sample (d) | 9 (5–14) | 8 (3–14) | .06 | 9 (4–14) |
| Time clinic-last news (d) | 12 (8–18) | 13 (7–17) | .90 | 12 (8–17) |

ALT, alanine aminotransferase; BMI, body mass index; IQR, interquartile range.
[a]*P* value severity WHO, stages <5 vs from 5 to 8.

The prognostic analysis was performed in all patients by combining the construction and validation subsets and called the "prognostic-subset" (Figure 1).

## Kinetic-Subsets

The kinetics were analyzed in the "kinetics-subset," which included the prospective integrated prognostic-subset and retrospectively severe cases admitted directly to the ICU (ICU patients).

In non-ICU patients, all cases with at least 3 repeated samples were analyzed. The aim was to describe the kinetics of A1HPV6 and its components, 8 and 14 days after hospital admission, that is around 16 and 24 days postinfection (Dpi). Only kinetics in NHP were able to assess the kinetics during the first 14 Dpi.

The severe cases were identified as ICU patients with persistent SARS-CoV-2 viremia and at least 9 samples repeated frozen plasma samples. The severe cases allowed us to identify the risks of A1HPV6 false negatives and its components, such as organ failure and aggressive treatment.[22]

## Controls Subset to Assess the Specificity of A1HPV6

We previously collected 5 cohorts, called "specificity-cohorts," which were used to retrospectively validate the

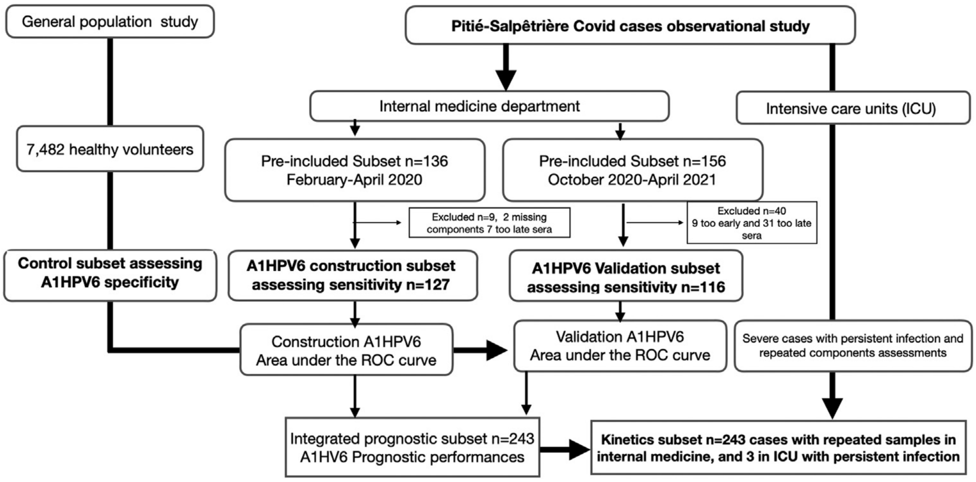


**Figure 1.** Flow chart of patients and controls.

specificity of A1 and HP in a large group of subjects without COVID-19 or pneumonia (Table A1). Specificity was assessed in the group of healthy volunteers that was representative of the French population before 2019.[23]

## Biochemical and Virological Methods

Measurements were all performed on fresh prospectively collected serum or plasma in the biochemistry unit of the APHP-PSL hospital. A1, HP, A2M, GGT, alanine aminotransferase and bilirubin were assessed according to BioPredictive (Paris, France) analytical recommendations.[3,24] Serum amyloid A (SAA) was performed by the ELISA method (Life Diagnostic, SAA-3). The virological methods used to diagnose SARS-CoV-2 in respiratory samples are described in File A2.

## Kinetic Study in NHPs

Briefly, 4 female and 3 male cynomolgus macaques and 2 female rhesus macaques, aged 3–6 years, were originating from Mauritian and Chinese Accreditation of Laboratory Animal Care-certified breeding centers,[9,25] respectively, and with the related NHP references described in File A2.

## Statistical Methods

The criterion for main prognostic endpoint was the accurate prediction of severe stage WHO 5–8 (Table 1) by A1HPV6 assessed by the odds ratio using regression analysis adjusted for the subset group. A1HPV6 values were also compared between WHO severities using median tests values and the percentage of high-risk A1HPV6 by Fisher Exact test. The variability of repeated samples of A1HPV6 and its components was assessed in the kinetic subsets by repeated analysis of variance using the Time F-Ratio. We assumed that in humans, the median presymptomatic infectious period across studies varied from <1 to 4 days.[26] The different repeated means were compared to the first assessment at baseline as control by Dunnett's 2-sided multiple-comparison test, and the mean differences significance was compared by Tukey-Kramer's all pairs simultaneous confidence intervals.

# Results

## A1HPV6 Diagnostic Performances

**Patients Included.** A total of 292 patients with suspected of COVID-19 infection were preincluded, 136 in the construction subset between January and June 2020, and 156 in the validation subset between October 2020 and May 2021. After noninclusion of 49 patients with no positive PCR or for noncontemporaneous assessment of A1HPV6 components, a total of 243 patients were included, 127 in the construction subset and 116 in the validation subset (Figure 1 and Table 1). Forty out of 156 patients (25.6%) were not eligible, due to the absence of contemporaneous serum samples, vs 9 out of 136 patients (6.6%) preincluded in the construction subset ($P < .001$) (Table A2).

Several characteristics were significantly different in the validation subset compared with the construction subset, including lower median age (interquartile range; $P$ value) 67 (53–77) years vs 71 (57–81; $P = .03$) years, a lower percentage of patients with European geographic origin (53% vs 59%; $P = .03$), and less need for oxygen support (78% vs 96%; $P < .001$).

**Construction and Validation of A1HPV6 (Table 2).** A1HPV6 was built including the 127 patients in the construction subset and the 7482 control patients in the general population, using logistic regression with and HP, GGT, and A2M, all adjusted for age and gender (patent pending).

The area under the receiver operating characteristic curve in the construction subset (n = 127) was 0.994 (0.982–0.998) with a sensitivity of 0.976 (0.933–0.995) and a specificity of 0.959 (0.955–0.964) using the cutoff of A1HPV6 ≥0.01 to define a high risk of infection.

The area under the receiver operating characteristic curve for the diagnosis of COVID-19 was 0.989 (0.971–0.991) in the validation subset (n = 116), with a sensitivity of 0.957 (0.902–0.986), which was nonsignificantly lower than that in the construction subset, with the same specificity of 0.959 (0.955–0.964) because the same control subset was used.

## Prognostic Performances

In the pooled population of patients with COVID-19, the prognostic value of A1HPV6 was significant on univariate analysis, with an odds ratio = 2.10 (1.27–3.50; $P = .004$) and a similar odds ratio of 2.07 (1.2–3.5; $P = .006$) in the construction subset after adjustment by regression analysis (Table 2). The prevalence of high-risk A1HPV6 was 99.3% in the 141 patients with severe WHO stages 5–8, which was greater than the 93.1% in the 102 patients with nonsevere WHO stages. A post-hoc analysis including geographical origins as the independent variable did not change the prognostic significance of A1HPV6, odds ratio = 2.14 (1.28–3.57; $P = .004$) (Table 2).

## Kinetic Studies

**Non-ICU Patients.** A total of 222 sera were assessed in 59 patients (Figure 2). No significant difference was between the mean A1HPV6 values on D7 and D14 (mean difference −0.04; −0.13 to 0.06; $P = .64$) compared with D2 values. The risk was only low in one case on D2 and in 4 cases thereafter (panel A). The A1 mean decreased from 1.24 on D2 to 0.98 g/L on D14 for a difference of 0.26 g/L (0.19–0.33; $P < .001$) (panel B). The HP means decreased from 4.15 on D2 to 2.99 g/L on D14, for a difference of 1.16 g/L (0.85–1.47; $P < .001$) (panel C). The kinetics of bilirubin and GGT were described in panels D and E, respectively. The A2M decreased during hospitalization from 2.04 on D2 to 1.85 and 1.77 g/L on D7 and D14, respectively. On D14, the mean difference was 0.27 g/L (0.20–0.34; $P < .001$) (panel F).

**ICU Patients.** Three ICU patients with persistent SARS-CoV-2 plasma viremia for more than 2 months who later died had 9 aliquots available each (Table A2). The repeated mean A1HPV6 values were not significantly different during follow-up compared with values at admission (D0) (Figure 3; panel A). The mean A1 on D0 and D80 were below 1 g/L1 D2, which were extremely low compared with normal values (>1.15 g/L), with a significant transient increase around D15 (panel B). HP values were severely decreased, with several values below detectable levels (0.08 g/L) during follow-up (panel C) and with a temporal association with cardiac arrests and extracorporeal membrane oxygenation (Table A2). Total bilirubin increased on D5 (panel D; $P = .004$). The mean GGT means was elevated from D5 to D20 (panel E, $P = .03$). A2M values were not significantly different (panel F). As expected in the presence of cardiac arrest, the 3 proteins were at the limit of detection on D2 in patient #2 (blue line).

## Kinetic Study in NHPs (Figure A2)

There was no significant change in A1 in NHP from 7 to 26 Dpi (panel A), unlike in COVID-19 patients (black box) whose values were lower ($P < .001$) than those in control (blue box) COVID-19 patients before, during, and after recovery in all repeated samples (panel B). HP peaked at 7 Dpi in NHP (panel C), close to the peak of nasopharyngeal SARS-CoV-2 viral loads and was still elevated at 20 Dpi.[19] Changes in HP were similar in patients, with a peak at D14 which then returned to normal values at D60 (panel D).

## Kinetic Study in NHPs, During the First Week After Infection (Figure 4)

A1 mean values fluctuated significantly (repeated time F-ratio = 5.1; $P = .008$) with lowest values on 2 and 3 Dpi and returned to baseline at 4 and 6–26 Dpi (panel A). It is interesting that we were not able to see this early decrease in the initial study because previous assessments were performed at 7 Dpi. The mean HP increased from 1.00 g/L at

**Table 2.** A1HPV6 Performances

| A1HPV6 outcome | Construction | Validation | Significant difference |
|---|---|---|---|
| Prevalence (%) of high risk | 127/7609 (1.7) | 116/7598 (1.5) | Not applicable |
| Diagnostic performance | | | |
| Area under ROC curve | 0.994 (0.982–0.998) | 0.989 (0.971–0.996) | <0.05 |
| Sensitivity % (95% CI) | 97.6 (93.3–95.9) | 95.7 (90.2–98.6) | <0.05 |
| Specificity | 95.9 (95.5–96.4) | 95.9 (95.5–96.4) | Not applicable same controls |
| Negative predictive value | 99.9 (99.8–100) | 99.9 (99.8–100) | <0.05 |
| Positive predictive value | 29.0 (24.7–33.5) | 26.8 (22.6–31.3) | <0.05 |

| Prognostic performance | Construction and validation pooled | Significance |
|---|---|---|
| WHO severe vs nonsevere | Stages 1–4 vs 5–8, n = 243 | |
| Log A1HPV6 n/median/SD | 87/−0.01/0.28 vs 40/−0.22/0.85 | <0.001 |
| Odds ratio univariate (95% CI) | 2.10 (1.27–3.50) | 0.004 |
| Odds ratio subset (validation vs construction) adjusted | 2.07 (1.24–3.48) | 0.006 |
| Odds ratio geographical origin adjusted | 2.14 (1.28–3.57) | 0.004 |

CI, confidence interval; ROC, receiver operating charecteristics; SD, standard deviation; WHO, World Health Organization.

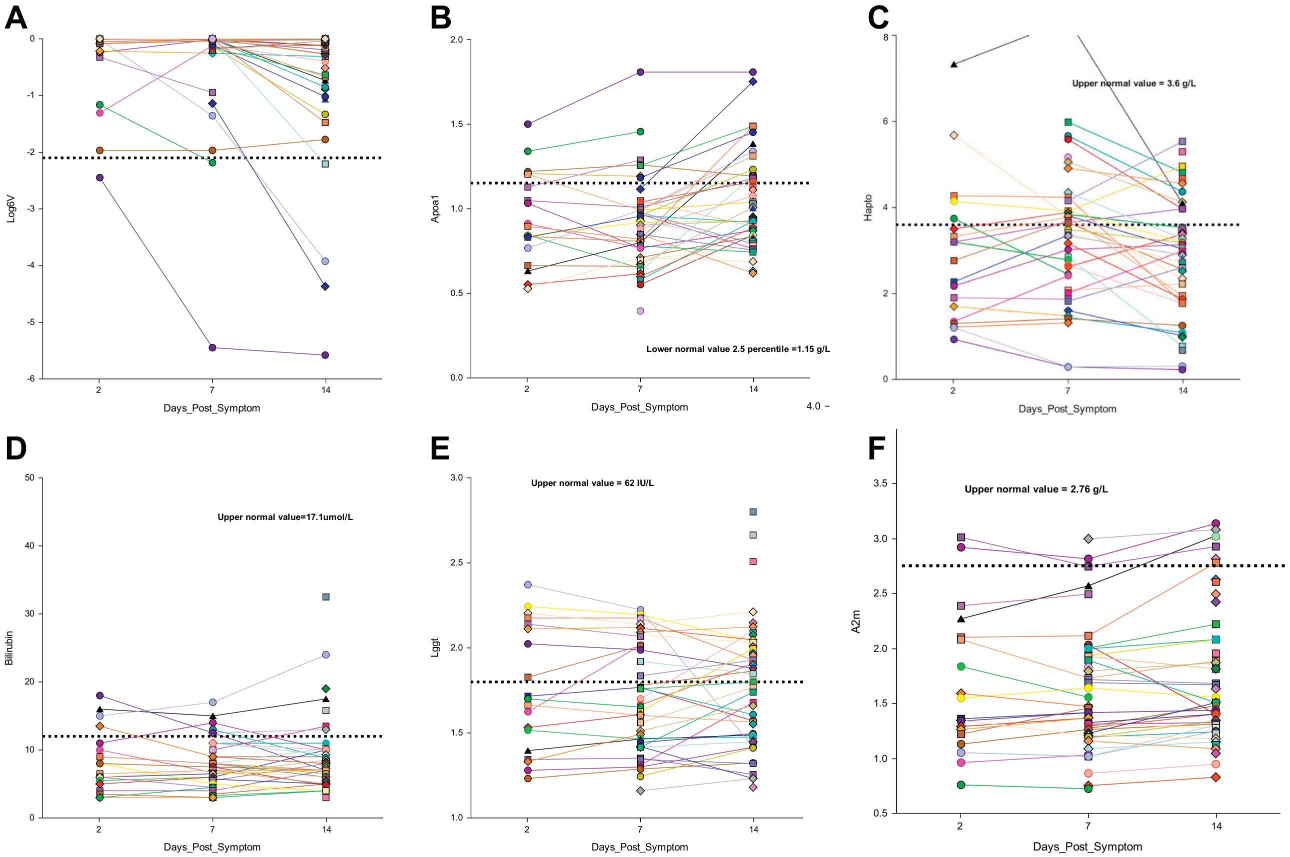


**Figure 2.** Kinetic in non-ICU patients. (A) A1HPV6. The dotted black line is the cutoff (Log = −2.1) defining the high risk of SARS-CoV-2 infection. (B) ApoA1. The dotted black line is the lower normal value (1.15 g/L). (C) Haptoglobin. The dotted black line is the upper normal value (3.60 g/L). (D) Total bilirubin. The dotted black line is the upper normal value (11.1 μmol/L). (E) GGT. The dotted black line is the upper normal value (62 IU/L). (F) A2M. The dotted black line is the upper normal value (2.76 g/L).

baseline to 0.60 g/L at 2 Dpi2 to, for a 0.40 g/L difference (0.14–0.66; $P = .002$) (panel B). The mean GGT decreased later ($P = .04$) (panel C). The mean A2M decreased regularly from 1.29 g/L at baseline to 1.04 g/L at 4 Dpi, with a difference of 0.25 g/L (0.16–0.33; $P < .001$) (panel D).

Mean CRP values did not fluctuate significantly during the week (panel E). SARS-CoV-2 viral loads in the trachea peaked at 2–3 Dpi 3, $8 \times 10^5$ copies/mL (1.9–5.4; $P < .001$) (panel F). Mean SAA values increased significantly very early from 195 to 1704 mg/L ($P = .04$) (Figure A3).

## Discussion

We constructed and prospectively validated A1HPV6, a multivariate risk calculator for the diagnosis of SARS-CoV-2 infection in hospitalized patients who did not require ICU at admission. We confirmed the prognostic value of A1HPV6 for the severity of SARS-CoV-2 infection, defined as COVID-19 WHO stages >4. To further our understanding of the 3 liver proteins included in A1HPV6, that is, A1, HP, and A2M, we evaluated their kinetics during hospitalization in non-ICU patients and in some ICU patients with persistent infection. Finally, we analyzed the kinetics of these components in a NHP model, which revealed a temporal association with viral load during infection, including viral recovery.

### *Diagnostic Performances*

Based on the results of our previous analyses of A1 and HP demonstrating their independent diagnostic value in patients with SARS-CoV-2 infection,[3] we constructed A1HPV6 by adding A2M and GGT, 2 other independent risk factors, to these proteins. A2M is a well-validated liver fibrosis biomarker in patients with chronic liver disease.[27,28] Recently a large cohort study showed that chronic liver disease plays a significant role in the burden of mechanical ventilation in severe COVID-19 patients.[29] GGT is a sensitive marker of liver injury[30] and an independent prognostic risk factor in patients hospitalized for COVID-19.[3,30] The main limitations were the relatively small size of the 2 subsets, with a non-contemporaneous control population, and the absence of independent external validation. Another limitation was the higher percentage of noneligible patients in the validation subset due to the too late serum assessment of tests' components. However, post-hoc comparisons found only a significant increase in patients with type-2 diabetes among the noneligible patients (18 out of 40, 45%, vs 29 out of 116,

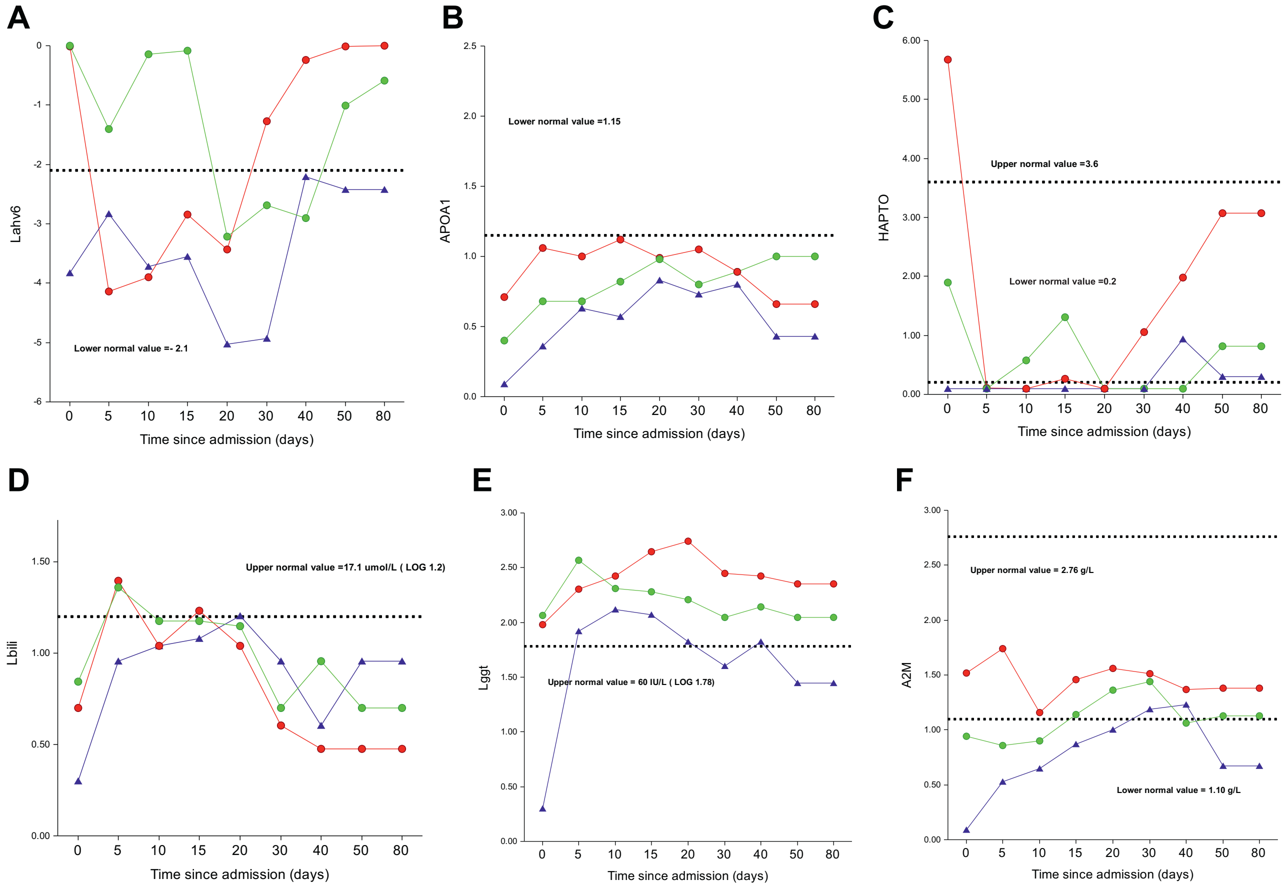


**Figure 3.** Kinetic in ICU patients with persistent plasmatic viremia. (A) A1HPV6. The dotted black line is the cutoff (Log = −2.1) defining the high risk of SARS-CoV-2 infection. (B) ApoA1. The dotted black line is the lower normal value (1.15 g/L). (C) Haptoglobin. The dotted black line is the upper normal value (3.60 g/L). (D) Total bilirubin. The dotted black line is the upper normal value (17.1 $\mu$mol/L). (E) GGT. The dotted black line is the upper normal value (62 IU/L). (F) A2M. The dotted black lines are the upper (2.76 g/L) and the lower (1.10 g/L) normal values.

25%; $P = .02$) compared with eligible patients. Most of the other characteristics were nonsignificant between the groups. The other significant differences were all expected in patients with late assessment as related to the recovery profile of the kinetics analyses: higher levels of A1, lower levels of HP, and a lower level of A1HPV6 score (Table A2).

However, the strengths include an assessment of the risk of false positive and negative in the A1HPV6 components since 2001 in a large number of patients including multiracial ethnic populations.[23,24,28] The kinetics of A1HPV6 and its components in ICU patients with persistent viremia (Figure 3) strongly suggests that the diagnostic value of HP, A1, and A2M should not be assessed in association with cardiac arrest or extracorporeal membrane oxygenation to avoid false positive or negative conclusions.

## *Prognostic Performance*

We used the COVID-19 WHO severity staging system to compare the performance of A1HPV6 with that of other blood tests. The main limitations were the small sample size, the limited number of events, and the short follow-up during hospitalization, which prevents a powerful multivariate analysis. There were 3 significant differences, in the validation subset vs the construction subset: median age 3 years younger, twice more patients from Asian and Subsaharan origin, and twice less high flow or ventilation. However, the A1HPV6 score already included age as a covariable, and at the inclusion, there were 3 significant differences, in the validation vs construction subset: median age 3 years younger, twice more patients from Asian and Subsaharan origin, and twice less high flow or ventilation. One regression analysis included the subset as an independent variable (validation vs construction 2.07 (1.24–3.48); $P = .006$), without a significant change in the prognostic performance of A1HPV6. A second regression analysis included geographical origin as an independent variable, which also did not change the prognostic significance of A1HPV6, odds ratio = 2.14 (1.28–3.57; $P = .004$) (Table 2).

## *Protein Kinetics*

This is the first time that similar results have been identified for liver protein kinetics in patients and in an NHP

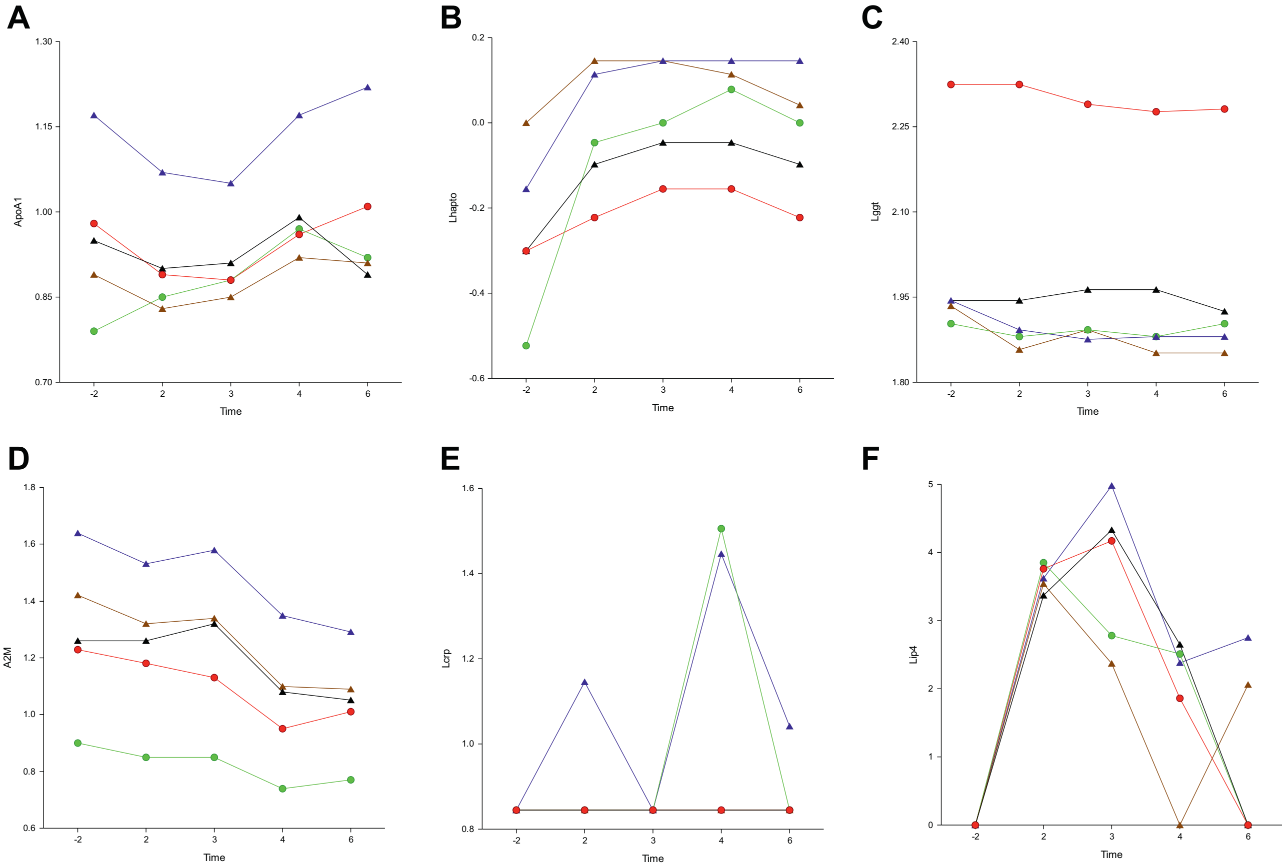


**Figure 4.** Early kinetics in Cynomolgus model. (A) ApoA1. (B) Haptoglobin. (C) GGT. (D) A2M. (E) CRP. (F) Tracheal SARS-CoV-2 viral loads.

model. Despite the limited number of cases, the numerous repeated sera analyses support the early kinetics of these liver proteins. In this study, early measurement of A1 during the first week of infection highlights the sensitivity of this protein to SARS-CoV-2 infection. Of note, in our initial NHP study, there was no significant variation detectable between 7 Dpi and 26 Dpi (Figure A2 panel A). However, an analysis of earlier samples shows a significant decrease in A1 on 2 Dpi associated with a peak in the virus load in the tracheal samples (Figure 4; panels A and F). On 6 Dpi, A1 returned to baseline while only traces of the virus were found in tracheal samples.

The second original result was the early sensitivity of serum HP (Figure 4, panel B), which was found to be more sensitive than CRP (Figure 4, panel E), the standard acute phase protein in human, or SAA (Figure A3). HP could also be better than CRP to evaluate inflammation recovery because of its sustained duration in SARS-CoV-2 infection. HP curves were similar in humans and NHP with abnormal values between 2 and 25 Dpi (Figure 2, panel C; Figure 3, panel C; Figure 4, panel B).

The third original and unexpected result was the highly significant decrease in A2M in both patients (Figure 2, panel F) and NHP (Figure 4, panel D). One hypothesis in line with these results is that A2M protects the endothelium from a variety of potentially harmful intravascular proteases during SARS-CoV-2 infection.[31] Our findings are also in line with the hypothesis that children are to some extent protected by higher A2M levels from severe COVID-19.[32] These results and hypotheses encourage new studies of A2M in COVID-19 patients. The interpretation of A2M variability must take into account the age of patients.

Finally, A1HPV6 score added diagnostic and prognostic information before, during, and after SARS-CoV-2 infection. These data could improve the appropriateness of vaccines, antivirals, and anti-inflammatory prescriptions.

In conclusion, these results validate the diagnostic and prognostic performances of A1HPV6 in patients infected by SARS-CoV-2, not hospitalized in ICU. These results are reinforced by similar kinetics of A1, HP, and A2M observed in the macaque model.

## Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2021.12.009.

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**Conflicts of Interest:**
These authors disclose the following: Thierry Poynard is the inventor of FibroTest and founder of BioPredictive, the patents belong to the public organization Assistance Publique-Hôpitaux deParis. Olivier Deckmyn, Valentina Peta, and Yen Ngo are full-time employees of BioPredictive. The remaining authors disclose no conflicts.

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The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

**Data Transparency Statement:**
Data, analytic methods, and study materials of this nonintervantional study will be made available to other researchers, at thierry@poynard.com.