


ORIGINAL ARTICLE

SARS-CoV-2 infection as a trigger of humoral response against apolipoprotein A-1

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Abstract

Background: Unravelling autoimmune targets triggered by SARS-CoV-2 infection may provide crucial insights into the pathophysiology of the disease and foster the development of potential therapeutic candidate targets and prognostic tools. We aimed at determining (a) the association between anti-SARS-CoV-2 and anti-apoA-1 humoral response and (b) the degree of linear homology between SARS-CoV-2, apoA-1 and Toll-like receptor 2 (TLR2) epitopes.

Design: Bioinformatics modelling coupled with mimic peptides engineering and competition experiments were used to assess epitopes sequence homologies. Anti-SARS-CoV-2 and anti-apoA-1 IgG as well as cytokines were assessed by immunoassays on a case-control (n = 101), an intensive care unit (ICU; n = 126) and a general

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population cohort (n = 663) with available samples in the pre and post-pandemic period.

Results: Using bioinformatics modelling, linear sequence homologies between apoA-1, TLR2 and Spike epitopes were identified but without experimental evidence of cross-reactivity. Overall, anti-apoA-1 IgG levels were higher in COVID-19 patients or anti-SARS-CoV-2 seropositive individuals than in healthy donors or anti-SARS-CoV-2 seronegative individuals ($P < .0001$). Significant and similar associations were noted between anti-apoA-1, anti-SARS-CoV-2 IgG, cytokines and lipid profile. In ICU patients, anti-SARS-CoV-2 and anti-apoA-1 seroconversion rates displayed similar 7-day kinetics, reaching 82% for anti-apoA-1 seropositivity. In the general population, SARS-CoV-2-exposed individuals displayed higher anti-apoA-1 IgG seropositivity rates than nonexposed ones (34% vs 16.8%; $P = .004$).

Conclusion: COVID-19 induces a marked humoral response against the major protein of high-density lipoproteins. As a correlate of poorer prognosis in other clinical settings, such autoimmunity signatures may relate to long-term COVID-19 prognosis assessment and warrant further scrutiny in the current COVID-19 pandemic.

KEYWORDS

anti-apolipoprotein A-1 autoantibodies, COVID-19, molecular mimicry, spike protein, toll-like receptor 2

1 | INTRODUCTION

Several lines of evidence point to a SARS-CoV-2-triggered maladaptive immune response as an important determinant of coronavirus disease 2019 (COVID-19) severity.^{1,2} Relying on a complex interplay between pathogens and host factors, the B-cell immune-mediated response is characterized by a polyclonal activation leading to the production of numerous antibodies which may cross-react with self-antigens when shared molecular homology between self and non-self-antigens occurs.³⁻⁵ Because infection-triggered autoantibodies have been shown to enhance tissue damage and the host inflammatory response, molecular mimicry between self and exogenous epitopes is considered to represent an important mechanism underlying the triad between infectious diseases, autoimmunity and poorer outcomes.³⁻⁵

Furthermore, concurring emerging data demonstrate that humoral autoimmune mechanisms are frequent in COVID-19, with several autoantibodies being detectable in up to 69% of COVID-19 acute cases.⁶⁻⁹ Sequence/structural homologies between SARS-CoV-2 immunodominant epitopes, the receptor-binding domain (RBD) and numerous host antigens have been proposed to underlie such phenomenon.¹⁰⁻¹² Recently, we identified three epitopes from the Spike(S) subdomains S1 and S2, and the C terminus (c-ter) of Spike as potential immunodominant epitopes of the Spike protein,¹³

the c-ter of Spike (amino acid region 1140-1170) having been independently confirmed.¹⁴⁻¹⁶ Previous unpublished observations indicated that this Spike c-ter region shares sequence homology with the c-ter of apolipoprotein A-1 (apoA-1), the major protein fraction component of high-density lipoprotein (HDL), while a more proximal RBD region (amino acid region 455-487), interacting with ACE2 receptor,¹⁵ displayed homology with Toll-like receptor 2 (TLR2). TLR2 engagement and subsequent activation have been shown to be required for autoantibodies against apoA-1 (anti-apoA-1 IgG) to mediate their pro-atherogenic effects.¹⁷⁻²⁰ Because anti-apoA-1 IgGs were shown to represent an independent cardiovascular (CV) risk factor associated with poor prognosis,²¹⁻²⁶ to be elevated after certain viral infections,^{27,28} and to be preferentially oriented against the c-ter part of apoA-1,^{29,30} we hypothesized that SARS-CoV-2 infection could elicit an anti-apoA-1 IgG response with substantial overlap with anti-SARS-CoV-2 IgG serology.

Therefore, we used bioinformatics modelling coupled with mimetic engineered peptides and competition assay to validate linear sequence homologies between apoA-1 and spike epitopes followed by the screening of three independent COVID-19 adult cohorts for the presence of anti-apoA-1 IgG, including a case-control (n = 101), a prospective intensive care unit (ICU) (n = 126) and a general population cohort (n = 663).

2 | MATERIAL AND METHODS

2.1 | Sequence homology analyses, Spike-apoA-1 and Spike-TLR2 mimic peptides synthesis

Homologies between Spike and nucleocapsid epitopes, TLR2 or c-ter apoA-1 were assessed using Clustal Omega and BlastP sequence alignment. These methods have been extensively described in the Supplementary Material.

2.2 | Study populations and sample collection

The case-control cohort, the ICU cohort and the general population cohort have been extensively described in the Supplementary Material.

2.3 | SARS-CoV-2 RT-PCR analyses

As previously reported,^{31,32} SARS-CoV-2 RT-PCR was performed according to the manufacturers' instructions on various platforms, including initially in house method using the BD SARS-CoV-2 reagent kit for BD Max system (Becton, Dickinson and Co, US) and Cobas 6800 SARS-CoV-2 RT-PCR (Roche, Switzerland).

2.4 | Anti-SARS-CoV-2 against Spike 1 domain IgG assessment

We used the Euroimmun IgG enzyme-linked immunosorbent assays (ELISA) (Euroimmun AG, Lübeck, Germany # EI 2606-9601 G; CE-marked) to assess SARS-CoV-2 IgG serology against the S1 domain of the Spike protein (anti-S1 IgG) as explained in the Supplementary Material.

2.5 | Assessment of total antibodies against N antigen of SARS-CoV-2

Total antibodies against the N antigen of SARS-CoV-2 were measured on a Cobas e801 analyser (Roche Diagnostics) according to the manufacturer's instructions. Results are reported as numeric values in form of a cut-off index (signal sample/cut-off or signal calibrator ratio) and are considered as positive when equal to or above 1. Inter-assay variation was 14.3% at a ratio of 2.97 (n = 17).

2.6 | Anti-apoA-1 IgG assessment

Anti-apoA-1 IgGs were measured as previously described.^{21,22,25-28,33} Those methods are explained in the Supplementary Material.

2.7 | Cytokines and anti-pneumococcal IgG (P14 serotype) assessment

All cytokines and the anti-pneumococcal IgG (P14 serotype) measurements were done using Meso Scale Discovery (MSD) platform on the SQ120 instrument. The methods are extensively described in the Supplementary Material.

2.8 | Cross-reactivity and competition experiments

To assess the degree of cross-reactivity between anti-SARS-CoV-2 and anti-apoA-1 IgG with their respective antigens and mimic peptides, two kinds of competition experiments were performed and are extensively reported in the Supplementary Material.

2.9 | Statistics

Analyses were performed with Statistica software (version 13.5.0.17; TIBCO Software, Inc). Statistical methods are explained in the Supplementary Material.

3 | RESULTS

3.1 | Sequence homology assessment, corresponding Spike mimic peptides synthesis and competition experiments

Capitalizing on prior findings indicating that: (a) anti-apoA-1 IgG has to bind to TLR2 due to molecular mimicry in order to generate a pro-inflammatory response by inducing the formation of a TLR2/TLR4/CD14 heterotrimer^{18,19} and (b) anti-apoA-1 IgG are preferentially oriented against the c-ter of apoA-1 in humans^{29,30}; we searched for linear sequence similarities between the Spike protein epitopes,¹³⁻¹⁶ apoA-1 and the extracellular part of TLR2.

As shown in [Figure 1](#), these analyses revealed that the amino acid (aa) sequence 1139-1162 of the Spike protein shares sequence homology with the c-ter part of apoA-1

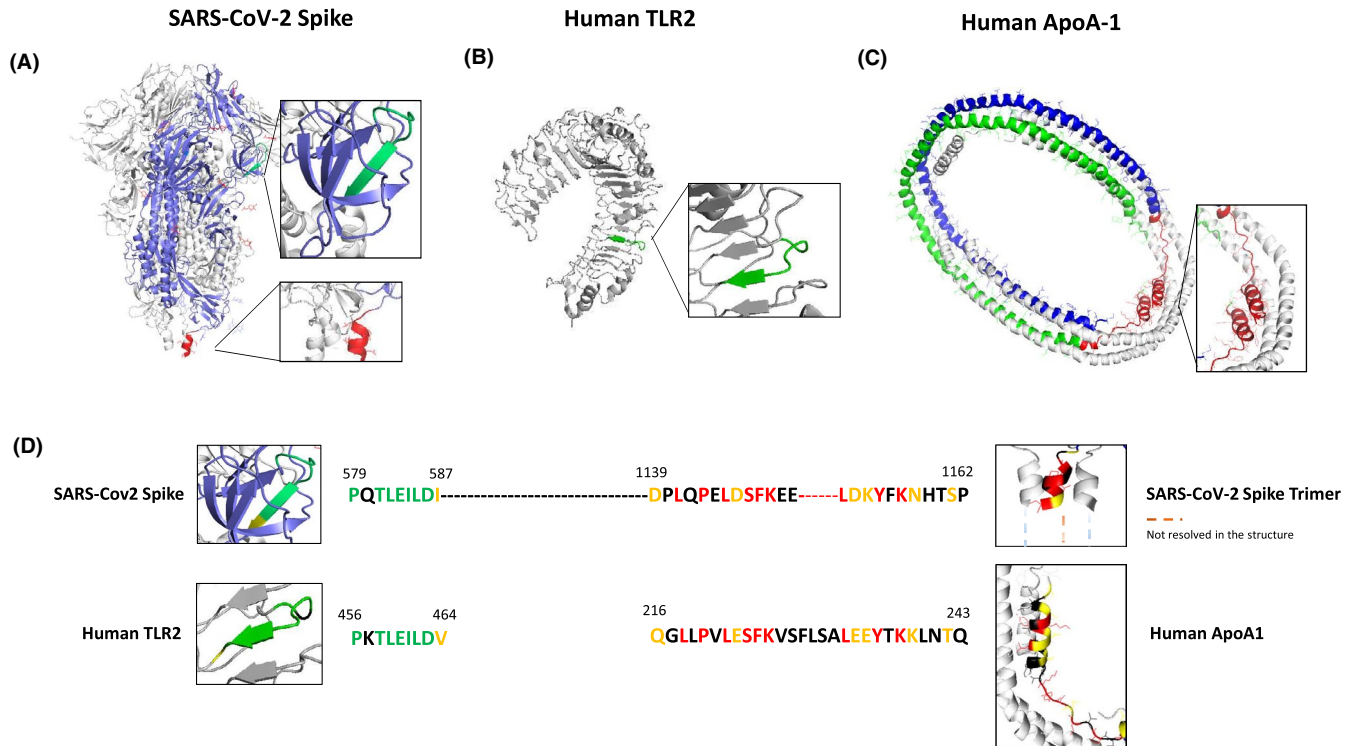


FIGURE 1 Localization of shared epitopes with apoA-1 and TLR2 on the crystal structure of SARS-CoV-2 Spike protein. Panel (A) crystal structure of SARS-CoV-2 spike protein homotrimer (PDB ID 6VXX). Panel (B) human TLR2 crystal structure (PDB ID 6NIG). Panel (C) human ApoA-1 tetramer crystal structure (PDB ID 1AV1). Epitope sequences conserved between Spike and TLR2 are highlighted in green (A and B) and conserved sequences between Spike and ApoA-1 are represented in red (A and C). Panel (D) sequence alignment of SARS-CoV-2 Spike protein (QIV65088.1) with human TLR2 (H33756AA.1) using Clustal W. Conserved residues are indicated in green and the semiconserved one in yellow. BlastP sequence alignment of SARS-CoV-2 Spike sequence with human apoA-1 (P02647.1). Conserved residues are shown in red and semiconserved (functional equivalent) ones in yellow

(amino acids 216-243; **Figure 1D**) and that the aa region spanning 579-587 of Spike protein had a good alignment with those of human TLR2 (aa 456-464; 7 out of 9 amino acids; **Figure 1D**). Closer inspection of the position of the sequence showed that the secondary structure of the peptide was comparable for both sequence matches (**Figure 1D**). Specifically, the aa sequence 1139-1162 in Spike is part of an alpha helical bundle with a portion of the peptide sequence unresolved, whereas the corresponding apoA-1 sequence is also part of an alpha helical bundle with an unstructured portion. The Spike aa 579-587 peptide is part of a beta turn and continues into a beta pleated sheet. There is remarkable structural homology with the structure of the corresponding peptide on TLR2, namely a beta turn followed by a beta pleated sheet segment (**Figure 1D**). The same analysis was performed to search for homology between TLR2, apoA-1 and the N-protein of SARS-CoV-2, using epitopes experimentally detected.^{15,16} A good alignment between apoA-1 (aa 131-143) and N (aa 400-412) was identified with 7 identical and 2 similar residues out of 11; however, the lack of structural data for the segment of the N-protein precluded further analysis. Likewise, a good match was identified between TLR2 (aa 549-557) and N (aa 217-225) with 5 identical and 2 similar

residues in a 8 amino acid stretch, but its structural homology could not be validated. In light of the lack of structural data for these N homology regions, further experimental validation of cross-reactivity was not pursued. Peptide sequences and structures are presented in Figures S1 and S2

Following the bioinformatics identification of common epitopes between Spike and apoA-1, we experimentally assessed the degree of cross-reactivity between anti-SARS-CoV-2 and anti-apoA-1 IgG with their respective antigens in our ELISA format. As shown in **Figure 2A,B**, we attempted to inhibit the anti-apoA-1 reactivity or the reactivity against the Spike protein incubating an anti-SARS-CoV-2 and anti-apoA-1 IgG double-positive sera with increasing concentration of Spike protein and C-ter apoA-1 peptides. As shown in **Figure 2A,B**, neither Spike protein nor c-ter apoA-1 competed for anti-apoA-1 IgG or anti-SARS-CoV-2 IgG signal, respectively. However, when polyclonal anti-Spike IgG pre-incubation onto the plate was performed, we observed a weak but significant decrease of the anti-apoA-1 IgG signal derived from a pool of anti-apoA-1 IgG/anti-SARS-CoV-2 IgG seropositive individuals (**Figure 2C**), while anti-apoA-1 IgG pre-incubation did not affect the anti-SARS-CoV-2 IgG signal of the pool of sera patients (**Figure 2D**).

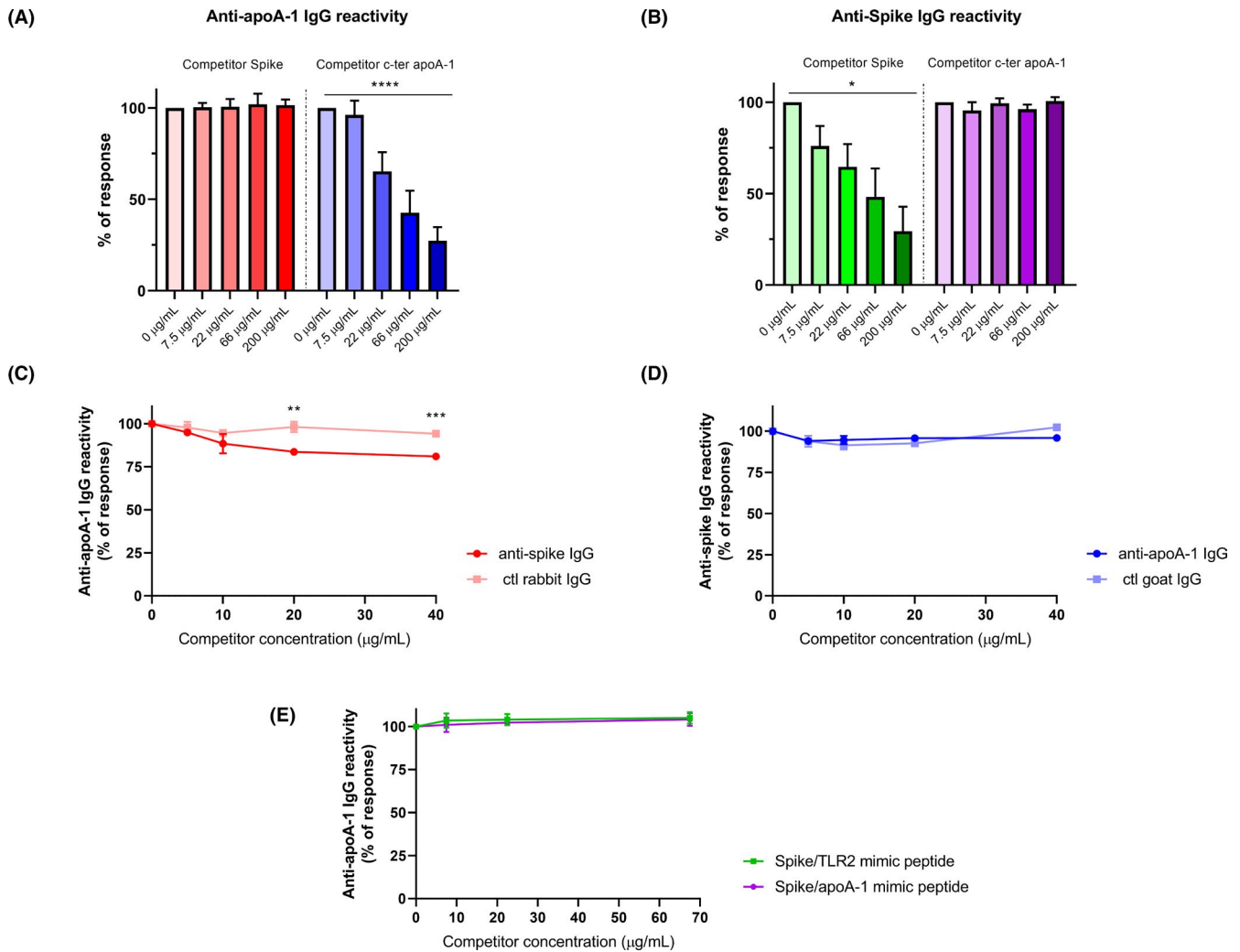


FIGURE 2 Absence of cross-reactivity between anti-apoA-1 IgG and anti-spike IgG. Panel (A and B). Four sera of COVID-19 patients positive for both anti-apoA-1 IgG and anti-spike IgG were pre-incubated with or without Spike or c-ter apoA-1 peptides as competitors at the indicated concentrations prior to addition to assay well for anti-apoA-1 IgG or anti-spike IgG measurements. Percentage of maximal ELISA signals were calculated as $100 \times \frac{[\text{signal in well}] - [\text{mean background signal (uncoated well)}]}{[\text{mean maximal signal (no peptide)}] - [\text{mean background signal}]}$. Results are expressed as mean \pm SD ($n = 4$). The statistical significance of the signal reduction was calculated by the one-way ANOVA test: $****P < .0001$ and $*P = .012$. Panel (C). Polyclonal anti-spike antibodies and not the control rabbit IgGs slightly but significantly compete for apoA-1 binding sites with anti-apoA-1 autoantibodies present in the pooled sera. Results are presented as the mean \pm SD of three independent experiments ($n = 3$). Student's t test was used to determine the significant difference between the means of the two groups. $**P = .002$ and $***P = .0002$. Panel (D). Anti-apoA-1 IgG did not compete for Spike protein. Results are presented as the mean \pm SD of three independent experiments ($n = 3$). Panel (E) absence of anti-apoA-1 IgG signal inhibition when the pool of four anti-apoA-1 IgG/anti-SARS-CoV-2 IgG seropositive individuals were pre-incubated with or without Spike/TLR2 and Spike/apoA-1 mimic peptides as competitors ($n = 3$)

To complete this set of experiment, Spike/ApoA-1 and Spike/TLR2 mimic peptides were used in similar experimental competition procedures. As shown in Figure 2E, none of these mimic peptides competes for the anti-apoA-1 IgG signal derived from a pool of anti-apoA-1 IgG/anti-SARS-CoV-2 IgG seropositive individuals.

Taken together, these results indicate that, despite the presence of common linear epitopes on Spike and apoA-1 according to bioinformatics, in vitro cross-reactivity between anti-apoA-1 IgG and S1 antigen seemed to be absent or modest at best if the results of Figure 5C would be predominantly considered.

3.2 | Anti-SARS-CoV-2 serologies and anti-apolipoprotein A-1 IgG associations

In order to validate our previous bioinformatics findings to humans, we explored the associations between anti-apoA-1 and anti-SARS-CoV-2 serologies on three different cohorts, including a case-control and the ICU cohort, as well as a general population cohort described in Figure S3 and S4. In the case-control and ICU cohort, we aimed at replicating the previously reported correlations between anti-apoA-1 IgG with cytokine and lipid profile^{20,34} as an additional

orthogonal assessment of closely related serologies. The general population cohort was instrumental to generalize the results observed in acute settings at the population level and to determine whether the pre-pandemic anti-apoA-1 serological status could modulate the anti-SARS-CoV-2 response.

3.2.1 | Results from the case-control cohort

The case-control cohort consisted 50 COVID-19 RT-PCR confirmed cases and 51 recruitment period-matched healthy donors recruited over the same period (Figure S3 panel a). The baseline demographic characteristics of participants are summarized in Table 1. Briefly, RT-PCR confirmed COVID-19 patients were older with an over-representation of male gender displaying a higher systemic pro-inflammatory state when compared to the healthy blood donors (Table 1). Among COVID-19 patients, the median delay between a positive SARS-CoV-2 RT-PCR diagnostic test and current biomarker assessment was 10 days (IQR 5-15 days). The proportion of patients within each days post-diagnosis subgroup (delta between their molecular testing and serological testing) was 43.5% for 0-6 days ($n = 20$), 30.4% for 7-14 days ($n = 14$) and 26.1% for >14 days ($n = 12$).

As shown in Table 1, COVID-19 patients had higher median levels of all the pro-inflammatory cytokines and serologies tested, with the exception of Pn14 pneumococcal IgG used as an unrelated serological control. The distribution of serological values between cases and controls is available in Figure S5. No difference between cases and controls was observed for circulating INF- γ levels. Furthermore, when the cohort was split according to anti-S1 IgG seropositivity status, identical differences were observed (Table 1, bottom panel).

These results were further corroborated by the significant and substantial correlations observed between anti-apoA-1 IgG and both (anti-S1 and anti-N) anti-SARS-CoV-2 serologies (Table 2). On the other hand, none of the aforementioned antibodies correlated with anti-pneumococcal IgG (Table 2). Furthermore, both anti-SARS-CoV-2 serologies, anti-apoA-1 IgG, displayed similar strength of associations with most of the cytokines measured. Finally, anti-pneumococcal IgG was not correlated to any of the cytokines tested (Table 2).

3.2.2 | Results from the ICU cohort

To extend and validate these findings in severe COVID-19 disease, we used a cohort of 126 consecutive patients admitted to the ICU for severe COVID-19 disease who completed a follow-up at 28 days, Figure S3 panel b. The baseline demographic and biological characteristics of ICU COVID-19

patients are summarized in Table 3. Anti-apoA-1 IgG seropositivity upon ICU admission was found in 26.9% of the ICU patients (34/126), while anti-S1 IgG and anti-N seropositivity was 36.5% (46/126) and 42% (53/126), respectively. When split according to anti-apoA-1 IgG seropositivity status upon patient admission at the ICU, seropositive patients tended to have a more severe *Simplified Acute Physiology Score II* score, a higher number of DPSO at ICU admission, displayed higher median D-dimers levels, but lower total cholesterol, LDL and triglycerides levels when compared to anti-apoA-1 seronegative individuals (Table 3). The proportion of anti-S1 and anti-N seroconversions were twofold higher in anti-apoA-1 IgG seropositive individuals compared to those tested negative for these autoantibodies (55.8% vs 28.2%, $P = .006$; and 64.7% vs 33.7%; $P = .01$, respectively). No other significant differences for the remaining parameters were identified between anti-apoA-1 IgG seropositive and seronegative individuals.

When split according to anti-S1 serological status, anti-S1 IgG seropositive patients were less likely to be known to have chronic kidney disease, they tended to have a shorter length of ICU stay and they were less likely to require mechanical ventilation (Table S1). On the other hand, anti-S1 IgG seropositive patients displayed higher number of DPSO before ICU admission, higher median levels of anti-apoA-1 IgG and D-dimers, but lower median levels of INF- γ and INF- α 2a. No significant differences were noted regarding the lipid profile and other biological parameters tested (Table S1). The proportion of anti-apoA-1 IgG seropositivity was increased by 2-fold in anti-S1 seropositive individuals compared to anti-S1 IgG seronegative ones (41.3% vs 18.8%, $P = .01$; Table S1). As shown in Table 4, very similar correlations and strength of associations were found between anti-apoA-1 IgG, anti-S1 and anti-N serologies compared to those observed in the case-control cohort. Furthermore, except for INF- γ and INF- α 2a, similar observations were made regarding the associations between the different serologies and cytokines levels (Table 4).

3.2.3 | One-week serological kinetics in ICU patients

In the subgroup of 54 ICU patients for which additional serum samples were available at day 3 and day 7 of ICU admission, significant increases in median values of anti-SARS-CoV-2 and anti-apoA-1 serologies were observed (Figure 3). Accordingly and as expected, anti-SARS-CoV-2 seropositivity rates for both anti-S1 and anti-N serologies were high and reached values above 92% at day 7. Anti-apoA-1 IgG seropositivity evolution displayed a very similar temporal trend to anti-SARS-CoV-2 serology, reaching 82.4% at day 7, indicating that anti-apoA-1 IgG serology kinetics closely

TABLE 1 Demographic and biological characteristics of the case-control cohort

The case-control cohort			
Patients clinical biological characteristics	COVID-19 cases (n = 50)	Healthy controls (n = 51)	P-value
Age	70 (61-76; 33-85)	47.0 (40-62; 22-62)	<.0001
Male Gender; % (n)	60.0 (30)	19.6 (10)	.0001
Cytokine			
IFN- γ , pg/mL	26.6 (18.3-63.2; 7.3-9589)	22.4 (16.3-35.8; 9.6-384.6)	.11
IL-6, pg/mL	12.0 (5.8-36; 3.02-3831.4)	1.1 (0.6-2.0; 0.1-22.3)	<.0001
TNF- α , pg/mL	9.1 (6.5-12.8; 2.2-13.4)	4.6 (3.7-7.3; 1.5-74.3)	<.0001
MCP-1, pg/mL	1875 (1080-2597; 404-1292)	1284.7 (949-1629; 440-3001)	.001
IFN- α 2a, pg/mL	2.8 (1.9-4.4; 0.7-39.4)	1.3 (0.3-1.9; 0.3-24.9)	<.0001
Serologies			
Anti-S1 IgG, ratio	15.8 (1.4-18; 0.2-20.6)	0.3 (0.3-0.4; 2-2.8)	<.0001
Anti-S1 IgG, seropositivity; %(n)	74 (37)	1.9 (1)	<.0001
Anti-N total ab, ratio	29 (2.5-73.3; 0.7-143)	0.07 (0.07-0.08; 0.06-53)	
Anti-N total ab, seropositivity; %(n)	74.0 (37)	1.9 (1)	
Anti-apoA-1 IgG, OD ₄₅₀	1.58 (1.11-1.9; 0.14-2.32)	0.7 (0.55-0.93; 0.3-1.30)	<.0001
Anti-pneumococcal (Pn14) IgG, mg/L	0.44 (0.15-1.36; 0.03-81.1)	0.76 (0.2-2.11-0.07-102)	.40
Splitting the derivation cohort according to anti-S1 IgG seropositivity status			
	Anti-S1 IgG seropositivity (n = 38)	Anti-S1 IgG seronegativity (n = 63)	P-value
Age	70 (61-76; 50-84)	48 (42-60; 22-85)	<.0001 <.0001
Male Gender; % (n)	78.9 (30)	15.8 (10)	<.0001
Cytokines			
IFN- γ , pg/mL	25.8 (18.3-63.2; 7.3-664.9)	22.6 (16.3-41.1; 9.6-9589)	.33
IL-6, pg/mL	8.6 (4.9-19.5; 1.6-3831)	1.6 (0.8-6.7; 0.1-1485)	<.0001
TNF- α , pg/mL	9.5 (6.5-12.8; 2.2-131.4)	5.5 (3.8-8.5; 1.5-74.3)	.0002
MCP-1, pg/mL	1810 (1078-2583; 538-9465)	1335 (958-1795; 404-12901)	.03
IFN- α 2a, pg/mL	2.7 (1.9-3.8; 0.7-10.9)	1.5 (0.9-2.5; 0.3-39.4)	<.0001
Serologies			
Anti-N total ab, ratio	47.2 (17.4-81.7; 0.26-147)	0.07 (0.07-0.08; 0.06-29)	<.0001
Anti-N total ab, seropositivity; % (n)	92.1 (35)	3.2 (2)	<.0001
Anti-apoA-1 IgG, OD ₄₅₀	1.65 (1.35-1.92; 0.14-2.32)	0.76 (0.57-1.00; 0.17-2.01)	<.0001
Anti-pneumococcal (Pn14) IgG, mg/L	0.46 (0.15-1.73; 0.03-81.1)	0.47 (0.17-1.49; 0.07-102)	.70

follow the occurrence of anti-SARS-CoV-2 antibodies over 7 days of severe COVID-19 disease (Figure 3). No such kinetics were observed for anti-Pn14 IgG.

3.2.4 | Results from the general population cohort

Because anti-apoA-1 IgG seropositivity has been shown to concern about one-fifth of the general population and to be

associated with a poorer prognosis over 5 years,^{22,35} our results prompted us to investigate whether such SARS-CoV-2-induced anti-apoA-1 IgG response in acute settings could be replicated in the general population. With this aim, we identified participants recruited in the 'Bus Santé' study between 2016 and 2018 and subsequently included in the SEROCO-V-POP study³² during the COVID-19 pandemic. Among these, we identified 663 individuals with available serum samples from both the pre- (2016-2018) and post-pandemic (2020) periods (Figure S4 and Table S2).

TABLE 2 Spearman correlations between serologies and cytokines profile in the COVID-19 cases of the case-control cohort

	Anti-S1 IgG r; P-value	Anti-N total ab r; P-value	Anti-apoA-1 IgG r; P-value	Anti-pneumococcal IgG r; P-value
Serologies				
Anti-S1 IgG; ratio	ND	0.70;<.0001	0.60;<.0001	-0.07; .50
Anti-N total ab; ratio	0.70; <0.0001	ND	0.54;<.0001	0.07; .47
Anti-apoA-1 IgG, OD ₄₅₀	0.60; <0.0001	0.54;<.0001	ND	-0.03; .77
Anti-pneumococcal (Pn14) IgG, pg/mL	-0.07; 0.50	0.07; .47	-0.03; .77	ND
Cytokines				
IFN- γ , pg/mL	0.15; 0.14	0.12; .25	-0.05; .63	0.01; .91
IL-6, pg/mL	0.41; <0.0001	0.45; <.0001	0.36;<.0001	-0.05; .59
TNF- α , pg/mL	0.38; 0.0001	0.40; <.0001	0.26; .001	-0.07; .47
MCP-1, pg/mL	0.30; 0.003	0.14; .18	-0.05; .73	0.09; .36
IFN- α 2a, pg/mL	0.43; <0.0001	0.37; .0001	0.26; .007	-0.05; .62

Abbreviations: Anti-N total ab, anti-N antigen total antibodies; ND, not determined.

The median age of this cohort was 50-year-old (range 24-78), 297 (44.7%) participants were male, while the baseline (pre-pandemic) anti-apoA-1 IgG seropositivity rate was 25.0% (166/663) and was not associated with any factors commonly ascribed to autoantibodies such as age, gender or smoking (data not shown). The median time between the first anti-apoA-1 IgG assessment in the pre-COVID-19 period and the anti-S1 IgG plus the second anti-apoA-1 IgG measurement during the post-COVID-19 period (after the first pandemic wave) was 3.2 years (IQR: 2.8-3.6; range: 2.3-4.3). In the post-COVID-19 period, 7.5% (50/663) were seropositive against SARS-CoV-2 according to anti-S1 IgG levels. The rate of anti-apoA-1 IgG seropositivity, as well as median anti-apoA-1 IgG levels (data not shown) were significantly lower than in the pre-COVID-19 period, despite remaining of the same order of magnitude than previously reported in the general population^{21,22} (Table S3). As shown in Table S3, in the post-COVID-19 samples, a modest but significant correlation was observed between anti-apoA-1 IgG and anti-S1 IgG levels. Furthermore, in the post-COVID-19 period, anti-S1 seropositive individuals displayed higher median anti-apoA-1 IgG levels (0.35 vs 0.57 OD; $P = .0002$) and higher median anti-apoA-1 seropositivity rates than anti-S1 seronegative individuals (34.0% vs 16.8%, $P = .004$) as shown in Table S3. Moreover, the strength of correlation between anti-apoA-1 IgG and anti-S1 IgG in anti-S1 seropositive individuals was of 0.31 ($P = .03$), whereas no association between these two serologies was found in anti-S1 seronegative individuals (Table S3). Cox regression analyses indicated that the pre-COVID-19 anti-apoA-1 IgG status was a significant predictor of post-COVID-19 anti-apoA-1 IgG status (HR: 1.95; 1.52-2.43; $P < .0001$), irrespective of age, gender and smoking status (HR: 1.57; 95%CI: 1.24-1.99; $P = .0001$), but did not predict post-COVID-19 anti-S1 seropositivity (HR:

1.30; 95%CI: 0.67-2.54; $P = .44$). Finally, adjusted logistic regression analyses indicated that post-pandemic anti-SARS-CoV-2 seropositivity was significantly associated with a 3-fold risk of post-pandemic anti-apoA-1 IgG seropositivity, independently of age, gender and smoking status (OR: 2.46; 95%CI: 1.31-4.60; $P = .005$).

4 | DISCUSSION

The major findings of this study can be summarized by the fact that SARS-CoV-2 infection triggers a humoral response against native apoA-1—the major HDL lipoprotein—in the vast majority infected individuals, displaying similar kinetics and marked correlations to anti-SARS-CoV-2 responses. In this context, the bioinformatics identification of common linear epitopes between SARS-CoV-2 and those of apoA-1 and TLR2 would have lend weight to the molecular mimicry hypothesis, especially as the known functions of identified epitopes would have been concordant with the correlations retrieved presently between anti-apoA-1 and anti-SARS-CoV2 responses, lipid profile and inflammation.

Indeed, the c-ter sequence identified in Spike, aa 1139-1162,¹⁵ is known to be conserved in HCoV-OC43 but not among other coronaviruses and was found to share linear homology with aa 216-243 of apoA-1. This c-ter region of apoA-1 structurally corresponds to an alpha helix bundle playing a key role in the cellular cholesterol efflux regulation by ATP-binding cassette transporter A1 (ABCA1) and in HDL maturation³⁶ and was shown to be preferentially targeted by the polyclonal anti-apoA-1 IgG response.^{29,30} Such regional targeting is therefore compatible with the previously reported inverse relationships between anti-apoA-1 IgG and HDL cholesterol,^{21,33,34} the similar ones retrieved presently between

TABLE 3 Baseline demographic and biological characteristics of ICU patients and according to anti-apoA-1 IgG serological status

Demographic and biological characteristics	Overall (n = 126)	Anti-apoA-1 IgG seropositive patients (n = 34)	Anti-apoA-1 IgG seronegative patients (n = 92)	P-value
Age, years	63.5 (57-73; 25-86)	65.5 (57-73; 28-86)	62.5 (57-72.5; 25-83)	.48
Female gender, % (n)	22.2 (28)	17.6 (6)	23.4 (22)	.63
BMI, kg/m ²	28.1 (25.5-32.1; 15.6-52.4)	28.3 (25.1-32.8-19.3-52.4)	28.1 (25.6-31.8; 15.6-50.8)	.92
Current smoking, % (n)	13.5 (17)	14.7 (5)	13.0 (12)	.78
DPSO and ICU admission	9.0 (7-11; 0-27)	9.5 (7.5-13.5; 3-27)	9.0 (7.0-10; 0-27)	.10
Comorbidities				
Hypertension, % (n)	47.6 (60)	44.1 (15)	48.9 (45)	.69
Dyslipidaemia, % (n)	58.3 (35)	23.5 (8)	29.3 (27)	.66
Diabetes, % (n)	26.7 (34)	29.4 (10)	26.0 (24)	.82
Previous IC and or HF, % (n)	23.8 (30)	23.5 (8)	23.9 (22)	1
Previous stroke, % (n)	5.5 (7)	0 (0)	7.6 (7)	.19
Known malignancy, % (n)	7.9 (10)	11.7 (4)	6.5 (6)	.46
Chronic kidney disease, % (n)	7.1 (9)	5.8 (2)	7.6 (7)	1
Severity upon admission				
APACHE II score	22 (14-29; 3-38)	22 (14-29; 3-38)	22 (13.5-27.5; 4-37)	.61
SOFA score	6 (4-7; 1-11)	5 (4-7; 2-10)	6 (4-7; 1-11)	.44
SAPS II score	53 (43-65; 6-82)	58 (46-69; 18-78)	52 (38.5-61.5; 6-82)	.09
28-day mortality, % (n)	16.7 (21)	23.5 (8)	14.1 (13)	.28
Length of stay at ICU, days	16 (10-21; 1-48)	14 (10-18; 1-42)	16 (10-22; 1-48)	.25
Mechanical ventilation, % (n)	96 (121)	91.1 (31)	97.8 (90)	.12
Cytokines and inflammation				
CRP, mg/L	154 (92-205; 23.1-402.8)	162.7 (114.1-219.5; 23.1-402.8)	144 (92.4-201; 31-311)	.35
IFN- γ , pg/mL	411.1 (112-988.1; 152.2-37747)	308.4 (112.1-691.5; 5.2-37747)	426.7 (175.1-1232.6; 175-17778)	.22
IL-6, pg/mL	155.0 (69.1-324.3; 6.7-7889.6)	212.4 (61.4-561.0; 6.7-7689.8)	140.8 (73.3-284.6; 13.9-2224.8)	.32
TNF- α , pg/mL	6.7 (4.0-16.6; 0.23-164.5)	9.3 (4.0-16.9; 0.3-71.2)	6.4 (4.2-14.8; 1.6-164.5)	.33
MCP-1, pg/mL	4199 (2509-8488; 254-36483)	3620 (2314-10281; 776-36483)	4300 (2634-7609; 254-30256)	.92
ifn- α 2a, pg/mL	7.1 (2.3-22.6; 0.2-468.5)	6.3 (1.5-19.1; 0.2-92.3)	8.9 (2.6-30.9; 0.3-468.5)	.13
D-dimers; ng/mL	1531 (961-2476; 220-10001)	1838 (1326-3245; 439-10001)	1433 (863-2174; 220-9999)	.03
Lipid profile				
Total cholesterol, mmol/L	2.8 (2.2-3.2; 1.1-5.8)	2.4 (1.9-3.1; 1.1-5.2)	2.9 (2.4-3.3; 1.1-5.8)	.03
HDL cholesterol, mmol/L	0.63 (0.47-0.76; 0.14-1.95)	0.52 (0.38-0.61; 0.27-0.88)	0.66 (0.54-0.79; 0.14-1.95)	.0001
LDL cholesterol, mmol/L	1.29 (0.87-1.76; 0.00-3.70)	1.00 (0.62-1.57; 0.08-3.70)	1.43 (1.05-1.79; 0.00-3.58)	.02

(Continues)

TABLE 3 (Continued)

Demographic and biological characteristics	Overall (n = 126)	Anti-apoA-1 IgG seropositive patients (n = 34)	Anti-apoA-1 IgG seronegative patients (n = 92)	P-value
Triglycerides, mmol/L	1.64 (1.18-2.24; 0.79-4.05)	1.75 (1.38-2.24; 0.81-4.00)	1.57 (1.15-2.23; 0.79-4.05)	.24
Cardiac biomarkers				
Hs-cTnT, ng/L	16.0 (9.7-34.9; 3.31-971)	13.0 (8.1-40.5; 3.7-665)	17.9 (9.7-33.5; 3.3-971)	.53
NT-proBNP, pg/mL	308 (95.6-1015; 15.1-18772)	363 (110-1437; 22-5928)	278.5 (93.3-909; 15.1-18772)	.42
Serologies				
Anti-S1 IgG, ratio	0.73 (0.4-1.9; 0.3-27.0)	2.1 (0.5-17.3; 0.4-27.0)	0.6 (0.4-1.15; 0.3-21.9)	.0009
Anti-S1 IgG, seropositivity; % (n)	36.2 (46)	55.8 (19)	28.2 (26)	.006
Anti-N total ab, ratio	0.46 (0.1-4.70; 0.1-36.3)	2.71 (0.2-11.3; 0.1-36.3)	0.31 (0.1-1.5; 0.1-29.1)	.0004
Anti-N total ab, seropositivity; % (n)	42.0 (53)	64.7 (22)	33.7 (31)	.002
Anti-apoA-1 IgG, OD ₄₅₀	0.43 (0.24-0.70; 0.00-2.60)	0.99 (0.83-1.70; 0.70-2.60)	0.33 (0.22-0.44; 0.0-0.67)	<.0001
Renal function				
Creatinine; μ mol/L	81.0 (66.5-105; 38-769)	81.5 (67.5-110.5; 47-173)	80.5 (65-98, 38-769)	.36

Note: All continuous variables are expressed as median (interquartile range; and range); *P-value derived from the comparison between anti-apoA-1 IgG seropositive versus seronegative individuals.

Abbreviations: APACHE II, Acute Physiology And Chronic Health Evaluation II; DPSO, days post-symptom onset; HF, heart failure; IC, ischaemic cardiopathy; SAPS, Simplified Acute Physiology Score; SOFA, Sequential Organ Failure Assessment.

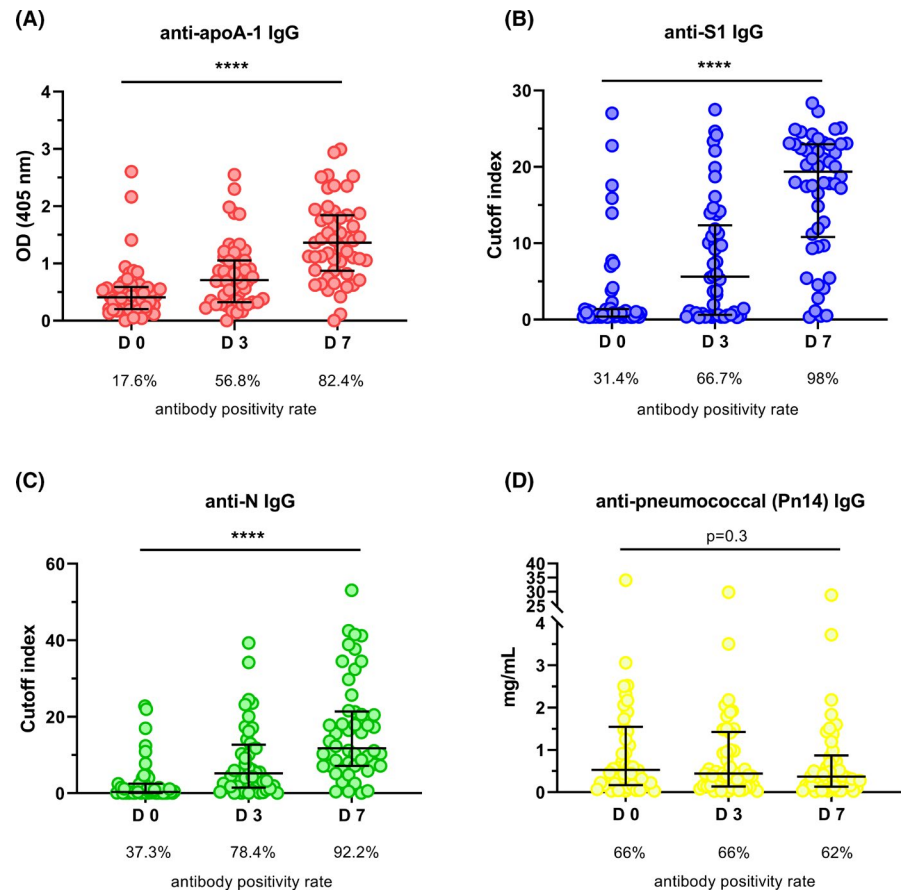
TABLE 4 Spearman correlations between serologies, cytokines and lipid profile in the ICU patients

	Anti-S1 IgG r; P-value	Anti-N total ab r; P-value	Anti-apoA-1 IgG r; P-value
Serologies			
Anti-S1 IgG; ratio	ND	0.77; <.0001	0.43; <.0001
Anti-N total ab; ratio	0.77; <.0001	ND	0.44; <.0001
Anti-apoA-1 IgG, OD ₄₅₀	0.43; <.0001	0.44; <.0001	ND
Cytokines			
CRP; mg/L	0.18; .05	0.16; .08	0.20; .02
IFN- γ , pg/mL	-0.25; .005	-0.25; .005	-0.19; .04
IL-6, pg/mL	-0.02; .81	-0.07; .47	0.11; .24
TNF- α , pg/mL	-0.02; .83	-0.09; .32	0.12; .19
MCP-1, pg/mL	0.04; .69	0.006; .94	0.01; .93
IFN- α 2a, pg/mL	-0.36; .0003	-0.44; <.0001	-0.30; .0005
D-dimers; ng/mL	0.29; .004	0.11; .26	0.05; .63
Lipid profile			
Total cholesterol, mmol/L	0.12; .21	0.04; .70	-0.16; .06
HDL cholesterol, mmol/L	-0.26; .004	-0.27; .003	-0.37; <.0001
LDL cholesterol, mmol/L	0.12; .21	0.09; .33	-0.16; 0.08
Triglycerides, mmol/L	0.24; .008	0.10; .29	0.20; 0.03

HDL levels and anti-SARS-CoV-2 serology titres in the ICU cohort. The second linear sequence homology identified concerned the aa 579-587 of Spike, located in the S1 domain and

close to the receptor-binding domain, which has very homology to the aa 456-464 sequence of TLR2. This sequence is part of the leucine-rich repeats (LRR) ectodomain of TLR2, known

FIGURE 3 One-week serological kinetics in ICU patients. In panels (A-C), ICU patients showed a significant increase (P -value of **** $P < .0001$ and $P = .025$) in antibody titre throughout seven days (days: 0, 3 and 7). In panel (D), the anti-pneumococcal (Pn14) antibody titre did not present any change over time ($P = .3$). Results are expressed as median with interquartile range and the Kruskal-Wallis test was used to compare the three groups. Samples were analysed in duplicate



to be key for proper pathogen-associated or damage-associated molecular pattern recognition and TLR2's function.³⁷ The anti-apoA-1 IgGs engagement of this region due to sequence homology with apoA-1²⁰ has been reported to mediate their pro-inflammatory/pro-atherogenic response,^{18-20,34,38} and the present correlations retrieved between anti-apoA-1 IgG, anti-SARS-CoV-2 serologies and pro-inflammatory cytokines on the case-control cohort would have lend further weight to the molecular mimicry hypothesis proposed to explain the occurrence of other cross-reacting pathogenic antibodies in COVID-19.^{39,40} However, by failing to clearly demonstrate any cross-reactivity between the different antigens and antibodies of interest, our experimental approach did not support the molecular mimicry hypothesis to explain the occurrence of COVID-19-induced anti-apoA-1 IgG response and its intricate relationship with anti-SARS-CoV-2 serologies. Such discrepancy between bioinformatics modelling and competition experiments can be explained by the possible existence of common conformational epitope(s) that our epitope mapping systems could not detect, and/or by the likely existence of additional mechanisms, such as intermolecular epitope spreading, allowing the initial targeted humoral response to quickly broaden to antigens other than the inducing epitope.⁴¹

From a physiopathological point of view, these results provide innovative perspectives. First of all, these results extend the coverage of virus-mediated anti-apoA-1 IgG induction to

SARS-CoV-2, as previously shown for HCV and HIV.^{27,28} The inverse associations reported here between anti-apoA-1 IgGs and HDL levels are similar to what has been observed in HCV²⁷ and are reminiscent of the concept that HCV could hijack the scavenger receptor B-1 (SR-B1)-mediated HDL uptake to infect hepatocytes^{27,42} which has recently been transposed to SARS-CoV-2 by the recent demonstration of Wei and colleagues who identified SR-B1 as an additional receptor facilitating the SARS-CoV-2 entry into cells.⁴³ SR-B1 being the canonical apoA-1/HDL receptor involved in reverse cholesterol efflux and HDL maturation, our results suggest that the molecular mimicry-based anti-apoA-1 IgG response in COVID-19 may concur with other established inflammatory factors to explain the low HDL and apoA-1 levels reported previously in COVID-19.^{44,45} Secondly and along the same line, our results lend further weight to the fact that host lipid metabolism may play an important role in COVID-19 severity by modulating the intensity of the immune response. Lee et al⁴⁶ recently demonstrated that COVID-19 activates regulatory element binding protein-2 (SREBP-2), a key transcription factory lying at the cross-roads of inflammation modulation and cholesterol biosynthesis. Given the requirement of cholesterol biosynthesis for SARS-CoV-2 budding-driven exocytosis, any factor modulating SREBP-2 pathway activation may influence the course of COVID-19 disease.⁴⁶ In this respect, recent findings in human macrophages indicate

that anti-apoA-1 IgGs increase the expression of SREBP-2 in a TLR2/4-dependent manner, culminating into enhanced foam cell formation, the hallmark of atherogenesis,³⁴ through anti-apoA-1 IgG-dependent ACAT activation leading to the redirection of cellular cholesterol towards intracellular esterified cholesterol pools and decreased membrane free cholesterol content.³⁴ Because membrane free cholesterol is a key regulator of membrane ACE2R trafficking into dedicated lipid rafts for optimal SARS-CoV-1 endocytosis,⁴⁷ knowing whether anti-apoA-1 IgG could influence the course of COVID-19 should be further investigated.

Although not designed to convey any actionable clinical implications, the results derived from this exploratory and hypothesis-generating study may bare the following potential clinical implications. From a pragmatic analytical standpoint, our results indicate that despite the intimate relationship between anti-apoA-1 and anti-S1 serology and the existence of linear sequence homology between defined apoA-1 and S1 epitopes; our results indicate that the risk of potential analytical interference between these serologies can safely be ruled out. This point is important to make as 30%–80% of COVID-19 individuals will have high levels of both anti-apoA-1 IgG and anti-SARS-CoV-2 antibodies, and the present associations would fuel such question. Importantly, although we could not assess the direct clinical implications of the COVID-19-induced anti-apoA-1 IgG response, our observations may well relate to patient prognosis for two reasons. Firstly, these pathogenic autoantibodies were shown to be active mediator of sterile inflammation^{18–20,34} and independent predictors of overall mortality and adverse CV events in numerous clinical settings, including general populations^{21–26}; these antibodies may well be of concern in COVID-19 too. Secondly and along the same line, functional antibodies against G-coupled receptors displaying identical biological activity to anti-apoA-1 IgGs were recently shown to be associated with prolonged symptoms persistence after COVID-19 infection,⁴⁸ further supporting to the suspected clinical relevance of COVID-19-induced pathogenic autoantibodies for long-term outcomes and potential enhanced patient risk stratification.⁴⁹ Until the formal demonstration of the harmlessness of such autoantibodies, such biological signature should be carefully evaluated, especially in the context of the long COVID syndrome.

We acknowledge several limitations of the present work. Firstly, we limited our analyses to autoantibodies directed against apoA-1 and did not consider other autoantibodies of possible relevance in COVID-19, such as anti-phospholipid or anti-platelet 4 autoantibodies.^{50,51} Secondly, due to the fact that our hospital became a COVID-19-only hospital during the first pandemic wave could not identify a COVID-19-free control population matched for usual factors impacting anti-SARS-CoV-2 serological response, such as age and gender during the recruitment period. However, because anti-apoA-1 IgG levels

have been shown to be independent of most age, gender, smoking and most comorbidities (except CV ones),^{20–26} and because of the kinetic observed on the ICU cohort together with the observations on the cohort population, we feel confident that using a recruitment-matched case-control cohort did induced a bias susceptible to blunt the conclusions of our present observations. Thirdly, in the context of the recent SARS-CoV-2 variants unknown during the first epidemic wave, we could not assess the possible impact of such variants on the anti-apoA-1 IgG response. However, as the linear sequence homologies identified between apoA-1 and Spike did not contain the characteristic epitope regions of the three main variants of concern (VOC) in Europe (United Kingdom, Brazilian and South-African strains), it is unlikely that our results could be specific of a defined and currently existing SARS-CoV-2 strain. Fourth, if our results indicate that an acute exposure to SARS-CoV-2 rapidly increases the anti-apoA-1 IgG response, they do not allow inferring any conclusions about the possible longer term persistence of anti-apoA-1 IgG levels after COVID-19 disease or the possible clinical relevance of phenomenon.

In conclusion, this report shows for the first time that in a substantial proportion of SARS-CoV-2-exposed individuals, a marked humoral autoimmune response against the major lipoprotein of HDL occurs for reasons other than molecular mimicry despite the linear sequence homologies retrieved between Spike and apoA-1 epitopes. Knowing whether the pre- or co-existence of anti-apoA-1 IgG may modulate the course of COVID-19 disease remains uncertain. However, as correlates of poorer prognosis in different settings, a better understanding of the possible clinical relevance of COVID-19-induced autoimmune biological signatures is warranted in the current COVID-19 pandemic and ongoing vaccination programmes.

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CONFLICT OF INTEREST

SP, OH and NV are named as co-inventors on a patent related to c-ter apoA-1 mimetic peptides ('Mimetic peptides for prognosis, diagnosis or treatment of a cardiovascular disease', N° P1347EP00). N. Vuilleumier, S. Pagano and O. Hartley are named as co-inventors of the patent related to

cterA1, peptide ('Mimetic peptides for prognosis, diagnosis or treatment of a cardiovascular disease', N° P1347EP00) but have no other conflict of interest to disclose. The remaining authors have no conflict of interest to declare. Funding sources played no role in the design and conduct of the study, nor in the collection, analysis and interpretation of the data, nor in the preparation, review and approval of the article or decision to submit for publication.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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