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Autoantibodies against apolipoprotein A-1 after COVID-19 predict symptoms persistence

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Abstract

Background: SARS-CoV-2 infection triggers different auto-antibodies, including anti-apolipoprotein A-1 IgGs (AAA1), which could be of concern as mediators of persistent symptoms. We determined the kinetics of AAA1 response over after COVID-19 and the impact of AAA1 on the inflammatory response and symptoms persistence.

Methods: All serologies were assessed at one, three, six and twelve months in 193 hospital employees with COVID-19. ROC curve analyses and logistic regression models (LRM) were used to determine the prognostic accuracy of AAA1 and their association with patient-reported COVID-19 symptoms persistence at 12 months. Interferon (IFN)-α and-γ production by AAA1-stimulated human monocyte-derived macrophages (HMDM) was assessed in vitro.

Results: AAA1 seropositivity was 93% at one month and declined to 15% at 12 months after COVID-19. Persistent symptoms at 12 months were observed in 45.1% of participants, with a predominance of neurological (28.5%), followed by general (15%) and respiratory symptoms (9.3%). Over time, strength of correlations between AAA1 and anti-SARS-COV2 serologies decreased, but remained

significant. From the 3rd month on, AAA1 levels predicted persistent respiratory symptoms (area under the curves 0.72-0.74; $p < 0.001$), independently of disease severity, age and gender (adjusted odds ratios 4.81–4.94; $p = 0.02$), while anti-SARS-CoV-2 serologies did not. AAA1 increased IFN-α production by HMDMs ($p = 0.03$), without affecting the IFN-γ response.

Conclusion: COVID-19 induces a marked though transient AAA1 response, independently predicting one-year persistence of respiratory symptoms. By increasing IFN-α response, AAA1 may contribute to persistent symptoms. If and how AAA1 levels assessment could be of use for COVID-19 risk stratification remains to be determined.

KEYWORDS

anti-apolipoprotein A-1 autoantibodies, COVID-19, long COVID-19, symptoms persistence, type I interferon response

1 | INTRODUCTION

Molecular mimicry between exogenous and endogenous epitopes can generate autoantibodies that can subsequently promote inflammation and secondary tissue damage.[1–3] More specifically, the humoral adaptative immune response characterized by a polyclonal activation generates numerous antibodies that may cross-react in case of shared molecular homology between self and non-self-antigens,[1–3] or with other structurally unrelated epitopes due to B-cell inter/intra molecular epitope spreading, a phenomenon occurring in inflammatory contexts where the initial humoral response is secondarily expanded to other antigens/epitopes than the triggering one.[4]

In coronavirus disease 2019 (COVID-19), a dysregulated immune response has been shown to contribute to disease pathobiology and severity.[5,6] Moreover, autoimmune mechanisms are frequent following SARS-CoV-2 infection, with several types of autoantibodies detectable in up to 2/3 of patients with acute COVID-19.[7–10] The sequence homology between several self-antigens and the SARS-CoV-2 immunodominant receptor binding domain (RBD) epitope of the Spike protein has been suggested as a potential explanation among several for this phenomenon [11–13]. In this line, it has been shown that the C-terminus (c-ter; amino-acid region 1140-1170) domain of Spike [14–17] shares sequence homology with the c-ter of apolipoprotein A-1 (apoA-1), the major protein fraction component of high-density lipoprotein (HDL). Providing that anti-ApoA-1 (AAA1) IgGs preferentially bind the c-ter part of ApoA-1,[18,19] this might explain why patients with SARS-CoV-2 infection trigger a humoral response against ApoA-1,[20] an independent cardiovascular (CV) risk factor for poor prognosis.[21–25] Among others, the possible clinical relevance of COVID-19-induced auto-antibodies have lately been suggested to be of significance for long-term outcomes.[26] In particular, functional antibodies against G-coupled receptors were shown to be associated with prolonged symptoms persistence after COVID-19 infection.[27] Because these autoantibodies share similar in vitro functional properties with AAA1 IgGs[28] whose persistence after COVID-19 is still unknown, we first evaluated the AAA1 IgGs persistence over time after COVID-19 aiming at defining the clinical and biological factors determining AAA1 IgG kinetics. Furthermore, we studied whether persistent symptoms at 12 months could be associated with the persistence of AAA1 IgG as well as anti-S1 antibodies, lipid profile parameters and serum cytokines. Finally, we assessed the impact of AAA1 IgG on macrophage interferons (alpha and gamma) production, whose differential temporal expression has been shown to be key drivers of COVID-19 severity and complications.[29–32]

2 | MATERIAL AND METHODS

2.1 | Study design and participants

This is an ancillary work from a previously published prospective single-centre observational longitudinal study enrolling staff from the Geneva University Hospitals' (HUG) aged ≥18 years, all with a nasopharyngeal reverse-transcription polymerase chain reaction (RT-PCR) confirmed SARS-CoV-2 infection.[33] Symptom density score was defined as the product of the total number of acute symptoms and the total duration (days) of each symptom. More details about the study procedures, exclusion

criteria, RT-PCR and viral load analysis are provided elsewhere.[33]

2.2 | Study end point

The predefined primary composite end point was defined by the persistence of any COVID-19 symptom at 12 months after SARS-CoV-2 infection, including (i) respiratory (dyspnoea and/or cough), (ii) and/or general (asthenia, and/or myalgia and/or arthralgia), (iii) and/or neurological (headache, and/or anosmia, and/or dysgeusia and/or memory losses and/or dizziness) symptoms. End points were defined by the study coordinator before biochemical analyses for AAA1 IgGs were performed.

2.3 | Ethical considerations

The study was approved by the regional ethics committee (CCER 2020-00516) and registered (NCT04329546) prior to initiation.

2.4 | AAA1 IgG assessment

AAA1 IgGs were measured as previously described,[21–25,34,35] in leftover sera after analyses for antibodies against SARS-CoV-2 antigens (see below). As previously published,[20] "Maxisorp plates (Nunc™, Roskilde,) were coated with purified, human-derived delipidated and unmodified apoA-1 (20 µg/mL; 50 µL/well) for 1 h at 37°C. After being washed, all wells were blocked for 1 h with 2% bovine serum albumin (BSA) in a phosphate buffer solution (PBS) at 37°C. Participants' samples were also added to a non-coated well to assess individual non-specific binding. After six washing cycles, a 50 µL/well of signal antibody (alkaline phosphatase-conjugated anti-human IgG; Sigma-Aldrich, ref: A-3150), diluted 1:1000 in a PBS/BSA 2% solution, was added and incubated for 1 h at 37 C. After washing six more times, phosphatase substrate p-nitrophenyl-phosphate-disodium (Sigma-Aldrich,) dissolved in a diethanolamine buffer (pH 9.8) was added and incubated for 30 min at 37°C (Molecular Devices™ Filter Max F3, Molecular Devices,). OD_{450} was determined at 450 nm, and each sample was tested in duplicate. Corresponding non-specific binding was subtracted from the mean OD_{450} for each sample. The specificity of detection against lipid-free and unmodified apoA-1 has been previously determined by conventional saturation tests, Western blot and LC-MS analyses.[23] At an intermediate ratio of 0.6 OD_{450}, the interassay coefficient of variation was 9% ($n = 5$), and the intra-assay CV was 5% ($n = 5$). For serum, the AAA1 IgG seropositivity cut-off was previously specified and validated and was set at an OD_{450} ratio >0.64 for the 97.5th percentile of AAA1 IgG of healthy blood donors."[21–25,34,35]

2.5 | Anti-SARS-CoV-2 antibody detection

Anti-SARS-CoV-2 antibodies measured in total immunoglobulin levels were analyzed using the Elecsys anti-S1 platforms on the cobas e801 analyzer (Roche Diagnostics Rotkreuz, Switzerland). Results for the quantitative Elecsys anti-S1 antibodies were reported as concentrations (U/ml) with the manufacturer's cuf-off >0·8 U/ml considered as positive. Quality controls and coefficients of variation for the anti-S1 assay are provided elsewhere.[33]

2.6 | Cholesterol levels assessment

At one month post-infection, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, non-HDL (nHDL) cholesterol and triglycerides (TG) were measured by standard chemistry assays (Roche 8000/H Cobas), whereas low-density lipoprotein (LDL) cholesterol values were calculated using the Friedwald formula. All results were provided in mmol/L.

2.7 | Cytokines multiplex assessment in blood samples

At one-month post-infection, the level of interferon (IFN)-α and IFN-γ was measured in the serum applying the Luminex MAP™ Technology using the Human ProcartaPlex Mix&Match 6-Plex Panel (Invitrogen,) using the Bio-Plex 100 (Bio-Rad Laboratories,) according to the manufacturer's instructions, with results provided in pg/mL. Lower limit of detection (LLOD) for IFN-α and IFN-γ was, respectively, 0.02 pg/mL and 0.25 pg/mL. IFN-α and IFN-γ values below the LLOD were attributed to half the LLOD value (0.01 pg/mL and 0.12 pg/mL, respectively).

2.8 | Human monocyte-derived macrophages (HMDMs) preparation

Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats obtained from healthy donors in the Geneva Hospital Blood Transfusion Center (Switzerland) as previously described[36] and differentiated into macrophages by incubation with 50 ng/ml of

human macrophage colony stimulating factor (M-CSF) (PeproTech,) in complete RPMI-1640-Glutamax I culture medium (10% heat-inactivated FBS, 50 µg/ml streptomycin, 50 U/ml penicillin, 2 mM L-glutamine) for 6 days. Macrophage preparation consisted of >90% CD68+ cells, as assessed by flow cytometry.

2.9 | Cytokines multiplex assessment in cell supernatants

HMDMs were treated with AAA1 IgG (Academy Biomedical Company, ref: 11A-G2) or respective control antibodies, goat IgG (Meridian Life Science, ref: A66200H) for 24 h. Supernatants were collected, centrifuged at 5000 *g* for 5 min at 4°C and frozen at −80°C. IFN-α and IFN-γ were measured, diluting supernatants 1:3, using the MesoScale Discovery (MSD) platform on the SQ120 instrument. Analyte concentrations were determined with Discovery Workbench® software 4.0, using a 4 parameters logistic fit model.

2.10 | Statistics

Logistical and funding considerations defined a target of 200 participants, and no sample size was calculated. AAA1 IgG levels at the different time points were compared using Wilcoxon signed rank tests. Correlations between AAA1 IgGs and anti-S1, lipid profile parameters and serum cytokines were performed using Spearman correlations. Latent growth modeling (LGM) was used to estimate growth trajectories[37] and predictors of AAA1 IgG initial levels and kinetics over time using R 4·0·3 (package lavaan version 0·6–7) program. More details about the LGM are provided in the Supplementary Methods of the main manuscript.[33] Prognostic accuracy of continuous AAA1 IgG levels, lipid profile parameters and serum cytokines for symptom persistence was assessed by receiver operating characteristic (ROC) analyses and expressed as the area under the curve (AUC) Excel Analyse-it software ™ (Microsoft Redmond,). We only considered significant predictors of any symptoms persistence upon ROC curve analyses for subsequent logistic regression analyses, and for covariates of interest in adjusted models. Univariate and adjusted logistic regression models were used to assess the association between AAA1 IgGs (as continuous values and according to the pre-defined seropositivity cut-off) and symptoms persistence at 12 months using Statistica ™ software (StatSoft,). Results are expressed as odds ratios (ORs) with their 95% confidence intervals (95% CI). Sensitivity (SN), specificity (SP), positive (PPV) and negative predictive values (NPV) were defined by ROC analyses at the pre-defined AAA1 IgG seropositivity cut-off. The remaining analyses were performed with SPSS software v23·0 (IBM Corp.,). The graphs were generated using GraphPad Prism 9.0 (GraphPad Software,). A value of $p < 0.05$ was considered statistically significant.

3 | RESULTS

Two hundred hospital employees were enrolled, among which 193 completed the follow-up at 12 months. Demographics and comorbidities of study participants are detailed in Table 1. More details about study inclusion and specific initial symptoms have been provided elsewhere.[33]

3.1 | Persistent symptoms at 12 months

Among the 193 participants who completed the follow-up at 12 months, neurological symptoms were reported in 55 participants (28.5%), whereas general symptoms and respiratory symptoms were reported in 29 (15.0%) and 18 (9.3%) participants, respectively (Table 1).

3.2 | Evolution of AAA1 IgG levels over time

AAA1 IgG seropositivity at one, three, six and 12 months after SARS-CoV-2 infection was respectively 92.7% (179/193), 68.9% (133/193), 52.8% (102/193) and 14.5% (28/193; Figure 1). Median AAA1 IgG levels at one, three, 6 and 12 months were, respectively, 0.99 (interquartile range [IQR] 0.79–1.34), 0.75 (IQR 0.57–0.95), 0.66 (IQR 0.52–0.87) and 0.36 (IQR 0.26–0.51), ($p < 00.001$ for all) (Figure 1).

In a LGM without covariates, AAA1 IgG initial levels were significantly different from zero (standardized estimate = 0.964, 95%CI: 0.913; 1.016, $p < 00.001$) and significantly differed between participants (standardized estimate = 0.116, 95%CI: 0.090; 0.143, $p < 00.001$). AAA1 IgG levels decreased over time (standardized estimate = −0.049, 95%CI: −0.053; −0.046, $p < 00.001$), and their change over time differed among participants (standardized estimate = 0.001, 95%CI: 0.001; 0.001, $p < 00.001$). Higher initial AAA1 IgG levels were associated with a faster decrease in levels over time (standardized estimate = −0.005, 95%CI: −0.006; −0.003, $p < 00.001$). In a LGM with covariates, older age, the presence of comorbidities and higher body mass index (BMI) were associated with higher initial AAA1 IgG levels (p = 0.001, 0.035, and 0.049 respectively) (Table 2). Similarly, AAA1 IgG levels decreased faster in older participants and in participants with comorbidities (p = 0.032 and 0.021, respectively) (Table 2). In a multivariate model including gender, age,

TABLE 1 Demographics of SARS-CoV-2-infected patients

	SARS-CoV-2 infection ($n = 193$)
Demographics	
Age, y, median (IQR)	40.6 (30.2–52.2)
Male sex, *n* (%)	58 (30.1)
Ethnicity, *n* (%)	
Caucasian	159 (82.4)
Hispanic	10 (5.2)
Mixed	8 (4.1)
African	6 (3.1)
Asian	4 (2.1)
Others	2 (1.0)
Not provided	4 (2.1)
Comorbidities, *n* (%)	
Obesity (BMI ≧30)	23 (11.9)
Asthma	17 (8.8)
Hypertension	9 (4.7)
Cancer	7 (3.6)
Autoimmune disease	6 (3.1)
Diabetes	3 (1.6)
Chronic lung disease	3 (1.6)
Eczema	3 (1.6)
Inflammatory bowel disease	2 (1.0)
Hepatic disease	2 (1.0)
Primary immune deficiency	1 (0.5)
No past medical history	133 (68.9)
Habits, *n* (%)	
Smoking	23 (11.9)
Vaping	9 (4.7)
Clinical management, *n* (%)	
Ambulatory care	188 (97.4)
Hospital admission to ward	5 (2.6)
Persisting symptoms at 12 months, n (%)#	87 (45.1)
General symptoms*	29 (15.0)
Respiratory symptoms**	18 (9.3)
Neurological symptoms***	55 (28.5)
Anti-S1 antibodies at 1 month post-infection, median U/ml (IQR)	96.1 (38.2–178.0)
Lipid profile parameters at 1 month post-infection, median (IQR)	
Total cholesterol	5.56 (4.86–6.47)
HDL	1.20 (0.98–1.49)
Non-HDL cholesterol	4.39 (3.59–5.24)
Triglycerides	1.86 (1.49–2.46)
LDL	3.40 (2.73–4.11)
Serum cytokines values at 1 month post infection	
IFN-α, median pg/mL (IQR)	0.07 (0.02–0.12)
Specimens below LLOD, n(%)	*46 (23.8)*
IFN-γ, median pg/mL (IQR)	0.90 (0.16–2.01)
Specimens below LLOD, n(%)	*48 (249)*

Abbreviations: Anti-S1, anti-Spike; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; IFN, interferon; LLOD, lower limit of detection.

*: Arthralgia, myalgia or fatigue.

**: Cough or shortness of breath.

***: anosmia, dys/agueusia, memory loss, dizziness or headache.

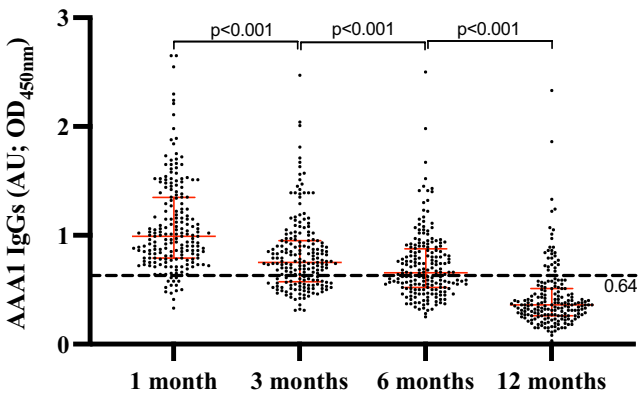

FIGURE 1 Evolution of AAA1 IgGs over time after COVID-19 in study participants ($n = 193$). AAA1: anti-apolipoprotein A-1. Every study time point was completed by all study participants

symptom density score, BMI and comorbidities, only age predicted higher initial AAA1 IgG levels ($p = 0.001$) (Table 2).

3.3 | Associations between AAA1 IgGs and anti-S1 antibody titers, lipid profile parameters and serum cytokines

3.3.1 | Anti-S1

Anti-S1 titers were moderately correlated with AAA1 IgG levels at one ($r = 0.412$; $p < 00.001$), three ($r = 0.236$; $p = 0.001$), six ($r = 0.219$; $p = 0.002$) and 12 months ($r = 0.169$; $p = 0.019$), even though the strength of the correlation decreased over time.

3.3.2 | Lipid profile parameters

There was no significant difference in the levels of TC, HDL, nHDL, TG and LDL between patients with or without

TABLE 2 Parameter estimates of latent growth models for AAA1 IgG levels

Covariates	Bivariate model						Multivariate model					
	Initial AAA1 levels*			Kinetics of AAA1 levels#			Initial AAA1 levels*			Kinetics of AAA1 levels#		
	Estimate	95% CI	*p*-value	Estimate	95% CI	*p*-value	Estimate	95% CI	*p*-value	Estimate	95% CI	*p*-value
Sex (ref. female)	−0.038	−0.150; 0.074	.511	0.006	−0.002; 0.004	.132	−0.001	−0.115; 0.112	.979	0.005	−0.003; 0.014	.236
Age	0.008	0.003; 0.012	.001	−0.001	−0.001; 0.000	.032	0.007	0.003; 0.012	.001	−0.001	−0.001; 0.000	.084
Any comorbidity	0.117	0.008; 0.227	.035	−0.009	−0.017; −0.001	.021	0.068	−0.051; 0.187	.264	−0.007	−0.016; 0.002	.105
BMI	0.011	0.000; 0.022	.049	−0.001	−0.002; 0.000	.105	0.007	−0.006; 0.019	.294	−0.001	−0.001; 0.001	.819
Log density score at baseline	0.107	−0.016; 0.231	.088	−0.008	−0.017; 0.001	.082	0.040	−0.086; 0.165	.537	−0.005	−0.015; 0.004	.277

Abbreviations: AAA1, anti-apolipoprotein A-1; CI, confidence interval; BMI, body mass index.

*: AAA1 levels at first study time point (1 month post SARS-CoV-2 infection).

#: Evolution of AAA1 levels between 1, 3, 6 and 12 months post SARS-CoV-2 infection.

positive AAA1 IgGs at one month after infection (data not shown). On the other hand, AAA1 IgGs levels were positively correlated with TC ($r = 0.142$; $p = 0.049$), nHDL ($r = 0.199$; $p = 0.006$) and LDL ($r = 0.247$; $p = 0.001$) and negatively correlated with HDL ($r = -0.192$; $p = 0.007$). There was no correlation between AAA1 IgGs and TG at 1 month ($r = 0.094$; $p = 0.195$).

3.3.3 | Serum cytokines

There was no significant difference in the serum levels of IFN-α and IFN-γ between patients with or without positive AAA1 IgGs at one month after infection (data not shown). Similarly, there was no correlation between serum levels of IFN-α and IFN-γ and AAA1 IgGs levels (data not shown).

3.4 | Association between patient-reported symptom persistence and AAA1 IgGs levels, lipid profil e parameters and serum cytokines

3.4.1 | AAA1 IgGs

Participants with persistent symptoms at 3 months were not more likely to have positive AAA1 IgGs at 3 months compared to participants with complete symptom resolution (71.6% [63/88] vs. 66.1% [72/109]; $p = 0.406$). However, participants with persistent symptoms at 6 months were more likely to have positive AAA1 IgGs at 6 months compared to participants with complete symptom resolution (61.5% [56/91] vs. 45.7% [48/105]; $p = 0.027$). The same was true at 12 months (20.7% [17/82] vs. 9.9% [11/111]; $p = 0.035$). Such associations were not observed with anti-S1 antibodies (data not shown).

AAA1 IgG levels at one month post-infection were predictive of persistent symptoms of any kind at 12 months (AUC 0.59 [95%CI 0.51-0.67]; $p = 0.01$), respiratory symptoms at 12 months (AUC 0.68 [95%CI 0.56-0.79]; $p = 0.01$), but did not predict persistent neurological and general symptoms at 12 months (Table 3). AAA1 IgG levels at 3 months post-infection were predictive of persistent respiratory symptoms at 12 months (AUC 0.72 [95%CI 0.59-0.87]; $p = 0.0005$) but failed to predict other symptoms at 12 months (Table 3). Also, AAA1 IgG levels at 6 months post-infection were predictive of persistent respiratory symptoms (AUC 0.72 [95%CI 0.60–0.85]; $p = 0.0002$), neurological symptoms (AUC 0.59 [95%CI 0.50-0.67]; $p = 0.03$) and symptoms of any kind (AUC 0.60 [95%CI 0.51–0.68]; $p = 0.01$) at 12 months (Table 3). Similarly, AAA1 IgG levels at 12 months post-infection were

TABLE 3 Performance of AAA1 IgG seropositivity and levels at 1, 3, 6 and 12 months in predicting persisting symptoms at 12 months

	AUC (continuous AAA1 IgGs)		Odds ratio (continuous AAA1 IgGs)		Odds ratio (dichotomic AAA1 IgGs)	
	AUC	*p*-value	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
AAA1 at 1 month post infection						
Any symptoms at 12 months	0.59 (0.51–0.67)	0.01				
Unadjusted			2.00 (1.001–3.99)	0.049	2.89 (0.78–10.74)	0.11
Adjusted			1.55 (0.72–3.32)	0.26	2.54 (0.64–10.0)	0.18
Respiratory symptoms at 12 months	0.68 (0.56–0.79)	0.01				
Unadjusted			2.51 (0.93–6.79)	0.07	*	ND
Adjusted			2.37 (0.77–7.31)	0.13	*	ND
Neurological symptoms at 12 months	0.56 (0.47–0.65)	0.08				
Unadjusted			1.74 (0.85–3.56)	0.13	2.52 (0.54–11.67)	0.24
Adjusted			1.34 (0.57–3.15)	0.5	2.15 (0.42–11.01)	0.36
General symptoms at 12 months	0.56 (0.43–0.79)	0.18				
Unadjusted			2.34 (1.003–5.45)	0.049	0.62 (0.16–2.39)	0.5
Adjusted			1.73 (0.68–4.46)	0.25	0.56 (0.13–2.41)	0.44
AAA1 at 3 months post infection						
Any symptoms at 12 months	0.57 (0.49–0.65)	0.05				
Unadjusted			1.73 (0.74–4.02)	0.2	*1.53 (0.81–2.88)*	*0.18*
Adjusted			1.47 (0.59–3.66)	0.4	*1.18 (0.59–2.35)*	*0.63*
Respiratory symptoms at 12 months	0.72 (0.59–0.87)	0.0005				
Unadjusted			4.06 (1.27–12.94)	0.01	*1.70 (0.44–6.54)*	*0.43*
Adjusted			4.94 (1.31–18.66)	0.02	*2.37 (0.66–8.53)*	*0.18*
Neurological symptoms at 12 months	0.53 (0.44–0.62)	0.24				
Unadjusted			1.53 80.63–3.72)	0.35	*1.26 (0.63–2.52)*	*0.51*
Adjusted			1.45 (0.50–4.25)	0.5	*0.82 (0.37–1.82)*	*0.63*
General symptoms at 12 months	0.57 (0.44–0.69)	0.15				
Unadjusted			1.90 (0.67–5.43)	0.23	*0.98 (0.41–2.31)*	*0.96*
Adjusted			1.35 (0.41–4.40)	0.62	*0.76 (0.29–1.97)*	*0.58*
AAA1 at 6 months post infection						
Any symptoms at 12 months	0.60 (0.51–0.68)	0.01				
Unadjusted			2.20 (0.86–5.76)	0.1	*1.77 (0.99–3.16)*	*0.05*
Adjusted			1.69 (0.63–4.53)	0.3	*1.40 (0.74–2.65)*	*0.29*
Respiratory symptoms at 12 months	0.72 (0.60–0.85)	0.0002				
Unadjusted			5.00 (1.41–17–78)	0.01	*8.27 (1.85–37.10)*	*0.005*
Adjusted			4.81 (1.22–19.03)	0.02	*6.81 (1.46–31.70)*	*0.01*

(Continues)

TABLE 3 (Continued)

	AUC (continuous AAA1 IgGs)		Odds ratio (continuous AAA1 IgGs)		Odds ratio (dichotomic AAA1 IgGs)	
	AUC	*p*-value	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
Neurological symptoms at 12 months	0.59 (0.50–0.67)	0.03				
Unadjusted			1.96 (0.74–5.19)	0.18	*1.85 (0.98–3.52)*	*0.06*
Adjusted			1.80 (0.58–5.59)	0.31	*1.42 (0.69–2.93)*	*0.34*
General symptoms at 12 months	0.55 (0.43–0.68)	0.2				
Unadjusted			1.46 (0.44–4.82)	0.53	*0.85 (0.40–1.98)*	*0.78*
Adjusted			0.97 (0.24–3.90)	0.97	*0.82 (0.34–1.96)*	*0.65*
AAA1 at 12 months post infection						
Any symptoms at 12 months	0.63 (0.55–0.71)	0.0008				
Unadjusted			2.44 (0.84–7.11)	0.1	*2.37 (1.04–5.40)*	*0.03*
Adjusted			2.02 (0.70–5.82)	0.2	*2.52 (1.04–6.10)*	*0.03*
Respiratory symptoms at 12 months	0.74 (0.62–0.85)	<0.0001				
Unadjusted			3.55 (0.99–12.69)	0.05	*3.47 (1.18–10.2)*	*0.02*
Adjusted			3.72 (0.91–15.17)	0.06	*3.58 (1.13–11.33)*	*0.03*
Neurological symptoms at 12 months	0.61 (0.52–0.70)	0.006				
Unadjusted			2.02 (0.71–5.75)	0.19	*1.77 (0.77–4.09)*	*0.17*
Adjusted			2.15 (0.67–6.89)	0.2	*1.97 (0.76–5.11)*	*0.15*
General symptoms at 12 months	0.60 (0.49–0.72)	0.05				
Unadjusted			1.46 (0.41–5.13)	0.56	*1.27 (0.44–3.68)*	*0.65*
Adjusted			1.15 (0.28–4.62)	0.85	*1.14 80.36–3.53)*	*0.81*

Abbreviations: AAA1, anti-apolipoprotein A-1; AUC, area under the curve; OR, odds ratio, CI, confidence interval; ND, not done.

symptoms at 12 months, hence no OR could be calculated.

Respiratory symptoms: persistent cough, dyspnoea (and/or).

Neurological symptoms persistent anosmia, dysgeusia, headache, memory impairment (and/or).

General symptoms: persistent fatigue, arthralgia, myalgia (and or).

Adjusted: adjusted for age, gender, density scoreV1 and presence of any comorbidity.

*: No participant with negative AAA1 a 1 month had persisting respiratory.

associated with persistent respiratory symptoms (AUC 0.74 [95%CI 0.62–0.85]; $p < 00.0001$), neurological symptoms (AUC 0.61 [95%CI 0.52–0.70]; $p = 0.006$) and symptoms of any kind (AUC 0.63 [95%CI 0.55–0.71]; $p = 0.008$) at 12 months (Table 3).

Using a logistic regression model and after adjusting for age, gender, symptom density score and any comorbidity, the presence of positive AAA1 IgGs or their levels at one month post-infection failed to predict persistent symptoms at 12 months (Table 3). At 3 months post-infection, AAA1 IgG levels predicted respiratory symptoms at 12 months (adjusted OR 4.94 [95% CI 1.31–18.66]; $p = 0.02$) (Table 3). Similarly, at 6 months post-infection, positive AAA1 IgGs as well as their levels predicted respiratory symptoms at 12 months (adjusted OR 6.81 [95% CI 1.46–31.70]; $p = 0.01$ and 4.81 [95% CI 1.22–19.03]; $p = 0.02$, respectively) (Table 3). Finally, positive AAA1 IgGs at 12 months post-infection were associated with the persistence of symptoms of any kind and respiratory symptoms at 12 months (adjusted OR 2.52 [95% CI 1.04–6.10]; $p = 0.03$ and 3.58 [95% CI 1.13–11.33]; $p = 0.03$, respectively) (Table 3).

At the pre-defined and previously validated AAA1 seropositivity cut-off, NPVs were found to be equal or above 95% for respiratory symptoms persistence at 12 months at any of the time points analysed (Table 4).

3.4.2 | Anti-S1

Unlike AAA1 IgGs, anti-S1 antibody levels at any time point were not associated with symptoms persistence at one year (data not shown).

3.4.3 | Lipid profile parameters

None of the lipid profile parameters was predictive of persistent symptoms of any kind at 12 months (data not shown).

3.4.4 | Serum cytokines

IFN-α and IFN-γ levels at one month both predicted the persistence of general symptoms at 12 months (AUC 0.62 [95%CI 0.51–0.73]; $p = 0.016$ and AUC 0.61 [95%CI 0.51–0.72]; $p = 0.014$, respectively). However, IFN-α and IFN-γ failed to predict the persistence of neurological symptoms, respiratory symptoms or the persistence of any symptoms (data not shown).

Because none of the lipid profile parameters nor IFNs were associated with any symptoms persistence, they were not further considered in regression logistic models

3.5 | Cytokine production in supernatant of stimulated HMDMs

Because of the association between serum IFN levels and symptoms persistence, and despite the lack of association between serum IFN levels and AAA1 IgGs, we evaluated to which extent those autoantibodies could modify the inflammatory response of HMDMs, as validated surrogate of alveolar macrophages.[38] As shown in Figure 2, AAA1 IgGs induced a significant increase of IFN-α production ($p = 0.03$; panel A), while a non-significant trends towards a decrease on IFN-γ production was observed ($p = 0.06$; Panel B).

4 | DISCUSSION

In this study, we evaluated the seropositivity and kinetics of AAA1 antibodies following COVID-19 and the association between persistent symptoms and AAA1 IgGs, anti-S1 antibodies, lipid profile parameters and serum cytokines. Persistent symptoms obviously become a medical challenge of clinical importance by affecting up to 80% of COVID-19 patients in certain cases.[33–36] In our cohort of participants with mostly mild COVID-19, 45% reported persistent symptoms 12 months after a documented infection. The reported symptoms were most frequently neurological, followed by general and respiratory symptoms. These symptoms are in line with a data on long-term symptoms following COVID-19 among healthcare workers.[39]

The first notable finding of our study was that 93% of participants had positive AAA1 IgGs 1 month after SARS-CoV-2 infection, which is significantly above what is expected in the general population where the prevalence of AAA1 IgGs is approximately 20%.[23,40] This finding is similar to what has been reported in COVID-19 patients admitted to the intensive care unit (ICU), where the AAA1 IgG seropositivity was found to swiftly raise from 18% upon ICU admission to 82% within 7 days of hospitalization and to closely mirror anti-SARS-CoV-2 serological levels and kinetics.[20] Extending previous investigations performed so far,[20] this the fourth independent cohort validating the proof of principle that SARS-CoV-2 infection increases the humoral response against apoA-1. Similarly, an increase in AAA1 IgGs has also been documented following COVID-19 mRNA vaccine.[41,42] Our results are in line with previous reports indicating that linear sequence between apoA-1 and other RNA viruses' epitopes, may underlie higher AAA1 IgG seropositvity rates observed in HIV, reaching 50% and being associated with surrogate biological markers of infection severity.[35] Similarly, HCV infection has been associated with an increase in AAA1 IgGs.[34] Taken together, these data suggest that infection with other RNA viruses, such as human coronaviruses, could also trigger an increase in AAA1 IgGs and possibly be associated with disease complications.

The second notable finding of this study that could not be addressed by previous investigations is that the COVID-19-induced AAA1 IgG response is transient over time, with a decrease kinetic influenced by older age and presence of comorbidities, both known host factors for COVID-19 pathogenesis and severity. Therefore, even if the seroprevalence of 15% at 12 months post-infection is similar to what has been retrieved in general populations,[23,40] knowing whether such seropositivity rate would represent the preinfection AAA1 IgG seroprevalence of our study population is still unknown.

The third and probably most important finding of this study resides in the demonstration that the COVID-19-induced AAA1 IgG response displays a substantial prognostic accuracy for the persistence of respiratory symptoms after SARS-CoV-2 infection. Although suspected due to the worse and independent prognostic value ascribed to these autoantibodies in other settings[22–25,40,43] and to the fact that autoantibodies against-G-coupled receptors sharing similar functional properties with AAA1 IgGs were associated with long COVID-19 symptoms,[27]

TABLE 4 Sensitivity, specificity and predictive values of predefined and validated AAA1 cut-off (≥0.64) in prediction of persisting symptoms

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
AAA1 at 1 month post infection				
Any symptoms at 12 months	94.30%	12.30%	47.00%	72%
Respiratory symptoms at 12 months	100%	9.10%	10.00%	100%
Neurological symptoms at 12 months	ND	ND	ND	ND
General symptoms at 12 months	ND	ND	ND	ND
AAA1 at 3 months post infection				
Any symptoms at 12 months	ND	ND	ND	ND
Respiratory symptoms at 12 months	83.30%	33.90%	16.70%	95%
Neurological symptoms at 12 months	ND	ND	ND	ND
General symptoms at 12 months	ND	ND	ND	ND
AAA1 at 6 months post infection				
Any symptoms at 12 months	59.80%	55.70%	53.00%	63.00%
Respiratory symptoms at 12 months	83.30%	52.00%	15.00%	97.00%
Neurological symptoms at 12 months	63.60%	53.60%	35%	79%
General symptoms at 12 months	ND	ND	ND	ND
AAA1 at 12 months post infection				
Any symptoms at 12 months	21.80%	91.50%	68.00%	59.00%
Respiratory symptoms at 12 months	33.30%	87.4	21.00%	93.00%
Neurological symptoms at 12 months	20.00%	87.70%	39.00%	73.00%
General symptoms at 12 months	ND	ND	ND	ND

Abbreviations: AAA1, anti-apolipoprotein A-1; AUC, area under the curve; OR, odds ratio, CI, confidence interval; ND, not done.

Respiratory symptoms: persistent cough, dyspnoea (and/or).

Neurological symptoms: persistent anosmia, dysgeusia, headache, memory impairment (and/or).

General symptoms: persistent fatigue, arthralgia, myalgia (and/or).

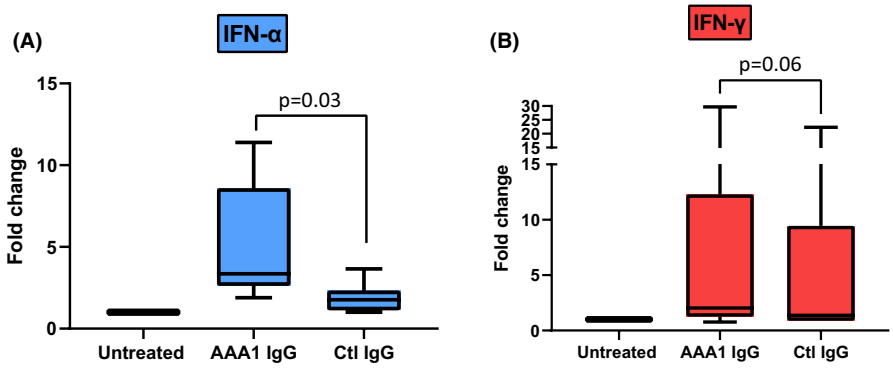

FIGURE 2 Effects of anti-apoA-1 IgG on IFN-α (A) and IFN-ɣ secretion by human monocyte-derived macrophages. AAA1: Anti-apolipoprotein A-1; IgG: immunoglobulin G; IFN: interferon; HMDM: human monocyte-derived macrophages. Cells were treated with AAA1 IgG or control IgG for 24 h and supernatants were harvested for IFN-α (A) and IFN-ɣ (B) secretion measurement. Data are presented as fold change in cytokine secretion of the median (IQR) of six independent experiments ($n = 6$). The fold change in cytokine production is relative to HMDM-untreated cells

such hypothesis remained to be formally demonstrated in the context of COVID-19. Our convergent results derived from LRM, ROC curve and logistic regression analyses concur to indicate that AAA1 IgG seropositivity at any time point studied independently predicted the persistence of respiratory symptoms at 12 months. The associations with other study end points were less consistent and prone to substantial variability according to follow-up duration. AAA1 IgG levels at 6 and 12 months also predicted the persistence of neurological symptoms at 12 months, whereas levels at 1, 6 and 12 months predicted the persistence of any type of symptoms at 12 months, even though the prognostic accuracy was lower when compared to predicting respiratory symptoms. Noteworthy, ROC curves analyses indicated that continuous AAA1 levels at 1, 3, 6 and 12 months predicted the persistence of respiratory symptoms at 12 months, with appealing AUC values above 0.72 for any time point considered. Using the previously defined and validated seropositivity cut-off of 0.64,[21–25, 34,35] which was not designed to predict COVID-19 persisting symptoms, AAA1 IgG seropositivity displayed negative predictive values ≥93% of for persistent respiratory symptoms and was overall associated with a 3–4-fold increased risk of respiratory symptom persistence at any time point considered, suggesting that such cut-off could well be relevant in the context of COVID-19. Based upon the high NPV associated to AAA1 seropositivity, our current results suggest that assessing these autoantibodies in COVID-19 patients could potentially represent an innovative way for COVID-19 risk stratification. Such hypothesis requires further validation before any clinical recommendations can be made.

The fourth important finding of this study resides in the observation that AAA1 IgG can increase the HMDM production of IFN-α (type I IFN response), recently reported to promote COVID-19 and respiratory tract disease severity through TNF-driven response,[29–32] while no significant effect was noted on IFN-γ (type 2 IFN response). Extending previous observations indicating that AAA1 IgGs elicit a broad cytokine response by stimulating macrophages toll-like receptor-2/4/CD14 complexes to promote atherogenesis, the present results indicate that AAA1 IgG could participate to COVID-19 pathogenesis by enhancing the host IFN-α response. As no associations between AAA1 IgGs and IFNs could be retrieved on our cohort, partly because IFNs levels were below the assay detection limit, our results lend weight to the hypothesis that AAA1 IgGs can locally increase the IFN-α production by macrophages. Transposed to the alveolar microenvironment,[38] these results provide a biological rational linking COVID-19-induced AAA1 IgG production with long-term respiratory symptom persistence.

Taken together, the present results add to the cumulating evidences indicating that the subacute/chronic phase of COVID-19 is characterized by a broad autoimmune response, leading to the production of numerous auto-antibodies whose pathogenicity will dependent upon a complex interplay between the specificity of the autoantibodies and some yet unidentified host predisposing factors. Molecular mimicry mechanisms between self-antigens and spike epitopes are considered as an important driver of such autoimmune response,[11–13] and currently more than 50 autoantibodies have been reported as being raised by COVID-19.[16, 44] Among these, pathogenic/functional auto-antibodies seems to be of utmost relevance for COVID-19 patients' prognosis,[44–48] and a deeper characterization of the COVID-19-induced humoral autoimmune response is likely to pave the way for innovative risk stratification strategies and hopefully therapeutic approaches.

This study has several limitations. First, given the study design, we were not able to have baseline sera to document

the prevalence of positive AAA1 IgGs before SARS-CoV-2 infection, and we had to rely on historical general population cohorts. However, because AAA1 seropositivity returned to 14.5% at 12 months post-infection, it is reasonable to assume that the baseline seroprevalence for AAA1 IgG was around 15%–20%, which is the expected prevalence in the general population.[23,40] A second limitation is the lack of a control group of uninfected patients to document the evolution of AAA1 IgGs over time in the absence of SARS-CoV-2 infection. Third, as most long-COVID-19-related studies, symptom persistence might be overestimated because of the non-specific nature of symptoms, even though we used conventional definitions. Moreover, as the association between AAA1 antibodies and respiratory symptoms persistence does not imply causality, further studies are required to understand the exact nature of such novel association. Furthermore, the predefined AAA1 seropositivity cut-off has been validated in the context of cardiovascular outcomes[22–25,40,43] and one could wonder the significance of this cut-off in a completely different context such as COVID-19. However, LRM and ROC curves in this study have shown that the seropositivity cut-off seem appropriate to capture symptom persistence following COVID-19, and post-hoc analyses indicated that an AAA1 IgG cut-off value of 0.53 OD would have been required to reach a NPV of 100%. Then, the fact that the first serum specimen was only collected one month after COVID-19 diagnosis, serum cytokine levels were low or below the assay detection limit in many patients. Then, due to the study design, we could not relate the presence of these autoantibodies to cardiovascular (CV) outcomes, where these autoantibodies have been extensively studied.[22–25,40,43] AAA1 IgG sharing similar pro-arrythmogenic properties with anti-G proteins-coupled receptors and antiheart autoantibodies retrieved in COVID-19,[25,27,28,49] knowing whether the AAA1 IgG response could be relevance for COVID-19-related CV complications, such as myopericarditis[50] and increased risk of arrhythmia[51] warrant further investigations. Then, we did not assess other auto-antibodies of possible interest in COVID-19, even though AAA1 IgG were the only relevant auto-antibodies regarding outcome prediction in acute coronary syndrome in a head-to-head comparison with of different auto-antibodies, including anti-phospholipid antibodies and autoantibodies related to the anti-oxLDL family.[52] Finally, even though our work suggests AAA1 IgGs could be a useful biomarker for long COVID risk stratification, the fact that only patients reported symptoms were available (and not independently adjudicated end points), it would be premature to provide any more clinical recommendation at this stage. Whether the association between AAA1 IgGs and persisting symptoms can be reproduced in more severe COVID-19 cases remains to be evaluated.

In conclusion, COVID-19 induces a marked though transient AAA1 IgG response, independently predicting one-year persistence of respiratory symptoms after SARS-CoV-2 infection. Moreover, those autoantibodies generate a predominant type I IFN response that could contribute to persistent symptoms. If and how AAA1 IgG levels assessment could be of use for COVID-19 risk stratification remains to be determined, and the present study indicates that such autoimmune signature requires a deeper understanding in the context of the COVID-19 pandemic.

AUTHOR CONTRIBUTIONS

Arnaud G L'Huillier, Stephanie Baggio, Christiane S Eberhardt, Angela Huttner, Klara M Posfay-Barbe, Claire-Anne Siegrist, Laurent Kaiser, Nicolas Vuilleumier designed and performed the study. Arnaud G L'Huillier, Benjamin Meyer, Diego O Andrey, Christiane S Eberhardt, Angela Huttner, Klara M Posfay-Barbe, Sabine Yerly, Claire-Anne Siegrist, Laurent Kaiser, Nicolas Vuilleumier contributed resources (reagents, dedicated time). Arnaud G L'Huillier, Sabrina Pagano, Diego O Andrey, Sabine Yerly, Laurent Kaiser, Nicolas Vuilleumier collected data. Arnaud G L'Huillier, Sabrina Pagano, Stephanie Baggio, Benjamin Meyer, Diego O Andrey, Mayssam Nehme, Idris Guessous, Sabine Yerly, Laurent Kaiser, Nicolas Vuilleumier analyzed data. All authors reviewed data. Arnaud G L'Huillier, Sabrina Pagano, Stephanie Baggio, Nicolas Vuilleumier wrote the manuscript. All authors critically reviewed the manuscript.

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CONFLICTS OF INTEREST

NV received restricted research grants unrelated to this study from Roche. All the other co-authors have no conflicts of interest to declare.

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