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Persistence of humoral response upon SARS-CoV-2 infection

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Summary

SARS-CoV-2 continues to leave its toll on global health and the economy. Management of the pandemic will rely heavily on the degree of adaptive immunity persistence following natural SARS-CoV-2 infection. Along with the progression of the pandemic, more literature on the persistence of the SARS-CoV-2-specific antibody response is becoming available. Here, we summarize findings on the persistence of the humoral, including neutralizing antibody, response at three to eight months post SARS-CoV-2 infection in non-pregnant adults. While the comparability of the literature is limited, findings on the detectability of immunoglobulin G class of antibodies (IgG) were most consistent and were reported in most studies to last for six to eight months. Studies investigating the response of immunoglobins M and A (IgM, IgA) were limited and reported mixed results, in particular, for IgM. The majority of studies observed neutralizing antibodies at all time points tested, which in some studies lasted up to eight months. The presence of neutralizing antibodies has been linked to protection from re-infection, suggesting long-term immunity to SARS-CoV-2. These neutralizing capacities may be challenged by emerging virus variants, but mucosal antibodies as well as memory B and T cells may optimize future immune responses. Thus, further longitudinal investigation of PCR-confirmed seropositive individuals using sensitive assays is warranted to elucidate the nature and duration of a more long-term humoral response.

KEYWORDS
Covid-19, humoral response, immunity, immunoglobulins, neutralizing antibodies, SARS-CoV-2

1 | INTRODUCTION

Despite ongoing social distancing and vaccination efforts around the world, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to leave its toll on global health and economy and may become endemic.[1,2] Management of the pandemic through epidemiological modelling and public health policies will heavily rely on the extent of immunity persistence following SARS-CoV-2 infection.[2,3] Like other viruses, SARS-CoV-2 elicits an adaptive immune response, including the development of SARS-CoV-2-specific T cells and antibodies.[4] Here, neutralizing antibodies (NAbs) are of special importance as they neutralize the virus and thereby prevent infection of cells.[3,5] NAbs bind to the spike protein on the surface of the virus. The main structural proteins of SARS-CoV-2 include the

transmembrane proteins M and envelope (E), the nucleoprotein (N) and the trimeric transmembrane glycoprotein spike (S). In particular, the S protein is of high interest, as it is responsible for attachment, fusion, and entry of SARS-CoV-2. The S protein is composed of an N-terminal S1 subunit and a C-terminal S2 subunit. S1 contains the receptor-binding domain (RBD) and an N-terminal domain (NTD).[6] The virus gains entrance into cells via the S1, which binds to the angiotensin-converting enzyme 2 (ACE2) receptor through RBD. Receptor binding triggers cleavage of S at the S2' site by cellular proteases such as TMPRSS2 either at the cell surface or within the endosome. This allows insertion of the fusion peptide into the cellular membrane and subsequent conformation changes in S2 which enable the fusion between the viral envelope and cellular membranes and, finally, the entry into the target cells.[2,6-8]

The viral load has been reported to peak in coronavirus disease 2019 (Covid-19) patients at symptom onset or shortly thereafter, before it begins to slowly decrease. The adaptive immune response is initiated as soon as the virus replicates, leading to the generation of cellular responses and antibody production, in most SARS-CoV-2 infected patients.[9,10] Antibodies generally play a crucial role in dealing with viral infection by different mechanisms such as neutralizing incoming virus, tagging viral antigens on the surface of infected cells, as well as modulating the activity of further immune components (e.g., phagocytes or natural killer cells).[2,11] For diagnostic purpose, the viral N and S protein are important for antibody detection, as they are targeted by most commercial serological asiksays. Findings reported on the early immune response indicate that SARS-CoV-2 induced a similar humoral response compared to SARS-CoV.[7] While the antibody response following SARS-CoV-2 infection in humans has been well documented for the initial phase, literature on antibody persistence beyond three months post infection is still scarce.[9,12] Several studies suggest an early decline of antibody production within weeks to months.[13-16] However, the decline of antibody production does not follow a linear pattern and thus cannot be predicted from earlier time points.[17] Preclinical findings[18,19] and rare reports of confirmed re-infections with SARS-CoV-2 in humans support the notion that immunity may last for some time after infection, but the persistence of immunity remains uncertain.[3,9,20,21]

Hence, a better understanding of the kinetics and the persistence of the humoral response is of high importance. This is especially true for the long-term persistence of NAbs, which are considered an important correlate of immunity.[3,5] Despite a plethora of research focussing on the short-term immune response after SARS-CoV-2-infection, data on the specific duration of immunity will only become available within the following years. Along with the progression of the pandemic, more literature on the persistence of the antibody response beyond a time span of 3 or more months post infection is becoming available. However, the quality of this literature varies and often does not allow direct comparison of findings due to their dependency on selected study populations, targeted antigens and the assays and positivity thresholds that were applied. Despite limited comparability, an overview of the current literature on the

kinetics of antibody positivity beyond three months post infection may help gain a better oversight on the current knowledge regarding the more long-term humoral and neutralizing response. Here, we summarize the current literature on the persistence of the humoral and NAb response at three to eight months post SARS-CoV-2 infection in non-pregnant adults. We provide an overview of study populations, assays used, targeted antigens and time points that yielded positive/negative findings for total immunoglobins, the major immunoglobulin classes (IgG, IgM, IgA) and NAbs.

2 | PERSISTENCE OF HUMORAL RESPONSE

Upon invasion of an infectious agent such as SARS-CoV-2 and its antigens, the host elicits a humoral response by producing antibodies, or immunoglobulins (Ig) from plasma cells. This immune response is orchestrated by an interplay of various antibody classes including immunoglobulins IgM, IgD, IgG, IgA and IgE. Each immunoglobulin class has specific constant regions, which have distinct biophysical qualities, functions, distributions and half-lives. The antigen binding sites can be found in the highly variable regions of the immunoglobulins; they are located at the top of the two arms of the Y-shaped antibodies.[2,22] Immunoglobulins that have been most frequently reported to be involved in the humoral response following SARS-CoV-2 infection are IgM, IgA and IgG. The first class to be activated through the entry of an infectious agent are immunoglobulins IgM, followed by IgA and IgG.[23] Dimeric IgA can be found on mucosal surfaces and in secretions, such as saliva, breast milk and nasal mucus. Hence, IgA also plays a crucial role for mucosal immunity.[2,22] IgG antibodies are the most frequent immunoglobulins to be found in serum, with approximately 75% of all serological antibodies being IgGs. They are usually elicited at a later stage of the humoral response and show a high neutralizing capacity. Moreover, IgG antibodies play a role for lasting immunity due to their durable half-life and their association with differentiated memory B cells.[2]

Current literature on the detectability of total immunoglobulins and immunoglobulins G total Ig, IgG at 12–35 weeks post symptom onset (PSO) or post positive polymerase chain reaction (PCR) test is summarized in Table 1. Table 2 summarizes current literature on the detectability of immunoglobulins M and A (IgM, IgA). Findings in both tables are presented in 2-week bins indicating when antibody levels were tested and whether test results were deemed positive according to assays and positivity thresholds used in these studies. Details on antibody titre values and sample sizes at various time points are not shown, and it should be mentioned that findings at a given time point may represent single cases only. In most studies, the timing of collecting follow-up samples PSO/positive PCR result was not standardized, but instead governed by availability of donors. Findings for different antigens are summarized if they did not differ in test assay or positivity outcome. In general, it should be noted that test procedures including test timing, assay types, targeted antibodies/antigens and positivity thresholds varied considerably across reviewed studies, as no standard procedures have yet been

TABLE 1 Overview of positive total Ig and IgG findings at 3–8 months post SARS-CoV-2 infection in non-pregnant adults

Study	Total population = N (% male), n = LTR population	PCR PCR+/N	Assay type (company)	Antibody (antigen)	12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	Baseline for time points
Findings for total antibodies																	
Bal et al.	Healthcare workers, N = 296 (17% male), n* = 296	170/296	ELISA (Wantai BioPharm)	Total Ig (RBD)							(+)	(+)	(+)				PSO
Choe, Kim et al.	Covid-19 patients, N = 58 (39.7% male), n = 58	58/58	ECLIA (Roche)	Total Ig (N)											+		PPCR
Deisenhammer et al.	Covid-19 patients and close contacts, N = 29 (51.7% male), n* = 29	NA	ELISA (Wantai BioPharm)	Total Ig (RBD)	+	+	+					+	+				PSO
Gudbjartsson et al.	Persons in Iceland, N = 30,576 (NA) Recovered Covid-19 patients, n* = 1215	1215/1215	ECLIA (Roche)	Total Ig (N)	+	+	+										PPCR
	Persons in Iceland, N = 30,576 (NA) Recovered Covid-19 patients, n* = 1215	1215/1215	ELISA (Wantai BioPharm)	Total Ig (RBD)	+	+	+										PPCR
Li et al.	Hospitalized Covid-19 patients, N = 1850 (50.2% male), n* = 11	1850/1850	MCLIA (Shenzen YHLO Biotech)	Total IgG/IgM (S, N)	+	+	+	+									PSO
Schaffner et al.	Covid-19 patients, N = 82 (51%), n* = 82	82/82	ELISA (Roche)	Total Ig (N)				(+)	(+)								PSO
Zhang et al.	Covid-19 patients, N = 112 (55.4% male), n* = 112	112/112	CMIA (In-house)	Total Ig (N)	(+)	(+)	(+)	(+)	(+)	+	+	+					PSO
Findings for IgG																	
Bal et al.	Healthcare workers, N = 296 (17% male), n* = 296	170/296	ELFA (bioMérieux)	IgG (RBD)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)				PSO
	Healthcare workers, N = 296 (17% male), n* = 296	170/296	CMIA (Abbott)	IgG (N)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)				PSO
Benotmane et al.	Covid-19 patients with kidney transplant, N = 29 (86% male), n* = 29	NA	ELISA (Dia.Pro)	IgG (S, N)	+	+	+	+	+	+	+	+					PSO
Bonifacius et al.	Recovered and active Covid-19 patients, N = 296 (NA), n = 204	296/296	ELISA (EUROIMMUN)	IgG (N)	+	+					−	−					PPCR or PSO
	Recovered and active Covid-19 patients, N = 296 (NA), n = 204	296/296	ELISA (EUROIMMUN)	IgG (S)	+	+			+		+	+					PPCR or PSO
Chen et al.	Recovered Covid-19 patients, N = 92 (28.3% male), n* = 76	91/92	ELISA (In-house)	IgG (S, RBD, N)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)				PSO
Choe, Kong et al.	Covid-19 patients N = 18 (NA), asymptomatic patients, n = 7, patients with pneumonia, n = 11	NA	ELISA (EUROIMMUN)	IgG (S1)						+							NA
Choe, Kim et al.	Covid-19 patients, N = 58 (39.7% male), n = 58	58/58	ELISA (Epitope Diagnostics)	IgG (N)										+			PPCR
	Covid-19 patients, N = 58 (39.7% male), n = 58	58/58	ELISA (InBios)	IgG (S)											+		PPCR
	Covid-19 patients, N = 58 (39.7% male), n = 58	58/58	ELISA (EUROIMMUN)	IgG (S1)											+		PPCR
Crawford et al.	Covid-19 patients, N = 32 (43.8%), n* = 23	32/32	ELISA (In-house)	IgG (S, RBD)	+	+	+	+	+								PSO
Dan et al.	Covid-19 cases, N = 188 (43% male), n* = 51	145/188	ELISA (In-house)	IgG (RBD, S)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+	PSO or PPCR
	Covid-19 cases, N = 188 (43% male), n* = 51	145/188	ELISA (In-house)	IgG (N)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	−	PSO or PPCR
De Donno et al.	Covid-19 patients, N = 54 (53.7% male), n* = 54	54/54	CLIA (Snibe)	IgG (S, N)			+	+			+						PSO
Deisenhammer et al.	Covid-19 patients and close contacts, N = 29 (51.7% male); n* = 29	NA	ELISA (EUROIMMUN)	IgG (S1)	+	+						+	+				PSO
Dispinseri et al.	Covid-19 pneumonia patients (26% with diabetes), N = 150 (69.3% male), n* = 72	150/150	LIPS (In-house)	IgG (S1, S2, RBD)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+	(+)			PSO
Dittadi et al.	Covid-19 patients, N = 55 (83.6% male), n* = 55	NA	CMIA (Abbott)	IgG	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)					PSO
	Covid-19 patients, N = 55 (83.6% male), n* = 55	NA	CMIA (Snibe)	IgG (S1, S2, N)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)					PSO

(Continues)

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TABLE 1 (Continued)

Study	Total population = N (% male), n = LTR population	PCR PCR+/N	Assay type (company)	Antibody (antigen)	Time points antibodies measured (weeks)												Baseline for time points
					12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	
Figueiredo-Campos et al.	Convalescent (potential) plasma donors, N = NA, n* = 356	NA	ELISA (In-house)	IgG (RBD)	+	+	+	+	+	+	+	+					PSO
Gaebler et al.	Covid-19 patients or close contacts, N = 87 (59.8% male), n* = 87	NA	ELISA (In-house)	IgG (RBD, N)						(+)	(+)	+	(+)	(+)			PSO
Gudbjartsson et al.	Persons in Iceland, N = 30,576 (NA)	1215/1215	ELISA (Epitope Diagnostics)	IgG (N)	+	+											PPCR
	Recovered Covid-19 patients, n* = 1215																
	Persons in Iceland, N = 30,576 (NA)	1215/1215	ELISA (EUROIMMUN)	IgG (S1)	+	+											PPCR
	Recovered Covid-19 patients, n* = 1215																
Han et al.	Covid-19 patients with severe conditions, N = 104 (71.4% male), n* = 104	104/104	ELISA (AnyGo Technology)	IgG (S, RBD, N)						(+)	(+)	(+)	(+)	(+)			PPCR
Hartley et al.	Covid-19 patients, N = 25 (68% male), n* = 25	25/25	ELISA (In-house)	IgG (RBD, N)	+	+	+	+	+	+	+	+					PSO
Isho et al.	Covid-19 patients, N = 439 (52% male), n* = 439	439/439	ELISA (In-house)	IgG (S, RBD, N)	+	+	+								+	+	PSO
Iyer et al.	Symptomatic Covid-19 patients, N = 343 (61.5% male), n* = 35	343/343	ELISA (In-house)	IgG (RBD)	+	+	+										PSO
Koutsakos et al.	Covid-19 patients, N = 85 (50.6% male), n = 25	85/85	ELISA (In-house)	IgG (RBD)	+	+											PSO
Lee et al.	Covid-19 patients, N = 57 (66.7% male), n* = 57	57/57	Flow cytometry (In-house)	IgG (S)	(+)	(+)	(+)	(+)	(+)								PSO
Li et al.	Hospitalized Covid-19 patients, N = 416 (NA), n* = 5	416/416	MCLIA (Nanjing RealMind Biotech)	IgG (S, RBD, N)	+			+	+								PSO
Liu et al.	Covid-19 patients, N = 52 (63.5% male), n* = 52	52/52	MCLIA (Bioscience Biotechnology)	IgG (RBD)	+							+					PSO
Lumley et al.	Health care workers, N = 3276 (NA), n* = 3123	245/3276	CMIA (Abbott)	IgG (S)	(+)	(+)	(+)	(+)	(+)	(+)	+						MAT
	Health care workers, N = 3276 (NA), n* = 3217	245/3276	CMIA (Abbott)	IgG (N)	(+)	(+)	(+)	(+)	(+)	(+)	+						MAT
Maine et al.	Covid-19 patients, N = 427 (NA), n* = 16,	427/427	CMIA (Abbott)	IgG (N)			+	+	+	+	+						PPCR
O'Nions et al.	Covid-19 patients with acute leukaemia receiving anti-cancer therapy, N = 9 (66.6% male), n* = 9	8/9	ELISA (In-house)	IgG (S1, N)	+	+											PSO
Orth-Höller et al.	Physicians with previous Covid-19 infection, N = 20 (NA), n* = 20	20/20	ELISA (EUROIMMUN)	IgG (S1, N)	+		+	+									PSO
Petersen et al.	Persons with previous Covid-19 infection, N = 2547 (65.3% male), n* = 2547	2547/2547	CLIA (Ortho Clinical Diagnostics)	IgG (S1, RBD)	+	+	+										PSO
Rippberger et al.	Covid-19 patients, N = 5971 (47.7% male), seropositive subjects with mild symptoms consistent with Covid-19, n* = 29	6/48 that were tested	ELISA (In-house)	IgG (S2, RBD, N)	+	+	+	+			+				+		PSO
	Covid-19 patients, N = 5971 (47.7% male), PCR-confirmed cohort, n* = 89	89/89	ELISA (In-house)	IgG (S2, RBD, N)	+	+											PSO
Röltgen et al.	Covid-19 patients, N = 240 (41.3% male), inpatients, n* = 79	79/79	ELISA (In-house)	IgG (S1, RBD)	+	+	+	+	+								PPCR
	Covid-19 patients, N = 240 (41.3% male), inpatients, n* = 79	79/79	ELISA (In-house)	IgG (N)	+	-	+	+	+								PPCR
	Covid-19 patients, N = 240 (41.3% male), outpatients, n* = 86	86/86	ELISA (In-house)	IgG (S1, RBD)	-	-	-	-	-								PPCR
	Covid-19 patients, N = 240 (41.3% male), asymptomatic/mildly ill individuals, n = 14	14/14	ELISA (In-house)	IgG (RBD)	+	+	+										PPCR
Sakharkar et al.	Covid-19 patients, N = 8 (62.5% male), n* = 8	8/8	ELISA (In-house)	IgG (S, RBD, NTD)	+	+	+	+	+	+	+						PSO
Sakhi et al.	Covid-19 patients on haemodialysis, N = 83 (71% male), n* = 65	64/83	CMIA (Abbott)	IgG (N)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+			PPCR

TABLE 1 (Continued)

Study	Total population = N (% male), n = LTR population	PCR PCR+/N	Assay type (company)	Antibody (antigen)	Time points antibodies measured (weeks)												Baseline for time points
					12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	
	Covid-19 patients on haemodialysis, N = 83 (71% male), n* = 65	64/83	CLIA (Ortho Clinical Diagnostics)	IgG (S)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+			PPCR
Sasisekharan et al.	Convalescent Covid-19 patients, N = 52 (40% male), n* = 28	19/52	ELISA (In-house)	IgG (S1, RBD, N)	(+)	(+)	(+)	(+)	+								PSO
Schaffner et al.	Covid-19 patients, N = 82 (51%), n* = 82	82/82	CMIA (Abbott)	IgG (N)			(+)	(+)	(+)								PSO
	Covid-19 patients, N = 82 (51%), n* = 82	82/82	ELISA (EUROIMMUN)	IgG (S)			(+)	(+)	(+)								PSO
Seow et al.	Covid-19 patients, N = 65 (male 78.5%), n* = 65	65/65	ELISA (In-house)	IgG (S, RBD, N)	+												PSO
	Health care workers, N = 31 (male 78.5%) n* = 31	0/31	ELISA (In-house)	IgG (S, RBD, N)	+												PSO
Wajnberg et al.	Covid-19 patients, high risk exposed individuals, and healthcare workers, N = 121 (47% male), n* = 121	NA	ELISA (In-house)	IgG (S)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)					PSO
Wang et al.	Convalescent Covid-19 patients, N = 30 (40% male), n* = 30	30/30	MCLIA (Bioscience)	IgG (NA)	+	+	+										PSO
Wheatley et al.	Recovered Covid-19 patients, N = 64 (56.2% male), n* = 64	54/64	Multiplex bead assay (In-house)	IgG (S, S1, S2, RBD, N)	(+)	(+)	(+)	(+)	+								PSO
Yamayoshi et al.	Covid-19 patients, N = 39 (69.2%), n* = 39	39/39	ELISA (In-house)	IgG (S, RBD, N)	+	+	+	+	+								PSO

* (LTR) n inconsistent across time points (could be as small as n = 1).

+ Ab levels found positive by assay and positivity threshold used in study.

− Ab levels found negative by assay and positivity threshold used in study.

(+) positive Ab findings reported for time range instead of time points.

Abbreviations: CMIA, chemiluminescence microparticle immunoassay; CLIA, chemiluminescence immunoassay; ECLIA, electrochemiluminescence immunoassay; ELFA, enzyme-linked fluorescent assay; ELISA, enzyme-linked immunosorbent assay; In-house, non-commercial assay; Ig, immunoglobulin; LIPS, luciferase immunoprecipitation system; LTR, long-term response (>3 months); MAT, maximum antibody titre; MCLIA, magnetic chemiluminescence enzyme immunoassay; N, nucleocapsid protein; NA, not available/unclear; NTD, anti-N-terminal domain; PCR+, positive PCR test result; PPCR, post positive PCR result; PSO, post symptom onset; RBD, receptor-binding domain; S, spike protein (S1 and S2 subunits).

TABLE 2 Overview of positive IgM and IgA findings at 3–8 months post SARS-CoV-2 infection in non-pregnant adults

Study	Total population = N (% male), n = LTR population	PCR PCR+/N	Assay type (company)	Antibody (antigen)	Time points antibodies measured (weeks)												Baseline for time points
					12–13	14–15	16–17	18–19	20–21	22–23	24–25	26–27	28–29	30–31	32–33	34–35	
Findings for IgM																	
Benotmane et al.	Covid-19 patients with kidney transplant, N = 29 (86% male), n* = 29	NA	ELISA (Dia.Pro)	IgM (S, N)	+	+	+	+	+	+	+	+					PSO
Crawford et al.	Covid-19 patients, N = 32 (43.8%), n* = 23	32/32	ELISA (In-house)	IgM (RBD)	+	+	+	+	+								PSO
De Donno et al.	Covid-19 patients, N = 54 (53.7% male), n* = 54	54/54	CLIA (Snibe)	IgM (S, N)	+		+				−						PSO
Dispinseri et al.	Covid-19 pneumonia patients (26% with diabetes), N = 150 (69.3% male), n* = 72	150/150	LIPS (In-house)	IgM (RBD)	(+)	(+)	(+)					(−)	(−)	(−)			PSO
Figueiredo-Campos et al.	Convalescent (potential) plasma donors, N = NA, n* = 356	NA	ELISA (In-house)	IgM (RBD)	+	+	+	+	+	+	+	+					PSO
Gaebler et al.	Covid-19 patients or close contacts, N = 87 (59.8% male), n* = 87	NA	ELISA (In-house)	IgM (RBD)						(+)	(+)	(+)	(+)	(+)			PSO
Gudbjartsson et al.	Persons in Iceland, N = 30,576 (NA) / Recovered Covid-19 patients, n* = 1215	1215/1215	ELISA (Epitope Diagnostics)	IgM (N)	−	−											PPCR
Isho et al.	Covid-19 patients, N = 439 (52% male), n* = 439	439/439	ELISA (In-house)	IgM (S, RBD, N)	+	+	+										PSO
Iyer et al.	Symptomatic Covid-19 patients, N = 343 (61.5% male), n* = 35	343/343	ELISA (In-house)	IgM (S, RBD)	−	−	+										PSO
Koutsakos et al.	Covid-19 patients, N = 85 (50.6% male), n = 25	85/85	ELISA (In-house)	IgM (RBD)	+	−											PSO
Lee et al.	Covid-19 patients, N = 57 (66.7% male), n* = 57	57/57	Flow cytometry (in-house)	IgM (S)	(+)	(+)	(+)	(+)	(+)								PSO
Li et al.	Hospitalized Covid-19 patients, N = 416 (NA), n* = 5	416/416	MCLIA (Nanjing RealMind Biotech)	IgM (S, RBD)	+			+	+								PSO
	Hospitalized Covid-19 patients, N = 416 (NA), n* = 5	416/416	MCLIA (Nanjing RealMind Biotech)	IgM (N)	−			+	+								PSO
Liu et al.	Covid-19 patients, N = 52 (63.5% male), n* = 52	52/52	MCLIA (Bioscience Biotechnology)	IgM (RBD)	+							+					PSO
Maine et al.	Covid-19 patients, N = 427 (NA), n* = 16,	427/427	CMIA (Abbott)	IgM (RBD)	+	−	−	−	−								PPCR
Orth-Höller et al.	Physicians with previous Covid-19 infection, N = 20 (NA), n* = 20	20/20	ELISA (EUROIMMUN)	IgM (N)			+	+									PSO
	Physicians with previous Covid-19 infection, N = 20 (NA), n* = 20	20/20	ELISA (Wantai BioPharm)	IgM RBD			+	+									PSO
Röltgen et al.	Covid-19 patients, N = 240 (41.3% male), inpatients, n* = 79	79/79	ELISA (In-house)	IgM (S1)	+	+		−	−								PPCR
	Covid-19 patients, N = 240 (41.3% male), inpatients, n* = 79	79/79	ELISA (In-house)	IgM (RBD)	−	+		−	−								PPCR
	Covid-19 patients, N = 240 (41.3% male), inpatients, n* = 79	79/79	ELISA (In-house)	IgM (N)	−	−	+	−	−								PPCR
	Covid-19 patients, N = 240 (41.3% male), outpatients, n* = 86	86/86	ELISA (In-house)	IgM (S1, RBD, N)	−	−	−	−									PPCR
	Covid-19 patients, N = 240 (41.3% male), asymptomatic/ mildly ill individuals, n = 14	14/14	ELISA (In-house)	IgM (RBD)	−		−										PPCR
Seow et al.	Covid-19 patients, N = 65 (male 78.5%), n* = 65	65/65	ELISA (In-house)	IgM (S, RBD, N)	+												PSO
	Health care workers, N = 31 (male 78.5%), n* = 31	0/31	ELISA (In-house)	IgM (S, RBD, N)	+												PSO

T A B L E 2 (Continued)

Study	Total population = N (% male), n = LTR population	PCR PCR+/N	Assay type (company)	Antibody (antigen)	12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	Baseline for time points
Wheatley et al.	Recovered Covid-19 patients, N = 64 (56.2% male), n* = 64	54/64	Multiplex bead assay (In-house)	IgM (S, S1, S2, RBD, N)	(+)	(+)	(+)	(+)	+								PSO
Yamayoshi et al.	Covid-19 patients, N = 39 (69.2%), n* = 39	39/39	ELISA (In-house)	IgM (RBD)	+		−	−	−								PSO
Findings for IgA																	
Crawford et al.	Covid-19 patients, N = 32 (43.8%), n* = 23	32/32	ELISA (In-house)	IgA (RBD)	+	+	+	+	+								PSO
Dan et al.	Covid-19 cases, N = 188 (43% male), n* = 51	145/188	ELISA (in-house)	IgA (RBD, S)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)		PSO or PPCR
Figueiredo-Campos et al.	Convalescent (potential) plasma donors, N = NA, n* = 356	NA	ELISA (In-house)	IgA (RBD)	+	+	+	+	+	+	+	+					PSO
Gaebler et al.	Covid-19 patients or close contacts, N = 87 (59.8% male), n* = 87	NA	ELISA (In-house)	IgA (RBD)						(+)	(+)	(+)	(+)	(+)			PSO
Gudbjartsson et al.	Persons in Iceland, N = 30,576 (NA) Recovered Covid-19 patients, n* = 1215	1215/1215	ELISA (EUROIMMUN)	IgA (S1)	+	+											PPCR
Isho et al.	Covid-19 patients, N = 439 (52% male), n* = 439	439/439	ELISA (In-house)	IgA (S, RBD, N)	+	+	+										PSO
Iyer et al.	Symptomatic Covid-19 patients, N = 343 (61.5% male), n* = 35	343/343	ELISA (In-house)	IgA (S, RBD)	+	−	−										PSO
Koutsakos et al.	Covid-19 patients, N = 85 (50.6% male), n = 25	85/85	ELISA (In-house)	IgA (RBD)	+	−											PSO
Orth-Höller et al.	Physicians with previous Covid-19 infection, N = 20 (NA), n* = 20	20/20	ELISA (EUROIMMUN)	IgA (S1)			+	+									PSO
Röltgen et al.	Covid-19 patients, N = 240 (41.3% male), inpatients, n* = 79	79/79	ELISA (In-house)	IgA (RBD)	+	+	+	+	−								PPCR
	Covid-19 patients, N = 240 (41.3% male), inpatients, n* = 79	79/79	ELISA (In-house)	IgA (S1)	+	+	−	+	−								PPCR
	Covid-19 patients, N = 240 (41.3% male), inpatients, n* = 79	79/79	ELISA (In-house)	IgA (N)	+	+	+	+	+								PPCR
	Covid-19 patients, N = 240 (41.3% male), outpatients, n* = 86	86/86	ELISA (In-house)	IgA (S1, RBD)	−		−	−									PPCR
	Covid-19 patients, N = 240 (41.3% male), outpatients, n* = 86	86/86	ELISA (In-house)	IgA (N)	−	−	−	−									PPCR
	Covid-19 patients, N = 240 (41.3% male), asymptomatic/ mildly ill individuals, n = 14	14/14	ELISA (In-house)	IgA (RBD)	−		−										PPCR
Schaffner et al.	Covid-19 patients, N = 82 (51%), n* = 82	82/82	ELISA (EUROIMMUN)	IgA (S)				(+)	(+)								PSO
Seow et al.	Covid-19 patients, N = 65 (male 78.5%), n* = 65	65/65	ELISA (In-house)	IgA (S, RBD, N)	+												PSO
Wheatley et al.	Recovered Covid-19 patients, N = 64 (56.2% male), n* = 63	54/64	Multiplex bead assay (In-house)	IgA1 (S, S1, S2, RBD, N)	(+)	(+)	(+)	(+)	+								PSO

* (LTR) n inconsistent across time points (could be as small as n = 1).

+ Ab levels found positive by assay and positivity threshold used in study.

− Ab levels found negative by assay and positivity threshold used in study.

(+) positive Ab findings reported for time range, instead of time points.

(−) negative Ab findings reported for time range, instead of time points.

Abbreviations: CMIA, chemiluminescence microparticle immunoassay; CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; In-house, non-commercial assay; LIPS, luciferase immunoprecipitation system; LTR, long-term response (>3 months); MCLIA, magnetic chemiluminescence enzyme immunoassay; N, nucleocapsid protein; NA, not available/unclear; PCR+, positive PCR test result; PPCR, post positive PCR result; PSO, post symptom onset; RBD, receptor-binding domain; S, spike protein (S1 and S2 subunits).

established.[23] Several immunoassays have not been approved by health and safety agencies such as the US Food and Drug Administration[2,23] and numerous studies reviewed here developed their own in-house tests. More in depth details such as diagnostic test accuracy and approvals for the most common tests have been summarized by previous more rigorous reviews.[2,23] Of note, a recent study,[24] comparing various antibody detection assays in PCR-confirmed Covid-19 patients, reported that while the majority of evaluated tests demonstrated high IgG/pan-Ig sensitivity and specificity to detect the serological response, IgM and IgA test performance was poor. Specifically, out of six IgM/IgA enzyme-linked immunosorbent assay (ELISA) or chemiluminescence immunoassay (CLIA)/electrochemiluminescence immunoassay (ECLIA) tests, only one (Snibe IgM CLIA) was deemed acceptable with a combined sensitivity above 80% and specificity of 99%. The majority of studies reviewed here focused on the persistence of IgG and only some studies reported findings for IgA, IgM or total Ig.

2.1 | Total immunoglobulins

All studies testing total Ig, employing pan-immunoglobulin assays measuring IgG, IgM and IgA isotypes, could demonstrate positivity at all test time points measured,[25–31] up to 32–33 weeks post positive PCR[26] despite a considerable variability of test time points, targeted antigens and assays used across studies. Gudbjartsson et al.[28] whose study cohort was one of the largest of all reviewed studies, even reported comparable findings for both antigens—RBD and N—in the same cohort.

2.2 | Immunoglobulin G

The majority of studies included in this review focused on the persistence of IgG. This is not surprising as IgGs have been described to represent the immunoglobulin class with the most consequential implications for serological testing and humoral responses including its capacity for viral neutralization and its involvement in immunity persistence after infection or vaccination.[2] A recent excellent systematic review on the immune response in Covid-19 patients reported the earliest detectability of IgG to occur at a median of 12 days PSO or PCR-confirmed diagnosis, to reach a peak at a median of 25 days and to start declining around 60 days.[23] Findings on IgG persistence are largely consistent across studies with positive IgG results being reported for all time points of serological testing,[12,15,17,20,21,25–28,31–59] up to 34–35 weeks PSO.[40,55] Whereas Hartley et al.[40] reported anti-N and anti-RBD IgG to be detectable at 34–35 weeks, Dan et al.[55] only confirmed positive findings for anti-S IgG, while anti-N IgG levels had declined toward threshold levels at this time point.

Some few negative or mixed IgG findings appear to be due to sample characteristics or the antigen that was targeted.[33,48] For instance, Röltgen et al.[48] assessed IgG (S1, RBD and N) in various

cohorts with an ELISA test format. While findings for IgG remained positive among inpatient and asymptomatic patients at time points up to 20–21 weeks post PCR, negative findings for all time points were reported for outpatients. Among these outpatients, the authors found lower and more rapidly decaying titres, whereas inpatients who required intensive care or died later in the study, developed the highest titres of IgG over time. Regardless of study cohort, participants without IgG production at earlier time points generally continued to test IgG-negative at later time points. Bonifacius et al.[33] reported negative anti-N findings at 24–27 weeks, while anti-S IgG values remained positive, with some values just above the positivity threshold.

Taken together, most studies report a durability of IgG responses, which in some studies lasted up to 8 months, despite the use of different assays, differences in study cohort composition and targeted antigen. A decline of IgG positivity was only reported in few studies.

2.3 | Immunoglobulin M

IgM immunoglobulins are the first immunoglobulin class produced following SARS-CoV-2 infection.[2,23] IgM has been reported to become detectable at a median of 7 days post infection, peaks at a median of 20 days and starts to decline as early as a median of 27 days.[23] Notably, recent studies also indicate a crucial role for IgM in neutralization capacities of SARS-CoV-2, as strong correlations between neutralization potency and the presence of RBD-specific IgM have been reported.[15,60]

Compared to IgG, the persistence of IgM was examined by a much smaller number of reviewed studies. The majority of studies reported durability of IgM responses for up to 30–31 weeks, the longest time point studied.[15,20,31,32,37,38,44,45,56,59] Five studies reported initially positive findings followed by negative ones[21,36,43,54,59] detected as early as 14–15 weeks[21,43] and as late as 26–31 weeks[36] post infection. Another study reported mixed findings for different cohorts and antigens tested.[48] Within a group of 79 inpatients, IgM antibodies against SARS-CoV-2 were reported above threshold at weeks 12–15 (anti-S1), 14–15 (anti-RBD) and 16–17 (anti-N) post PCR. However, positive findings for anti-RBD and anti-N IgM were preceded and succeeded by negative findings at earlier and later time points.[48] Such inconsistent patterns may occur if test results at different time points pertain to different subjects, indicating that durability of IgMs was different among patients. In contrast to the inpatient group, a group of 86 outpatients tested negative for IgM against all three antigens at all time points tested (12–19 weeks post PCR), as did a small group of 14 asymptomatic or mildly ill individuals who were tested negative for IgM anti-RBD at 12–13 weeks and 16–17 weeks post PCR. One study did not report any IgM response within the time scope targeted by our review[28] and two studies reported negative findings at early time points, followed by positive results later on.[31,42] In at least one of the two studies, the initial negative finding is most likely caused by the circumstance that the

subject who tested positive at later time points was not tested at the earlier time point.[31] In addition, recent findings regarding the potential insufficient performance of IgM and IgA assays should be taken into consideration.[24] Overall, it appears that more studies are needed to evaluate the specific long-term kinetics of IgM at this point.

2.4 | Immunoglobulin A

The limited number of studies we identified to examine IgA is in line with a general scarcity of literature on IgA production following the initial phase of SARS-CoV-2 infection.[2,9,61] More literature is needed given the crucial function of IgA to act as a neutralizing barrier to infectious agents invading the respiratory and digestive system, as it has been described that secretory dimeric IgA may neutralize SARS-CoV-2 before binding epithelial cells.[61] IgA has been reported in the literature to appear at a median of 11 days PSO or post PCR. A recent living systematic review by Arkhipova-Jenkins et al. reported peak prevalence for IgA at a median of 23 days and onset of decline at a median of 30 days.[23] Of note, the authors report low confidence in these findings due to a low number of studies and varying test time points.

Only about a third of the reviewed studies examined IgA response and most of them detect SARS-CoV-2-specific IgAs at all time points measured,[15,20,28,29,37,38,41–43,48,55,56,58] with the most durable persistence being reported at 34–35 weeks PSO for anti-S and anti-RBD IgA.[55] The study with the largest test cohort of 1215 recovered Covid-19 patients to investigate IgA levels was conducted in Iceland by Gudbjartsson et al.[28] They reported IgA anti-S1 levels to remain detectable up to 14–15 weeks, which was the final test time point of their study.

IgA positivity decay, that is, a change from initially positive to negative findings at later test time points, was reported by three studies.[42,43,48] One of these studies found highly variable results which appear to be associated with study cohort characteristics and targeted antigen.[48] For instance, whereas no anti-S1/anti-RBD IgAs were detected among a group of outpatients across all time points, among inpatients, negative findings were limited to intermediate and final (20–21 weeks post PCR) test time points. Moreover, IgA anti-N findings were found to remain positive across all time points measured within this group of inpatients. This group, which included patients who required intensive care or died later in the study, developed and maintained the highest levels of IgA. Notably the authors reported higher viral loads in patients with more severe disease than patients with milder illness, indicating that larger initial amounts of viral antigen may contribute to the higher serological responses in this group of patients.[48]

Taken together, despite the lower number of studies investigating the persistence of IgA, most studies reviewed here report a homogenous picture, with IgA remaining detectable at most time points measured. The latest time point investigated was 8 months post infection. Negative findings or variable detectability after three months post infection were discussed by authors to be attributable to differences in study cohort or targeted antigen, although the reported insufficient performance of IgM and IgA assays[24] may also be a contributing factor that should be taken into consideration.

3 | NEUTRALIZING HUMORAL RESPONSE

While serological tests may provide information about exposure to SARS-CoV-2, neutralization assays are needed to indicate whether antibodies detected after infection are indeed capable of neutralizing the virus and hence may provide immunity upon subsequent exposure to SARS-CoV-2.[2,5] Consequently, an insufficient stimulation of NAbs may give rise to a potential re-infection with SARS-CoV-2.[3] In line with reports on other virus infections, it has been shown in Covid-19 patients that titres of NAbs are lower than total binding antibodies.[62] The neutralizing humoral response shows a similar pattern for most acute-disease inducing viruses, with the NAbs inhibiting the binding of the virus to cellular receptors. Hence, the induction of virus-neutralizing antibodies is one main goal of current anti-viral vaccines.[3,62] Upon SARS-CoV-2-infection, antibodies have been described to target structural and non-structural viral antigens. Two structural proteins, the N protein and S protein, are target for most commercial serological assays and are therefore used to characterize the immune response to SARS-CoV-2.[7,52] The S protein, as surface glycoprotein responsible for receptor binding, is known from other coronaviruses to be the main, and potentially the only, target for NAbs and the same circumstance has been suggested for SARS-CoV-2.[52] Within the S protein the RBD and the adjacent NTD have been identified to contain the major epitopes for NAbs,[3,63] although also the fusion peptide in S2 has been discussed to be targeted by some NAbs.[64] RBD appears to be particularly vulnerable because blocking the binding of RBD to the ACE2 receptor represent a major mechanism of neutralization.[62,65] Current neutralization studies employ a wide variety of different SARS-CoV-2 neutralization assays with different methodological approaches, and it is crucial to bear in mind those differences when interpreting results. Conventional virus neutralization tests that use live virus, and hence require biosafety level 3 laboratories, are still considered the gold standard of neutralization testing.[5] However, those assays are labour intense and not always readily available, so that alternative tests such as pseudovirus virus-neutralizing tests (pVNTs) have been developed and validated[2,5] Additionally, more recently surrogate neutralization assays (sVNTs) have been described.[66] These assays are based on an antibody-mediated blockage of RBD/ACE2 binding.

The early timeline of the NAb response upon SARS-CoV-2 infection has been systematically reviewed by Arkhipova-Jenkins et al.[23] They report that NAbs can be detected as early as a median of 6 days PSO or post PCR, peak at a median of 31 days and start declining around the same time when peak prevalence is observed, approximately at a median of 30 days. Notably, the authors report a lower confidence in these findings compared to IgG or IgM reports, due to a low number of studies, the high variance in neutralization test used and test time points used. Our findings on neutralizing humoral response are shown in Table 3. Analogously to

TABLE 3 Overview of positive neutralizing antibody findings at 3–8 months post SARS-CoV-2 infection in non-pregnant adults

Study	Total population = N (% male), n = LTR population	PCR PCR+/N	Assay type	Time points antibodies measured (weeks)												Baseline for time points
				12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	
Bal et al.	Healthcare workers, n = 296 (17% male), n* = 296	170/296	PRNT							(+)	(+)	(+)				PSO
Chen et al.	Recovered Covid-19 patients, N = 92 (28.3% male), n* = 76	91/92	pVNT	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)			PSO
	Recovered Covid-19 patients, N = 92 (28.3% male), n* = 76	91/92	ACE2-binding inhibition assay	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)			PSO
Choe, Kong et al.	Covid-19 patients N = 18 (NA), asymptomatic patients, n = 7, patients with pneumonia, n = 11	NA	cVNT						+							NA
	Covid-19 patients, N = 58 (39.7% male), n = 58	58/58	sVNT											+		PPCR
Crawford et al.	Covid-19 patients, N = 32 (43.8%), n* = 23	32/32	pVNT	+	+	+	+	+								PSO
Dan et al.	Covid-19 cases, N = 188 (43% male), n* = 51	145/188	pVNT	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)		+	PSO or PPCR
Deisenhammer et al.	Covid-19 patients and close contacts, N = 29 (51.7% male), n* = 29	NA	pVNT							+						PSO
Dispinseri et al.	Covid-19 pneumonia patients (26% with diabetes), N = 150 (69.3% male), n* = 72	150/150	pVNT	(+)	(+)	(+)					(+)	(+)	(+)			PSO
Figueiredo-Campos et al.	Convalescent (potential) plasma donors, N = NA, n* = 84	NA	pVNT	+			+	+								PSO
Gaebler et al.	Covid-19 patients or close contacts, N = 87 (59.8% male), n* = 87	NA	pVNT						(+)	(+)	(+)	(+)	(+)			PSO
Han et al.	Covid-19 patients with severe conditions, N = 104 (71.4% male), n* = 104	104/104	Micro-neutralization assay						(+)	(+)	(+)	(+)	(+)			PPCR
Hartley et al.	Covid-19 patients, N = 25 (68% male), n* = 25	25/25	pVNT	+	+	+	+		+		+			+	−	PSO
Isho et al.	Covid-19 patients, N = 439 (52% male), n* = 439	439/439	sVNT	+	+	+										PSO
Koutsakos et al.	Covid-19 patients, N = 85 (50.6% male), n = 3	85/85	Micro-neutralization assay		+											PSO
Lee et al.	Covid-19 patients, N = 57 (66.7% male), n* = 44 (D614 virus variant)	44/44	pVNT	(+)	(+)	(+)		(+)								PSO
	Covid-19 patients, N = 57 (66.7% male), n* = 6 (G614 virus variant)	6/6	pVNT	(+)	(+)											PSO
O'Nions et al.	Covid-19 patients with acute leukaemia receiving anti-cancer therapy, N = 9 (66.6% male), n* = 9	8/9	pVNT	+	+											PSO
Orth-Höller et al.	Physicians with previous Covid-19 infection, N = 20 (NA), n* = 20	20/20	cVNT			+	+									PSO

TABLE 3 (Continued)

Study	Total population = N (% male), n = LTR population	PCR PCR+/N	Assay type	12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	Baseline for time points
Rippberger et al.	Covid-19 patients, N = 5971 (47.7% male), seropositive subjects with mild symptoms consistent with Covid-19, n* = 29	6/48 that were tested	PRNT	+	+	+	+							+		PSO
Röltgen et al.	Covid-19 patients, N = 240 (41.3% male), inpatients, n* = 79	79/79	pVNT	+	+	+	+	+		+						PPCR
	Covid-19 patients, N = 240 (41.3% male), asymptomatic/mildly ill individuals, n = 14	14/14	pVNT	+		+										rRT-PCR
	Covid-19 patients, N = 240 (41.3% male), inpatients, n* = 79	79/79	Competitive ELISA	+	+	+	+	−								PPCR
	Covid-19 patients, N = 240 (41.3% male), outpatients, n* = 86	86/86	Competitive ELISA	−	−	−	−									PPCR
	Covid-19 patients, N = 240 (41.3% male), asymptomatic/mildly ill individuals, n = 14	14/14	Competitive ELISA	+		−										rRT-PCR
Sakharkar et al.	Covid-19 patients, N = 8 (62.5% male), n* = 8	8/8	pVNT	+	+			+								PSO
Sasisekharan et al.	Convalescent Covid-19 patients, N = 52 (40% male), n* = 28	19/52	sVNT	(+)	(+)	(+)	(+)	+	+	+						PSO
Seow et al.	Covid-19 patients, N = 65 (male 78.5%), n* = 65	65/65	pVNT	(+)												PSO
	Health care workers, N = 31 (male 78.5%), n* = 31	0/31	pVNT	(+)												PSO
Wajnberg et al.	Covid-19 patients, high risk exposed individuals, and healthcare workers, N = 121 (47% male), n* = 121	NA	Micro-neutralization assay	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)					PSO
Wang et al.	Convalescent Covid-19 patients, N = 30 (40% male), n* = 30	30/30	pVNT	+	+	+										PSO
Wheatley et al.	Recovered Covid-19 patients, N = 64 (56.2% male), n* = 64	54/64	Microneutralisation	(+)	(+)	(+)	(+)	+								PSO
	Recovered Covid-19 patients, N = 64 (56.2% male), n* = 64	54/64	Multiplex bead assay	(+)	(+)	(+)	(+)	+								PSO
Yamayoshi et al.	Covid-19 patients, N = 39 (69.2%), n* = 39	39/39	PRNT	+	+	+	+	+								PSO

* (LTR) n inconsistent across time points (could be as small as n = 1).

+ Ab levels found positive by assay and positivity threshold used in study.

− Ab levels found negative by assay and positivity threshold used in study.

(+) positive Ab findings reported for time range instead of time points.

Abbreviations: cVNT, conventional virus neutralization assay; LTR, long-term response (>3 months); NA, not available/unclear; PCR+, positive PCR test result; PPCR, post positive PCR result; PRNT, Plaque-reduction Neutralizing Test; PSO, post symptom onset; pVNT, Pseudovirus Neutralization Test; snELISA, Surrogate Neutralization ELISA; sVNT, Surrogate Virus Neutralization Test.

Tables 1 and 2, findings presented in 2-week bins indicate time points at which NAb levels were assessed and whether observed levels exceeded the cut-off threshold used by studies. Findings shown may represent single samples only, as in most studies, the timing of follow-up samples PSO/positive PCR result was not standardized, but instead governed by availability of donors. Findings on NAbs were quite consistent across reviewed studies, with the majority studies observing positive NAbs at all time points tested.[15,17,20,25–27,34–41,43,44,47–49,51–56,58] Dan et al. using a pVNT found the most durable NAb response to last up to 34–35 weeks post PSO/PCR.[55] Three studies reported the detectability of NAbs for up to 32–33 weeks PSO[17,40] and post positive PCR test[26] despite different neutralization tests were used, including a plaque-reduction neutralizing test,[17] a pVNT,[40] and a sVNT.[35] Waning of the detectability of NAbs was only observed by two studies. One study, using a pVNT, identified NAbs for up to 33 weeks and only the final sample taken at 34–35 weeks PSO tested negative.[40] The second study,[48] using a competitive ELISA, was unable to confirm NAbs among outpatients (12–13 weeks post PCR) and among asymptomatic patients (16–17 weeks post PCR), whereas inpatients' NAbs remained positive for up to 19 weeks post PCR. The same study also conducted a pVNT that yielded positive results among inpatients for up to 20–21 weeks post PCR and among asymptomatic patients for up to 16–17 weeks post PCR. For outpatients, pseudotype neutralizing results were only obtained for time points prior to the ones reviewed here. A number of studies suggest that neutralizing activity may be correlated with the presence of IgG, in particular binding titres for anti-S and anti-RBD.[5,23,27,34,39,42,52] Of note, recent studies have also indicated potential roles for IgA or IgM in neutralization of SARS-CoV-2. One study reported, for example, that SARS-CoV-2 neutralization appears to more be closely correlated with IgA than IgM or IgG in the first weeks after infection.[67] This finding may be dependent on the dimeric, form of IgA, as it was reported to have a higher potency than its respective monomer against authentic SARS-CoV-2.[68] In contrast, another study reported a strong correlation between neutralization potency and the presence of RBD-specific IgM.[60] Nevertheless, despite the growing evidence for a correlation between binding and NAbs, neutralizing assays are still considered the gold standard for assessing potential immunity in patients.[5]

4 | CONCLUSIONS

Here, we reviewed current peer-reviewed literature on humoral and NAb response at 3+ months PSO in non-pregnant adults. Available studies including such more long-term time points of sampling remain limited and, due to considerable methodological heterogeneity, their findings generally lack comparability.

Most consistent are outcomes on the IgG response, with positive antibody titres reported for up to 8 months post infection,[40,55] regardless of targeted antigens and assays used across studies. Given those consistent findings, it seems likely that future studies may establish IgG responses at even later time points. Likewise, IgA, even

though it was studied by a much smaller number of studies, was reported to remain fairly persistent with time points later than 7 months PSO,[55] similar to IgG these findings were observed despite different assays and antigens tested. Findings on the IgM response were much more inconsistent, and more research is needed. Consistent findings, with few exceptions, were also reported for the persistence of NAbs which have been shown so far in some studies to persist up to 32–33 weeks.[17,26,40] Regarding results for IgM and IgA titres it is important to note that while most IgG/pan-Ig assays have been found to show a reliable performance, IgM and IgA test performance was recently reported to be poor for most assays.[24]

A key issue in the current state of the pandemic is the question about the potential strength and duration of immunity after SARS-CoV-2 infection and vaccination, as these factors will strongly impact decisions on current restrictions.[16] Regarding the question of long-term immunity to SARS-CoV-2, seropositivity and in particular the presence of NAbs has been linked to several months of protection from re-infection. Within a study describing a SARS-CoV-2 outbreak at a summer school retreat, no individual in a sub-group of 24 persons, who had been seropositive three months prior to the retreat, developed any symptoms.[69] A prospective study in the United Kingdom found among a large sample of 12,541 healthcare workers that those who were anti-S IgG positive at baseline (n = 1265) were also less likely to have a preceding or succeeding positive PCR test result.[46,70]

Another factor that should be taken into consideration when speculating about potential long-term immunity, are the recently emerging SARS-CoV-2 variants such as B.1.1.7 (United Kingdom), B.1.351 (South Africa), P.1 (Brazil) and P.2 (Brazil, Japan). The mutation of these variants affects the S protein, which is the main target for NAbs. Consequently, concerns have been voiced that neutralizing capacities may be reduced due to the S protein mutation of some of these virus variants such as B.1.351, P.1 and P.2.[71–73] Neutralizing activity appears to be most strongly affected in individual monoclonal antibodies and less affected in polyclonal antibodies involved in serum neutralization.[72] Another study employed pseudoviruses representing currently circulating strains of SARS-CoV-2 to examine neutralization potency in vaccinated individuals. While some variants (e.g., B.1.1.7, B.1.1.298, or B.1.429) continued to be neutralized despite their respective mutations, other variants seemed to evade vaccine-induced humoral immunity. The P.2 variant was reported capable of significantly reducing neutralization potency of fully vaccinated individuals. Likewise, P.1 and B.1.351 were found to effectively evade neutralization.[73] Further research on the impact of recent SARS-CoV-2 mutations on neutralization capacities will hopefully shed more light on the question whether the neutralizing humoral response induced by natural SARS-CoV-2 infection or current vaccines remains effective in promoting longer lasting immunity.

Research available for SARS-CoV suggests that NAbs can still be detected up to 17 years later[74,75] and only a few cases of confirmed SARS-CoV-2 re-infections were reported.[76,77] Similarly, to other human coronaviruses, this leaves room for hope that re-infections

may follow milder disease trajectories than the initial infection.[48,78,79] Finally, waning serological antibodies may not equate to loss of immunity.[48] Mucosal antibodies located in the respiratory tract may prevent SARS-CoV-2 infection. Moreover, memory B and T cells stimulated during the initial infection may optimize future response to the virus.[48] At this point, further longitudinal investigation of PCR-confirmed seropositive individuals using sensitive assays is warranted to elucidate the nature and duration of a more long-term humoral response.

CONFLICT OF INTEREST
We declare no competing interests.

ETHICS APPROVAL
Not applicable.

AUTHOR CONTRIBUTIONS
Andrea Knies and Miriam Schneider designed the review, searched, screened and extracted literature and wrote the manuscript. Dennis Ladage and Ralf J. Braun contributed to the development of the review design and reviewed interpretation of findings. Janine Kimpel reviewed and edited the manuscript. All authors reviewed and approved the manuscript before submission.

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