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Dynamic Change and Clinical Relevance of Postinfectious SARS-CoV-2 Antibody Responses

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Background. Although reports suggest that most individuals with coronavirus disease 2019 (COVID-19) develop detectable antibodies postinfection, the kinetics, durability, and relative differences between immunoglobulin M (IgM) and immunoglobulin G (IgG) responses beyond the first few weeks after symptom onset remain poorly understood.

Methods. Within a large, well-phenotyped, diverse, prospective cohort of subjects with and without severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction (PCR)–confirmed infection and historical controls derived from cohorts with high prevalence of viral coinfections and samples taken during prior flu seasons, we measured SARS-CoV-2 serological responses (both IgG and IgM) using commercially available assays. We calculated sensitivity, specificity, and relationship with disease severity and mapped the kinetics of antibody responses over time using generalized additive models.

Results. We analyzed 1001 samples from 752 subjects, 327 with confirmed SARS-CoV-2 (29.7% with severe disease) spanning a period of 90 days from symptom onset. Sensitivity was lower (44.1%–47.1%) early (<10 days) after symptom onset but increased to >80% after 10 days. IgM positivity increased earlier than IgG-targeted assays, but positivity peaked between days 32 and 38 post–onset of symptoms and declined thereafter, a dynamic that was confirmed when antibody levels were analyzed, with a more rapid decline observed with IgM. Early (<10 days) IgM but not IgG levels were significantly higher in those who subsequently developed severe disease (signal/cutoff 4.20 [0.75–17.93] vs 1.07 [0.21–5.46]; $P = .048$).

Conclusions. This study suggests that postinfectious antibody responses in those with confirmed COVID-19 begin to decline relatively early postinfection and suggests a potential role for higher IgM levels early in infection in the prediction of subsequent disease severity.

Keywords. antibody; COVID-19; SARS-CoV-2; serology.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), was first identified in December 2019 in Wuhan, China, before rapidly becoming pandemic. Over and above the significant proportion of asymptomatic cases, the majority of symptomatic COVID-19 cases are mild. However, up to 20% of infections progress to severe disease, as classified by the World Health Organization (WHO) [1], with comorbidities, male sex, and older age associated with poorer outcomes [2]. It remains unclear to what extent infection with SARS-CoV-2 confers postinfectious immunity, either through humoral (antibody-mediated) or cellular (T-cell-mediated) mechanisms.

Emerging data suggest that many individuals, particularly with severe COVID-19, mount detectable anti-SARS-CoV-2 immunoglobulin G (IgG) responses within 2 weeks after infection [3], with many factors influencing antibody responses including age, disease severity, and time from onset of symptoms, with variable intensity and durability of serologic responses reported [4, 5]. Serology plays an important role in the diagnosis of many infections, both at an individual and population levels, with early immunoglobulin M (IgM) responses used to detect recent infections and more persistent memory IgG responses used to estimate seroprevalence. However, given the uncertainties surrounding the development and persistence of antibody responses to SARS-CoV-2 [6], the role of serology in the diagnosis or surveillance of COVID-19 remains to be fully clarified.

A number of commercial anti-SARS-CoV-2 serological assays report high sensitivity and specificity. However, their validation in real-world settings, taking into account the range of factors that affect serologic responses, including cross-reactivity against other chronic infections, has been limited [7].

Although several studies have compared commercially available serological assays in COVID-19, many have small sample sizes [8] or lack non-SARS-CoV-2-infected controls

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[7, 9]. Additionally, inclusion of uninfected controls, defined as not detected on SARS-CoV-2 PCR [10], raises the potential for false-positive antibody tests to be misinterpreted in those with previous infection, particularly where detailed clinical information is lacking. Many other studies either have limited data on disease severity [11, 12] or have over-representation of hospitalized patients with severe disease. In a systematic review, only 4 of 40 studies included nonhospitalized patients [13], which limits the generalizability of some observations, such as associations between higher antibody titers and disease severity [14]. In one of the largest studies to date, analyzing 976 prepandemic blood samples and 536 blood samples from patients with SARS-CoV-2 infection, severity data were only available for 29% [15]. Lastly, although SARS-CoV-2 serological responses are dynamic, not all studies either report or account for time since symptom onset; in a recent Cochrane systematic review, 19 of 57 included studies did not stratify by time since symptom onset [16]. The same review found very little data beyond 35 days post–onset of symptoms.

To address these data gaps, we aimed to compare several different commercial SARS-CoV-2 serological assays in demographically, clinically diverse and well-phenotyped clinical cohorts in order to define the dynamic change in qualitative and quantitative antibody responses over time since symptom onset and delineate the relative role of IgM vs IgG antibodies in relation to onset and severity of infection in clinical samples from individuals with and without COVID-19 infection.

METHODS

Study Design

The All Ireland Infectious Diseases (AIID) Cohort study is a prospective, multicenter cohort enrolling patients attending clinical services for Infectious Diseases in Ireland. Subjects provide data on demographics (age, sex, ethnicity, smoking status), clinical characteristics (hospitalization, date of symptom onset, underlying conditions, eg, diabetes, malignancies), and laboratory results. COVID-19 disease severity was defined according to the WHO guidance [1] and collapsed into severe and nonsevere for analysis. Subjects also provided blood samples for bio-banking on up to 3 occasions.

For this analysis, we included AIID Cohort participants who presented to the Mater Misericordiae University Hospital and St Vincent's University Hospital with symptoms consistent with COVID-19 between March 26, 2020, and July 10, 2020, with available biobanked samples. In addition, as controls, we included subjects with plasma samples biobanked prior to 2020 from the AIID Cohort and another longitudinal study of subjects with and without HIV infection [17], including samples specifically taken during previous flu seasons from 2016 to 2019, as outlined in national statistics [18, 19].

Patient Consent Statement

All subjects provided written, informed consent, and the study was reviewed and approved in line with national and European regulations on health research by the St Vincent's Hospital Group Research Ethics Committee and the National Research Ethics Committee for COVID-19 in Ireland.

Laboratory Analysis

Plasma, stored at −80°C and thawed in batches, underwent same-day serological testing in the Core Laboratory in the Clinical Research Centre, University College Dublin, Ireland, by blinded technicians using 4 assays according to the manufacturers' instructions: the Elecsys anti-SARS-CoV-2 electrochemiluminescence immunoassay (Roche Diagnostics, Penzberg, Germany) run on the Cobas e411 automated platform (Roche Diagnostics), the SARS-CoV-2 IgG chemiluminescent microparticle immunoassay (CMIA; Abbott Laboratories, IL, USA) run on both the Architect i2000SR platform (Abbott Diagnostics) and the Abbott Alinity ci platform (Abbott Diagnostics), and the Abbott SARS-CoV-2 IgM assay run on the Abbott Architect i2000SR platform. The Elecsys assay is a sandwich immunoassay that detects immunoglobulin A (IgA), IgM, and IgG antibodies to SARS-CoV-2, so a positive result may reflect reactive, non-IgG antibody responses. The Abbott SARS-CoV-2 IgG and IgM assays are 2-step immunoassays targeting the SARS-CoV-2 nucleocapsid protein.

The Elecsys assay presents results as a cutoff index (COI), derived from comparison of electrochemiluminescence signals from the sample to positive and negative calibration signals with both qualitative (reactive or nonreactive) and quantitative (COI) results provided. The Abbott assays also provide automated qualitative and quantitative (signal/cutoff [S/CO] ratios) results. For the IgG and IgM assays, S/CO $\geq$1.4 and $\geq$1.0, respectively, were interpreted as reactive. The Alinity and Architect versions of the Abbott IgG assay are similar; both use the same capture antibody, conjugate material, and formulations of calibrator and QC material, with corresponding ranges.

Statistical Analysis

Continuous and categorical variables are summarized using median with interquartile range (IQR) and frequency/percentage, respectively. Sensitivity and specificity along with their binomial exact 95% CIs were used to describe the performance characteristics of the assays. Sensitivity was calculated based on samples from subjects who tested detected on SARS-CoV-2 PCR (SARS-CoV-2 Pos). Specificity was derived for 2 distinct groups (i) samples from subjects who presented for hospital care during the 2020 pandemic but who tested "not detected" on SARS-CoV-2 PCR (SARS-CoV-2 Neg), and (ii) historical controls–subjects (Controls Pre-2020), who included subjects with and without chronic infections such as HIV and hepatitis C as well as subjects with biobanked serum samples taken during prior flu seasons between 2016 and 2019.

Within the SARS-CoV-2 Pos group, assay sensitivity was also evaluated at different time periods after the date of symptom onset: 0–10, 11–21, 21–42, and >42 days. We used scatter plots with superimposed curves fitted using generalized additive mixed models (GAMMs), with either a Gaussian or binomial link function and time since symptom onset fitted as a spline, to depict the nonlinear relationship between time from symptom onset and either (i) quantitative antibody levels or (ii) seropositivity rate as dependent variables, respectively. GAMM models were fitted using the *mgcv* package in R and incorporated individual participants as a random effect and also included an autocorrelation error structure. We compared quantitative antibody responses (COI for Elecsys or S/CO for Abbott IgG and IgM assays, referred to as antibody "levels") and positivity rate for the first 2 time periods post–symptom onset (0–10 and 11–21 days) between subjects categorized into severe and nonsevere maximal disease stage, attained using the Wilcoxon rank-sum test and the chi-square test, respectively.

Overall sensitivity and specificity were compared between assays using McNemar's chi-square test as previously described [20, 21]. Overall concordance between the assays was evaluated using the Cohen's Kappa and percentage agreement. Cross-reactivity was assessed in the Controls Pre-2020 group in samples from subjects with and without known chronic viral infections (HIV, hepatitis C or B) and samples from the 2016–2019 flu seasons. All analyses were conducted using Stata 15 (College Station, TX, USA) and R, version 4.0.2.

RESULTS

A total of 752 subjects provided 1001 samples for analysis. The SARS-CoV-2 Pos group comprised 202 individuals who provided 327 samples between March 26 and July 10, 2020, and the SARS-CoV-2 Neg group included 149 subjects who provided 222 samples. Among these 2 groups, 76 (37.6%) and 49 (32.9%) provided ≥2 samples, respectively. Samples were collected a median (IQR) of 19 (11–41) and 8 (5–17) days post–symptom onset in the SARS-CoV-2 Pos and Neg groups, respectively. The Controls pre-2020 group comprised 401 subjects who provided 452 samples collected before 2020, including 116 samples taken during previous flu seasons. Within the Controls pre-2020 group, 19 (4.8%) were hepatitis B surface antigen positive and the majority (80%) were HIV antibody positive; of these, 40 (12.5%) were also hepatitis C antibody positive (Table 1).

The median (IQR) ages of the SARS-CoV-2 Pos and SARS-CoV-2 Neg groups were similar (57 [45–68] years and 60 [42–75] years, respectively), while members of the Controls

Table 1. Characteristics of the Study Population

	SARS-CoV-2 Pos	SARS-CoV-2 Neg	Controls Pre-2020
Characteristic	(n = 202)	(n = 149)	(n = 401)
Age, y	57 (45–68)	60 (42–75)	45 (40–53)
Gender, male, No. (%)	108 (54.0)	87 (58.4)	234 (58.4)
Ethnicity, No. (%)			
Caucasian	135 (66.8)	126 (84.5)	217 (54.1)
Asian	21 (10.4)	4 (2.7)	7 (1.8)
African	2 (1.0)	1 (0.7)	143 (35.7)
Unknown	44 (21.8)	18 (12.1)	34 (8.4)
Current smoker, No. (%)	12 (5.9)	20 (13.4)	-
Diabetes, yes, No. (%)	26 (12.9)	15 (10.1)	-
Underlying malignancies, No. (%)	17 (8.4)	4 (2.7)	-
Hospital admission, yes, No. (%)	145 (71.8)	119 (79.9)	-
COVID-19 disease severity, No. (%)[a]			
Mild	117 (57.9)	-	-
Moderate	25 (12.4)	-	-
Severe	60 (29.7)	-	-
Coinfections, No. (%)			
HIV mono-infected	-	-	270 (67.5)
HIV/HCV	-	-	40 (10.0)
HIV/HBV	-	-	11 (2.8)
HBV			8 (2.0)
CRP, mg/L[b]	23.9 (1.7–76)	27.8 (4–89)	-
Ferritin, μg/L[c]	376 (141–1085)	205 (83–447)	-

Data are median (IQR) unless otherwise stated. HBV status defined as sAg positive.

Abbreviations: COVID-19, coronavirus disease 2019; CRP, C-reactive protein; HBV, hepatitis B virus; HCV, hepatitis C virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

[a]Disease severity assigned according to WHO criteria [1].

[b]CRP missing in 4 and 15 SARS-CoV-2 Pos and SARS-CoV-2 Neg, respectively.

[c]Ferritin missing in 7 and 18 SARS-CoV-2 Pos and SARS-CoV-2 Neg, respectively.

Pre-2020 group were younger (45 [40–53] years). Among all 3 study groups, males and those of Caucasian ethnicity were the most represented (Table 1). Compared with the SARS-CoV-2 Neg group, the SARS-CoV-2 Pos group was more likely to be diabetic, more likely to have an underlying malignancy, and less likely to smoke. Although the majority of the SARS-CoV-2 Pos and SARS-CoV-2 Neg groups were admitted to the hospital (71.8% and 79.9%, respectively), within the SARS-CoV-2 Pos group, most (58%) experienced only mild disease, 12% moderate, and 30% severe disease, respectively.

Sensitivity and Specificity

Overall, the sensitivity for all 4 assays was relatively low, ranging from 74.3% to 77.1% (Table 2), with no significant difference in sensitivity between assays (Supplementary Table 1). In contrast, all 3 IgG-targeted assays and the IgM assay were highly specific, ranging from 92.7% to 100% (Table 2), with the Elecsys assay (100%; 95% CI, 99.2%–100%) having marginally but significantly higher specificity than the Abbott IgG assays (IgG Architect, 99.1%; 95% CI, 97.7%–99.8%; IgG Alinity, 99.1%; 95% CI, 97.7%–99.8%; % difference, +0.0009; 95% CI, 0.0002–0.018; $P = .046$). Specificity did not differ between the Abbott IgG assays (Architect, 99.1%; 95% CI, 97.7%–99.8%; Alinity, 99.1%; 95% CI, 97.7%–99.8%; % difference, +0.00; 95% CI, –0.006 to 0.006). The percentage of agreement between the 3 IgG-targeting assays ranged between 96.8% and 99.7% (Supplementary Table 2).

The sensitivity of the 3 IgG-targeted assays increased considerably with time after onset of COVID-19 symptoms: 44.1% to 47.1% in samples collected ≤10 days post–symptom onset, increasing to a maximum sensitivity ranging from 86.5% to 90.5% in samples collected at >42 days post–symptom onset, with no significant differences between the 3 assays (Figure 1, Table 3). In contrast, the Abbott IgM assay sensitivity increased from a low of 57.6% in the early (≤10 days) period to a high of 89.0% at 11–21 days post–symptom onset, but notably declined to 68.5% >42 days post–symptom onset (Figure 1).

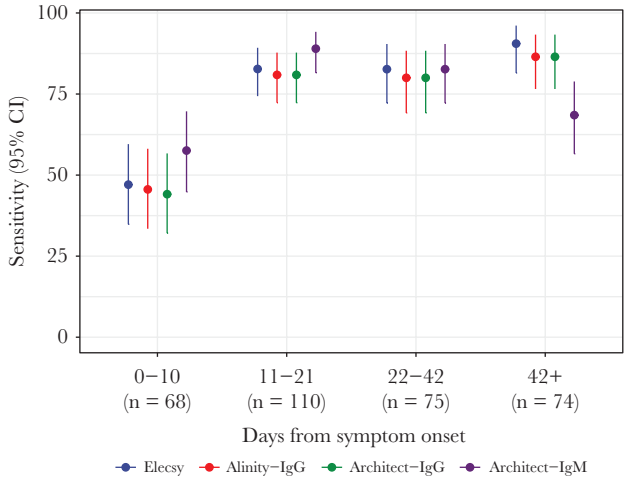

Figure 1. Sensitivity of serological assays based on time periods from onset of symptoms. Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M.

Merging Abbott Architect IgM- and IgG-positive responses into a single variable did not appreciably alter overall sensitivity (82.9%; 95% CI, 78.3%–86.8%) or specificity (98.3%; 95% CI, 93.9%–99.8%) at any of the time points analyzed (data not shown).

From fitted generalized estimating equation longitudinal curves, estimated positivity rates peaked at day 38 post–symptom onset for the Elecsys assay (positivity rate, 92.2%; 95% CI, 85.2%–96.0%), day 36 for the Abbott Alinity IgG assay (positivity rate, 89.1%; 95% CI, 81.6%–93.8%), and day 36 for the Abbott Architect IgG assay (positivity rate, 89.1%; 95% CI, 81.6%–93.8%) (Supplementary Figure 1). There was a more rapid increase in the positivity rate with the Abbott Architect IgM assay, with an earlier peak at day 23 post–symptom onset (positivity rate, 88.7%; 95% CI, 82.8%–92.8%) (Supplementary Figure 2). In addition, after day 23 post–symptom onset, there was a more rapid decline in positivity rate with the Abbott IgM assay compared with the IgG assay (Figure 2).

Table 2. Overall Sensitivity and Specificity of the 3 Immunoassays to Detect Antibodies Against SAR-CoV-2

	No. of Serum Samples								
	Sensitivity			Specificity					
Type of Assays	SARS-CoV-2 Pos	Antibody Positive	% (95% CI)	SARS-CoV-2 Neg	Antibody Negative	% (95% CI)	Controls Pre-2020[a]	Antibody Negative	% (95% CI)
Elecsys	327	252	77.1 (72.1–81.5)	222	213	95.9 (92.4–98.1)	450	450	100 (99.2–100)
Alinity-IgG	327	244	74.6 (69.5–79.2)	222	214	96.4 (93.0–98.4)	450	446	99.1 (97.7–99.8)
Architect-IgG	327	243	74.3 (69.2–79.0)	222	214	96.4 (93.0–98.4)	450	446	99.1 (97.7–99.8)
Architect-IgM[a]	323	247	76.5 (71.5–81.0)	222	206	92.7 (88.6–95.8)	116	115	99.1 (95.3–100)

When measured in all available samples, sensitivity for all assays was lower than specificity.

Abbreviations: Ab, antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; SAR-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]IgM was only measured in the control samples from prior flu seasons (n = 116), and data were available for 150 of 152 samples for each of the other 3 assays.

Table 3. Performance of the 3 Serological Assays in Different Periods From Date of Onset of Symptoms

	Elecsys		Alinity-IgG		Architect-IgG		Architect-IgM	
Days From Symptom Onset	Ab Positive	Sensitivity (95% CI)	Ab Positive	Sensitivity (95% CI)	Ab Positive	Sensitivity (95% CI)	Ab Positive	Sensitivity (95% CI)
0–10	32/68	47.1 (34.8–59.6)	31/68	45.6 (33.5–58.1)	30/68	44.1 (32.1–56.7)	38/66	57.6 (44.8–69.7)
11–21	91/110	82.7 (74.3–89.3)	89/110	80.9 (72.3–87.8)	89/110	80.9 (72.3–87.8)	97/109	89.0 (81.6–94.2)
22–42	62/75	82.7 (72.2–90.4)	60/75	80.0 (69.2–88.4)	60/75	80.0 (69.2–88.4)	62/75	82.7 (72.2–90.4)
>42	67/74	90.5 (81.5–96.1)	64/74	86.5 (76.5–93.3)	64/74	86.5 (76.5–93.3)	50/73	68.5 (56.6–78.9)

The proportion of individuals with a SARS-CoV-2 PCR-detected diagnosis of COVID-19 with positive antibody responses was higher 10 days after symptom onset for all assays.
Abbreviations: Ab, antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; PCR, polymerase chain reaction; SAR-CoV-2, severe acute respiratory syndrome coronavirus 2.

Dynamics of Antibody Levels in the SARS-CoV-2 Pos Group

Overall, the median (IQR) antibody levels for the 3 IgG-targeted assays were 11.93 (1.66–35.64) for Elecsys, 6.47 (1.35–10.43) for Abbott Alinity, and 4.71 (1.17–6.53) for Architect; the median (IQR) antibody level was 6.35 (1.17–15.88) for the Abbott Architect IgM. The Abbott Alinity and Architect assay levels were highly correlated (repeated-measures correlation coefficient $r = 0.99$; 95% CI, 0.98–0.99), but less strongly between Elecsys and Architect ($r = 0.60$; 95% CI, 0.47–0.70) and Ececsys and Alinity ($r = 0.62$; 95% CI, 0.50–0.72), respectively.

IgG-targeted antibody levels increased after onset of symptoms, peaking at 47, 35, and 36 days post–symptom onset for the Elecsys (peak COI of 32.9), Abbott Alinity (peak S/CO 8.2), and Abbott Architect (peak S/CO 5.4), respectively (Figure 3A–C). The IgM antibody titers peaked earlier at 26 days and followed a more rapid decline thereafter relative to the other 3 assays (Figure 3D).

There were no significant differences in early IgG-targeted antibody levels (0–10 or 11–22 days post–symptom onset) between subjects who developed severe vs nonsevere COVID-19 infection (Figure 4; Supplementary Table 2). In contrast, early IgM antibody levels were significantly higher in subjects who developed severe COVID-19, with an almost 4-fold higher IgM at days 0–10 post–symptom onset (4.20; 95% CI, 0.75–17.93; vs 1.07; 95% CI, 0.21–5.46; $P = .048$). This difference persisted when measured at days 11–22 post–symptom onset (severe disease, 17.30; 95% CI, 6.82–27.32; vs nonsevere disease, 8.66; 95% CI, 4.25–14.80; $P = .016$) (Figure 4D; Supplementary Table 3).

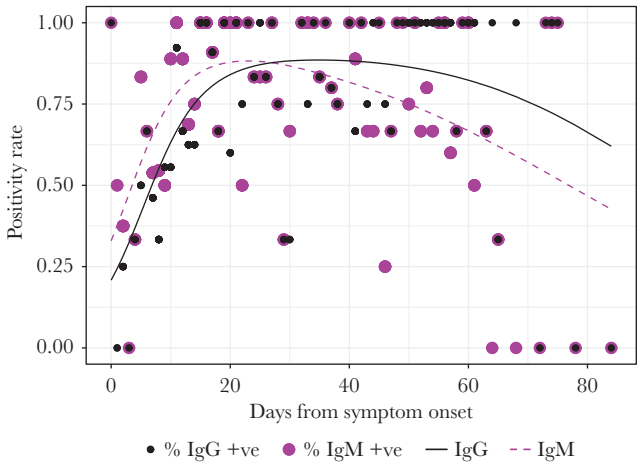

Figure 2. Dynamic change in seropositivity over time since onset of symptoms. We modeled change in seropositivity rates over time using fitted probability of positive IgG and IgM antibody test results. The trend lines were estimated using GEEs with logit link and time as a natural spline. IgM positivity rates increase more rapidly but fall off earlier than positivity rates from IgG-directed serological assays. Abbreviations: GEE, generalized estimating equation; IgG, immunoglobulin G; IgM, immunoglobulin M.

Cross-Reactivity

Within the SARS-CoV-2 Neg group, 9 (4.02% overall) returned a positive result on the Elecsys assay, of whom 8 (3.57% overall) were also positive on both Abbott IgG assays. Detailed clinical review of these 9 subjects revealed that the majority had a clinical presentation suggestive of COVID-19 despite having a negative SARS-CoV-2 PCR; 6 presented with an influenza-like illness, 2 presented with a systemic inflammatory response, 4 had history of close contact with a confirmed COVID-19 case, and 1 was diagnosed with a viral myocarditis (Supplementary Table 4).

Within the Controls Pre-2020 group, only 1 (0.9%) sample derived from previous flu seasons 2016–2019, and 3 samples (1.1%) from historical controls with chronic HIV monoinfection were positive on the Abbott IgG assays. We observed no cross-reactivity with the Elecsys assay for samples from the Controls Pre-2020 group (Supplementary Table 5).

DISCUSSION

This is among the largest and most comprehensive studies to analyze the performance and dynamics of both IgG and IgM anti-SARS-CoV-2 assays in well-phenotyped, diverse populations with and without confirmed COVID-19 infection. Our results show a clear delineation between development of IgM and IgG responses, with IgM responses developing earlier after onset of symptoms and predicting development of more severe COVID-19 infection. Furthermore, we demonstrate declines in antibody levels of all assays after a peak that occurs quite early (5–6 weeks) post–symptom onset. These data provide important insights into both the clinical utility of serology in managing SARS-CoV-2 infection

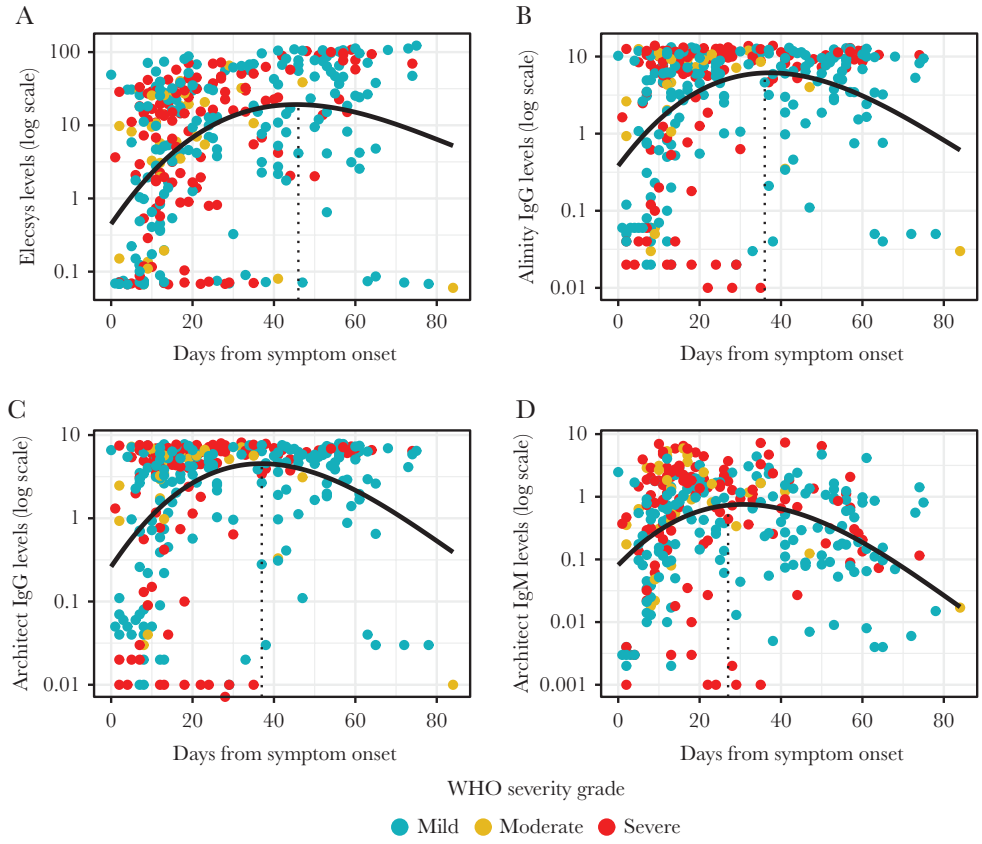

Figure 3. Dynamic change in antibody levels over time since onset of symptoms. We modeled change in antibody levels over time using scatter plots with superimposed curves fitted using GAMMs, with a Gaussian link function and time since symptom onset fitted as a spline. Graphs depict change in antibody levels over time of (A) IgG Roche assay, (B) Alinity IgG-targeted assay, (C) Architect IgG-targeted assay, and (D) Architect IgM assay. Abbreviations: GAMM, generalized additive mixed model; IgG, immunoglobulin G; IgM, immunoglobulin M; log, logarithmic.

and the limited longevity of antibody responses, which may have implications for postinfection immunity to SARS-CoV-2.

Although overall assay sensitivity for all 4 assays was <80%, sensitivity was lower early after symptom onset and increased to levels consistently >80% after day 11 and maintained beyond day 42 for all but the Abbott IgM assay, which decreased notably after day 42 to 68.5% (Table 3). These data are in keeping with previous studies and metanalyses that demonstrated lower assay sensitivity early after onset of symptoms [7, 11, 13, 16] and other studies that demonstrated high sensitivity in samples taken more than 2 weeks after symptom onset [9, 15]. Data on IgM responses are lacking and limited to relatively small studies [4, 22], with 1 study (n = 74) showing overall sensitivity of 70% in samples taken at least 3 weeks post–exposure to SARS-CoV-2 [4], similar to that seen in the later time periods of our analysis (>42 days), when the proportion with positive IgM was notably reduced.

To our knowledge, this is the first study to map dynamic changes in antibody levels against date of symptom onset within a large, diverse cohort. Consistent across all 3 IgG-targeted assays, antibody levels peaked just over 5 weeks after symptom onset and decreased thereafter. The dynamics of IgM titers followed an earlier peak and more rapid subsequent decline, which is biologically plausible. The declines in antibody levels observed across all assays support earlier data from a small cohort that demonstrated loss of both antibody levels and neutralizing antibody responses in the early convalescent period [4] and suggest the potential for waning of postinfectious immune responses that may explain the recent increase in reported reinfections with SARS-CoV-2 [23].

In our analysis, significantly higher IgM levels early in infection (before day 10), but not levels of the other antibodies tested, were observed in subjects who developed severe COVID-19. This is in contrast to a previous smaller study that showed higher IgG levels (but not IgM) in subjects with severe compared with asymptomatic SARS-CoV-2 infection; however, this previous study did not include as heterogeneous a study population as our analysis. Interestingly, the only other study to report on kinetics of IgM and IgG early in infection (n = 23, 11 with moderate and 12 with severe infection) also demonstrated higher early IgM but not IgG levels in more severe disease [22]. These data, confirmed within our large, diverse population, suggest a potential role for early measurement of IgM in identifying those presenting with symptoms who are at greater risk of developing severe COVID-19.

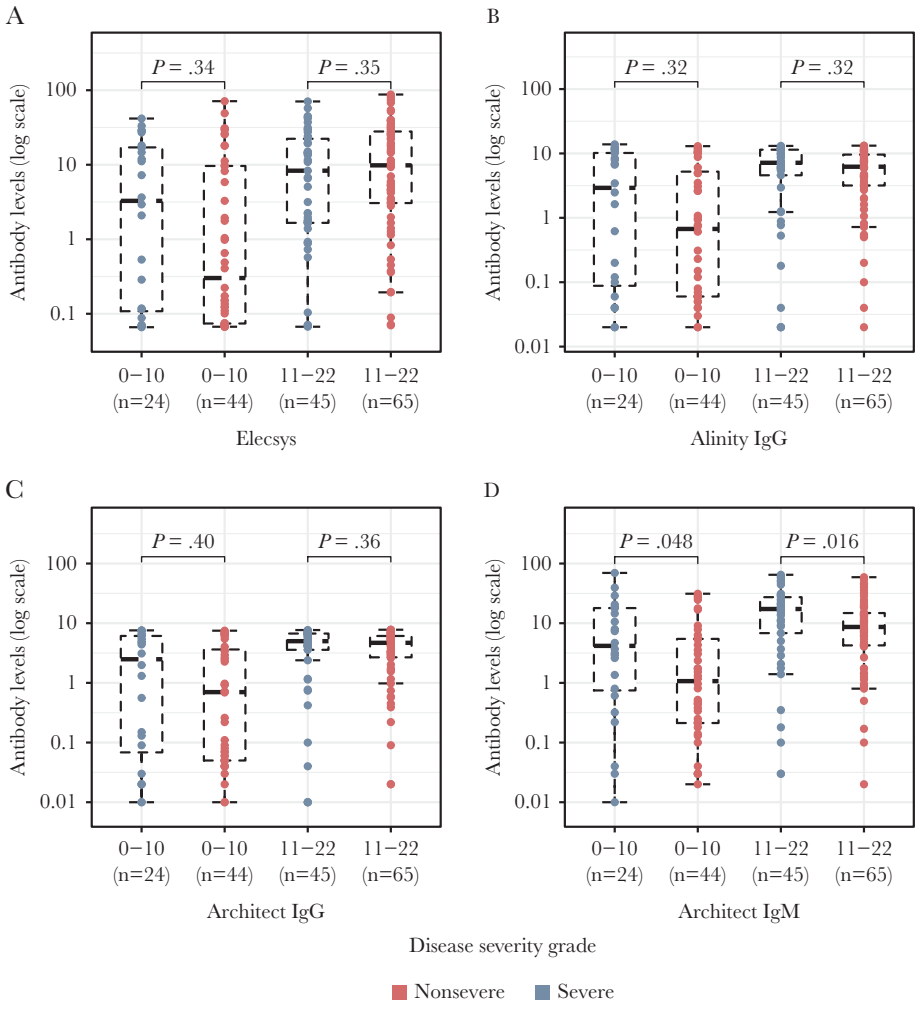

Figure 4. Early antibody levels in subjects who developed severe vs nonsevere COVID-19. We compared antibody levels taken early after symptom onset (<10 days or between 11 and 22 days) in those who developed severe and nonsevere disease. We found higher IgM in those with severe disease (D), an effect not seen with any of the IgG-targeted assays (A–C). Abbreviations: COVID-19, coronavirus disease 2019; IgG, immunoglobulin G; IgM, immunoglobulin M; log, logarithmic.

Our results add to the body of data showing the high specificity of the serological assays tested. In particular, we showed little cross-reactivity with historical samples from populations with a high prevalence of common viral co-infections as well as those taken during previous outbreaks of reported community influenza-like illnesses in Ireland. Of note, when we analyzed cases of positive antibody responses in subjects hospitalized but not detected by SARS-CoV-2 PCR, we identified a majority that presented with symptoms consistent with COVID-19 where no alternative diagnosis was established. This suggests an additional clinical use for serological testing in aiding clinical diagnosis in these circumstances.

Our study has limitations. The AIID Cohort is a prospective, observational cohort, but biobanking is not conducted at set time points. This results in a spread of results over time that makes analyses less conventional and potentially more difficult to interpret but does enable modeling over time from subjects with SARS-CoV-2 infection from a variety of sources and can provide insights into pathogenesis that may not be readily apparent from conventional studies with fixed sampling. There were some differences in characteristics between those with and without SARS-CoV-2 in terms of sampling time, reflected in the choice of SARS-CoV-2-negative population from predominantly hospital-admitted cases while SARS-CoV-2-positive cases were recruited from both hospitals and outpatient clinics. Although we measured serological responses, we did not have data on corresponding functional immunity, which is important when interpreting the clinical relevance of the observed decline in antibody levels. We chose to model kinetics in all those with positive SARS-CoV-2 infection to provide overall population kinetics in a symptomatic population. However, although the majority of our cohort presented with mild SARS-CoV-2 infection, we did not examine individuals who were asymptomatic but SARS-CoV-2 positive, in which some reports suggest that serological responses may be lower again than what we observed [24]. Although we analyzed historical samples, we did

not have data on confirmed influenza in these subjects, nor did we routinely test the SARS-CoV2 Pos and Neg groups for other co-infections. Lastly, we used SARS-CoV-2 diagnosed by PCR as our reference for diagnosis but acknowledge that no test has perfect characteristics as a "gold standard."

Despite these limitations, this study, one of the largest and most detailed analyses of the performance and kinetics of anti-SARS-CoV-2 antibody responses, suggests higher, early IgM responses in those who develop more severe COVID-19. The early decline in antibody levels, as early as 5 weeks post–symptom onset, contributes to an increasing concern that postinfectious immunity to SARS-CoV-2 infection may be time-limited.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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