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Cross-sectional Characterization of SARS-CoV-2 Antibody Levels and Decay Rates Following Infection of Unvaccinated Elderly Individuals

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Background. SARS-CoV-2 infections have disproportionally burdened elderly populations with excessive mortality. While several contributing factors exists, questions remain about the quality and duration of humoral antibody–mediated responses resulting from infections in unvaccinated elderly individuals.

Methods. Residual serum/plasma samples were collected from individuals undergoing routine SARS-CoV-2 polymerase chain reaction testing in a community laboratory in Canada. The samples were collected in 2020, before vaccines became available. IgG, IgA, and IgM antibodies against SARS-CoV-2 nucleocapsid, trimeric spike, and its receptor-binding domain were quantified via a high-throughput chemiluminescent enzyme-linked immunosorbent assay. Neutralization efficiency was also quantified through a surrogate high-throughput protein–based neutralization assay.

Results. This study analyzed SARS-CoV-2 antibody levels in a large cross-sectional cohort (N = 739), enriched for elderly individuals (median age, 82 years; 75% >65 years old), where 72% of samples tested positive for SARS-CoV-2 by polymerase chain reaction. The age group ≥90 years had higher levels of antibodies than that <65 years. Neutralization efficiency showed an age-dependent trend, where older persons had higher levels of neutralizing antibodies. Antibodies targeting the nucleocapsid had the fastest decline. IgG antibodies targeting the receptor-binding domain remained stable over time, potentially explaining the lack of neutralization decay observed in this cohort.

Conclusions. Despite older individuals having the highest levels of antibodies postinfection, they are the cohort in which antibody decay was the fastest. Until a better understanding of correlates of protection is acquired, along with the protective role of nonneutralizing antibodies, booster vaccinations remain important in this demographic.

Keywords. COVID-19; elderly; neutralization; SARS-CoV-2; serology.

Since its emergence, SARS-CoV-2 has disproportionally affected elderly populations through increased COVID-19 disease severity, poor clinical outcomes, and excess mortality [1–3]. In fact, by April 2020, half of COVID-19–related deaths in Canada were individuals aged ≥69 years who were living in long-term care (LTC) [1]. By May 2021, seniors aged ≥65 years in Canada accounted for 93% of COVID-19–related deaths [4].

Factors influencing this disproportionate effect on the elderly remain unclear but are likely mediated by epidemiologic and biological factors. The link between LTC and infectious disease outbreak susceptibility has been studied and established [5, 6]. This link is thought to be explained in part by various attributes of the LTC setting. For example, seniors residing in LTC settings are frequently exposed to numerous other residents through shared rooms and common areas and indirectly through contact with LTC personnel [5, 7]. In addition, maintaining physical distancing was complicated by residents requiring high levels of care, resulting in prolonged close contact with LTC personnel and a decreased ability to follow infection control measures [8].

In addition, elderly individuals present distinct comorbidity profiles that inherently make them more at risk of poor outcomes following COVID-19 infection [2, 9]. In Canada, an estimated 37% of seniors aged ≥65 years and close to 50% of seniors >85 years reported living with ≥2 chronic conditions,

including cancer, cardiovascular disease, diabetes, hypertension, respiratory disease, and others [10].

In contrast to younger populations, elderly individuals display distinct differences in innate and adaptive immune responses. Two phenomena act synergistically and are believed to affect immune responses in older people: immunosenescence and inflammaging [11]. Inflammaging refers to the low but chronic presence of proinflammatory mediators, which increases with age [12, 13], while immunosenescence refers to the reduced effectiveness of the immune system as we age, such as the hyporesponsive nature of some immune cells [11, 14]. For example, macrophages/monocytes and dendritic cells can have reduced expression and function of various Toll-like receptors with age [15]. In the case of neutrophils, impaired formation of neutrophil extracellular traps was reported in aging [16] as well as diminished chemotaxis [17]. Reduced infiltration was noted in macrophages and a dendritic cell population, as was a reduction in phagocytosis [15, 18]. Impairment in innate cell functions, such as defects in antigen presentation, in combination with an inflammaging environment can affect adaptive response initiation [19]. In fact, CD4+ and CD8+ T cells display significant reduction in T-cell receptor repertoire with age, a process most likely linked with thymic involution [20]. In addition to the contracted repertoire, a reduction in naive T cells is documented with age [21], and the ones that remain display reduced expansion and T effector functions [14, 22]. B cells in older persons have impairments in undergoing somatic hypermutation, which can lead to lower-affinity antibodies [11]. Like T cells, it appears that naive B cells are reduced in numbers and the overall repertoire is affected with age [23]. In addition to intrinsic age-related defects in B-cell development and function, it is believed that the lower quality of T- and B-cell interaction caused by aging T cells extrinsically affects B-cell differentiation, expansion, and antibody production [14].

The impact of the aging immune system has been highlighted in immunization response studies against various pathogens. For example, a reduction in vaccine responsiveness has been documented for influenza A [24], hepatitis B [25], pneumococcal disease [26], and more recently SARS-CoV-2 [27, 28] in older individuals.

Given the rapid development and implementation of vaccine programs in most countries, vaccine-induced humoral responses have been described. However, few groups have focused on humoral responses in elderly cohorts who are vaccine naive. Tut et al showed in a cohort of 152 individuals that the elderly generate high levels of antibodies following infection [29]. Others have characterized elderly humoral responses in various study designs [30–36] and highlighted the fact that elderly people generate higher levels of antibodies following infection than those who are younger [33–36]. Despite these studies, the complete evaluation of all antibody isotypes (IgG, IgM, and IgA) and neutralization efficiency has not been done in large elderly cohorts. Furthermore, a significant proportion of the elderly in some countries is not yet protected and remains unvaccinated. While the decline rate of vaccine-induced antibodies has been extensively characterized, antibody decay rates from natural infection have not been described, to our knowledge, in an elderly-enriched vaccine-naive cohort.

METHODS

The complete method description is available in the Supplementary Material.

RESULTS

Establishment of an Elderly Unvaccinated Cohort

Between 28 April 2020 and 21 September 2020, residual serum and plasma samples from a community laboratory in Canada were collected from 766 individuals who had undergone polymerase chain reaction (PCR) testing for SARS-CoV-2 within the previous 180 days. Nine samples were excluded due to low sample volume. In addition, 18 were removed due to a lack of metadata or errors within that could not be corrected. The final cohort was composed of 739 participants, among which the median age was 82 years (SD = 18.9; Table 1, Supplementary Figure 1A); 252 (34.1%) were male; and 533 (72.1%) had received a positive diagnosis for SARS-CoV-2 through PCR testing. After PCR testing, a serum/plasma sample was collected when contributors provided serum or plasma at the request of their health care providers, resulting in various time points after the onset of COVID-19 symptoms, ranging from 1 to 162 days with a median of 40 (SD = 33.3; Supplementary Figure 1B).

Comparison between results from PCR testing and serologic analyses showed the following: 454 (61.4%) participants had received a positive diagnosis for SARS-CoV-2 through PCR and had detectable levels of antibodies; 194 (26.5%) had received a negative diagnosis for SARS-CoV-2 through PCR and had no detectable levels of antibodies; 79 (10.7%) had received a positive diagnosis for SARS-CoV-2 through PCR but had no detectable levels of antibodies; and 12 (1.6%) had received a negative diagnosis for SARS-CoV-2 through PCR but had detectable levels of antibodies (Table 1).

Elderly Individuals Generate Higher Levels of Antibodies Against SARS-CoV-2

The effect of age on the antibody response following SARS-CoV-2 infection was investigated. To do so, the cohort of 513 participants who were SARS-CoV-2 positive with data on age was divided into 3 age groups—hereafter, <65 (n = 126), 65–89 (n = 252), and ≥90 (n = 135) years—given the overall age distribution of our cohort (Supplementary Figure 1A). The <65 group had significantly lower anti-RBD (anti–receptor-binding domain), anti-S, and anti-N IgGs than the 65–89 and

Table 1. Cohort Demographics and Collected Information Based on Molecular and IgG Seroconversion. Demographic data on individuals and sample information used in subsequent analyses were compiled and presented based on seroconversion to IgG (Sero+/-) and results of molecular (PCR) testing for SARS-CoV-2 (PCR+/-).

	Total (N = 739)	PCR+/Sero+ (n = 454)	PCR−/Sero− (n = 194)	PCR+/Sero− (n = 79)	PCR−/Sero+ (n = 12)
Age, y					
Mean (SD)	75.2 (18.9)	76.3 (18.6)	73.2 (18.8)	71.1 (20.5)	83.0 (10.5)
Median	82.0	83.0	78.0	79.5	85.0
Min–max	21–105	21–105	23–100	22–98	60–97
Sex, No. (%)					
Male	252 (34.1)	147 (32.4)	74 (38.1)	25 (31.7)	6 (50.0)
Female	486 (65.76)	307 (67.6)	119 (61.3)	54 (68.4)	6 (50.0)
Indeterminate	1 (0.14)	0 (0.00)	1 (0.52)	0 (0.00)	0 (0.00)
Days between symptom onset and collection date					
Mean (SD)	45.5 (33.3)	49.8 (34.8)	33.6 (20.9)	40.5 (37.2)	25 (13.9)
Median	40.0	43.0	38.0	33.0	33.0
Min–max	1–162	4–162	1–72	1–153	9–33
No. of patients with date of collection	233	199	22	12	0
Collection site, No. (%)					
Long-term care	442 (59.8)	262 (57.7)	121 (62.4)	48 (60.8)	11 (91.7)
Hospital–intensive care unit	1 (0.14)	1 (0.22)	0 (0.00)	0 (0.00)	0 (0.00)
Not reported	296 (40.1)	191 (42.1)	73 (37.6)	31 (39.2)	1 (8.30)

≥90 groups (Figure 1, Supplementary Table 2). Significance was tested with a Mann-Whitney *U* test instead of an analysis of variance due to the nonnormal distribution. The same trend was observed for IgA levels, with the <65 group having the lowest overall levels for anti-RBD ($P < .05$), anti-S ($P < .01$), and anti-N ($P < .01$) IgA of all 3 age groups (Figure 1B). The difference in IgM levels between the <65 group and the 65–89 and ≥90 groups was not as pronounced as the one for IgG and IgA, although the level of anti-N IgM of the <65 group was significantly lower than that from 65–89 ($P = .0025$) and ≥90 ($P = .027$; Figure 1C). Neutralization efficiency also varied across age groups. Indeed, the <65 group had a significantly lower median neutralization efficiency than the 65–89 group ($P = .000005$) and the ≥90 group ($P = .00019$; Figure 1D), which correlated with its lower overall antibody levels as compared with the 65–89 and ≥90 groups.

Relationship Between IgG Level and Neutralization Over Time
The relationship between the time of serum/plasma collection, calculated as the number of days between symptom onset and sample collection, and antibody levels was characterized through generalized linear models with a gamma regression. This analysis used only the 216 SARS-CoV-2–positive samples containing information about the time of symptom onset and serum/plasma collection. The data set was further trimmed to contain the 185 samples that were collected between 10 and 100 days after symptom onset, as antibody production is limited during the first days following symptom onset and the few samples that were collected before 10 days (n = 10) and after 100 days (n = 21) were not sufficient for analysis over that period. The specificity of the antigen was added as an interacting

variable in the model so that the slope would vary depending on the antigen target, meaning that anti-S, anti-RBD, and anti-N antibodies were allowed to vary differently from one another across time. Interestingly, anti-RBD IgG levels did not show a decrease in samples up to 100 days after onset of symptoms and remained relatively constant over the time observed (slope = 0.00066 relative light units [RLU]/d). Levels of anti-S IgG (slope = −0.00456 RLU/d) and anti-N IgG (slope = −0.00682 RLU/d) displayed higher decay with anti-N IgG having a faster decay rate than anti-S IgG (Figure 2A, Supplementary Table 4). IgA and IgM decay rates were overall faster than the ones for IgG, as expected (Supplementary Figure 5A–C). This suggests an antigen/isotype-specific decay rate after infection.

To determine if this decrease in antibody levels affects the neutralization efficiency, the relationship between the neutralization efficiency and antibody levels was modeled on the whole cohort via a 4-parameter log-logistic regression (Figure 2B). The levels of anti-RBD IgG (median effective dose [ED_{50}] = 1.18), anti-S IgG (ED_{50} = 1.7) and anti-N IgG (ED_{50} = 1.01) all positively correlated with neutralization. Similar trends were observed for IgA and IgM (Supplementary Figure 5C and D, Supplementary Table 3). The relationship between neutralization efficiency and time of serum/plasma collection was then modeled through a linear model. Although antibody levels were lower in samples collected later after symptom onset and the neutralization efficiency positively correlated with antibody levels, neutralization efficiency varied very little with the time of serum/plasma collection (slope = −0.0036; Figure 2C).
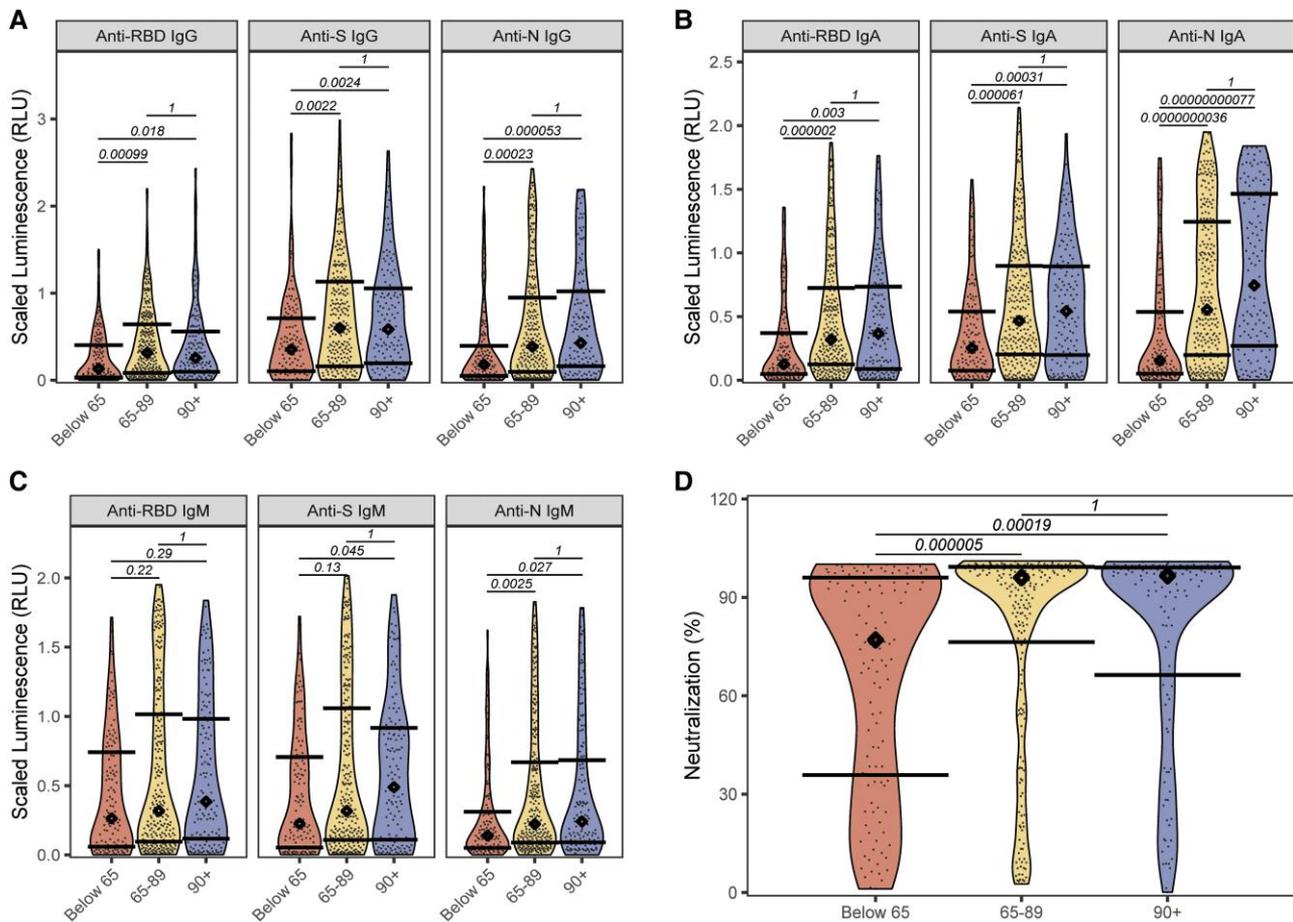
Figure 1. Antibody levels and neutralization efficiency relative to age groups. *A–C,* Levels of anti-RBD, anti-S, and anti-N IgG, IgA, and IgM relative to their corresponding age groups (<65, 65–89, and ≥90 years). *D,* Neutralization efficiency relative to the age group. For all panels, medians are indicated by diamonds and IQRs by horizontal segments. Serum/plasma was diluted by a factor of 100 for IgA and IgM and 10 000 for IgG. *P* values presented above the violin plots were calculated with a Mann-Whitney *U* test (n = 513). RBD, receptor-binding domain; RLU, relative light units.

Modeling of Antibody Decline Reveals That Decline Rate Is Increased With Age

The effect of age on the decrease of antibody levels was investigated. The relationship among antibody levels, time of serum/plasma collection, and age was modeled with a generalized linear model with a gamma regression on the 216 SARS-CoV-2–positive samples with data for the time of serum/plasma collection after symptom onset. The data set was further trimmed to the 185 samples that were collected between 10 and 100 days after symptom onset. Interestingly, the ≥90 group had faster decrease rates for anti-S IgG and anti-N IgG than the <65 and 65–89 groups (Figure 3*A*, Supplementary Figure 6*A* and *B*, Supplementary Table 4). This suggests that although the ≥90 group had overall higher anti-S IgG and anti-N IgG levels when compared with the <65 and 65–89 groups, they appeared to decay at a faster rate to reach similar or even lower levels than the ones in the younger groups by day 100 after symptom onset. This faster decay rate for the ≥90 group was also observed for all IgAs and IgMs, although the rate of

decrease was faster than IgGs. Interestingly, the IgG, IgA, and IgM levels for the <65 group did not vary much with time, with the exception of anti-N IgG, anti-S IgA, and anti-N IgA, which decreased with time (Figure 3, Supplementary Figures 6 and 7). These results suggest that although younger populations mounted a less potent immune response following SARS-CoV-2 infection, their antibody levels were more stable than those of older individuals during the 100-day period that was studied.

DISCUSSION

Disease-related comorbidities, aging immune system, and changing living circumstances (home care, LTC, hospital stay) are a few important factors that can render elderly people more susceptible to poor clinical outcomes. During the COVID-19 pandemic, questions were raised regarding how the humoral immune system responds following SARS-CoV-2 infection in the elderly. Considerable research efforts have been dedicated to elucidate
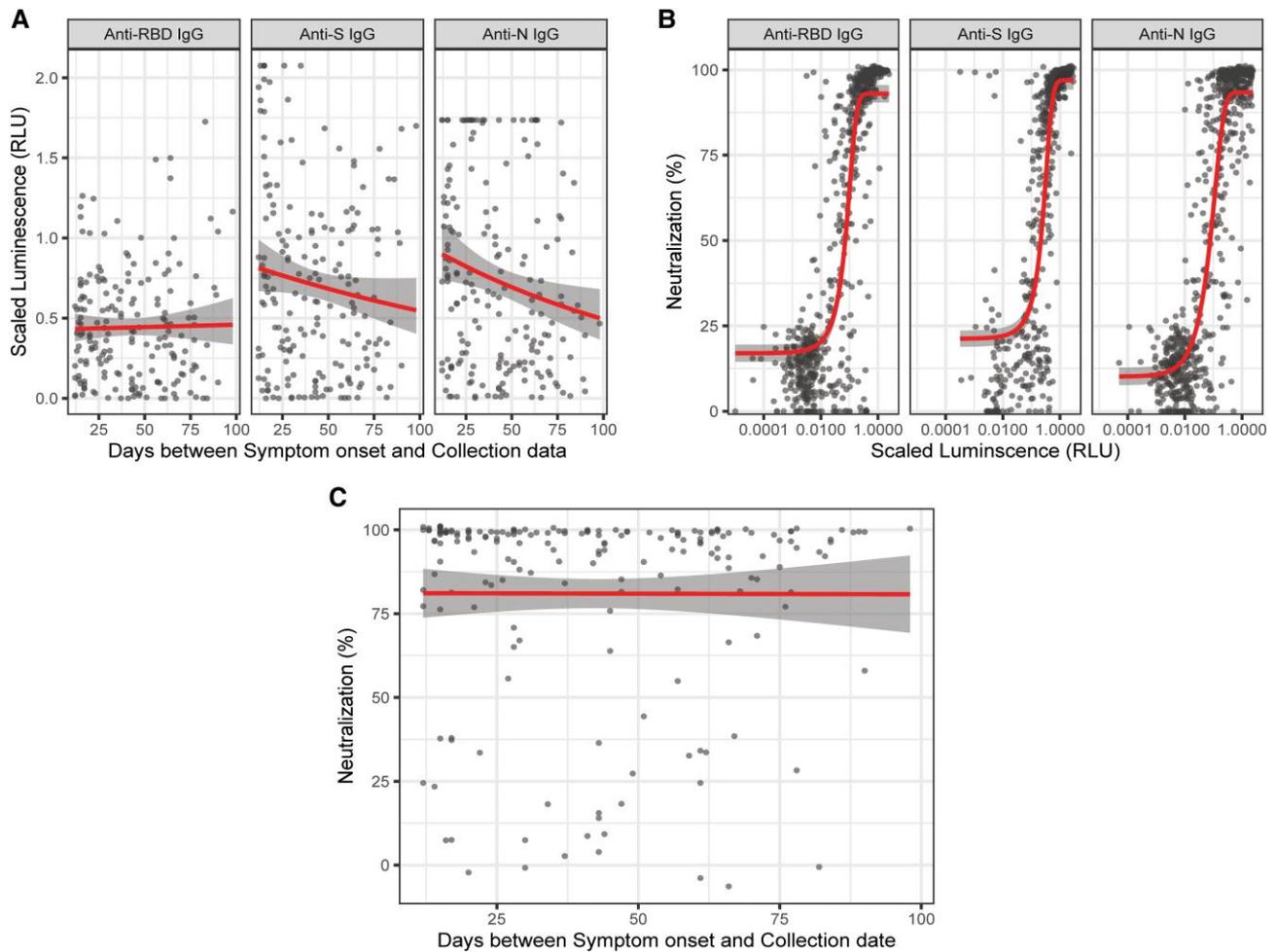
Figure 2. Antibody levels and neutralization efficiency relative to the time of serum/plasma collection. *A*, Levels of IgG raised against RBD, S, and N relative to the number of days between the onset of symptoms and serum/plasma collection. A generalized linear model of the gamma family was generated to model IgG-level variations as a function of time per the following formula: scaled luminescence ∼ number of days × IgG antigen (line). The SE of the model is displayed (shaded area) (n = 185). *B*, The relationship between neutralization efficiency and IgG levels was modeled via a 4-parameter log-logistic regression per the following formula: neutralization ∼ scaled luminescence for each IgG antigen (line). The SE of the model is displayed (shaded area) (n = 739). *C*, Neutralization efficiency was based on the number of days between symptom onset and serum/plasma collection. The relationship was modeled with a linear model per the following formula: neutralization ∼ number of days (line). The SE of the model is displayed (shaded area) (n = 185). Serum/plasma was diluted by a factor of 100 for IgA and IgM and 10 000 for IgG. RBD, receptor-binding domain; RLU, relative light units.

characteristics of antibody responses to SARS-CoV-2, as these represent the best known correlate of protection [37].

In this study, we collected serum/plasma from 739 individuals, 75% of whom were >65 years old, between April and September 2020, prior to vaccine availability in Canada (Table 1, Supplementary Figure 1). A retrospective study that examined mortality during our recruitment period reported that elderly populations aged ≥65 years accounted for 93% of deaths attributed to COVID-19 [4]. Molecular testing was initially performed and identified 533 people with active SARS-CoV-2 infection. From these, 79 (10.7%) did not seroconvert to IgG or IgA/IgM despite being positive by PCR. Upon further analysis of the 79 who had cycle threshold (Ct) data available, a large proportion had Ct values >36 for the N gene, which suggests a low viral load at the time of testing or

a false-positive PCR result. Low levels of viral RNA, which could be associated with a very mild infection, may not be sufficient in certain persons to elicit a detectable antibody response. In addition, cross-reactive seasonal coronavirus T cells have been reported to abrogate SARS-CoV-2 infection, resulting in a lack of seroconversion [38]. A few groups have noted an increased lack of seroconversion in those with asymptomatic and mild infection, in patients with a body mass index >30, and in children [39, 40]. In addition, the decreased prevalence of naive B cells and weaker interactions with T cells, for example, are likely contributors to higher frequencies of non-seroconversion in older people [14, 22].

The majority (85.2%) of individuals with positive PCR tests seroconverted (Tables 1 and 2). Serology profiling identified an additional 12 who tested negative by PCR but had detectable levels of
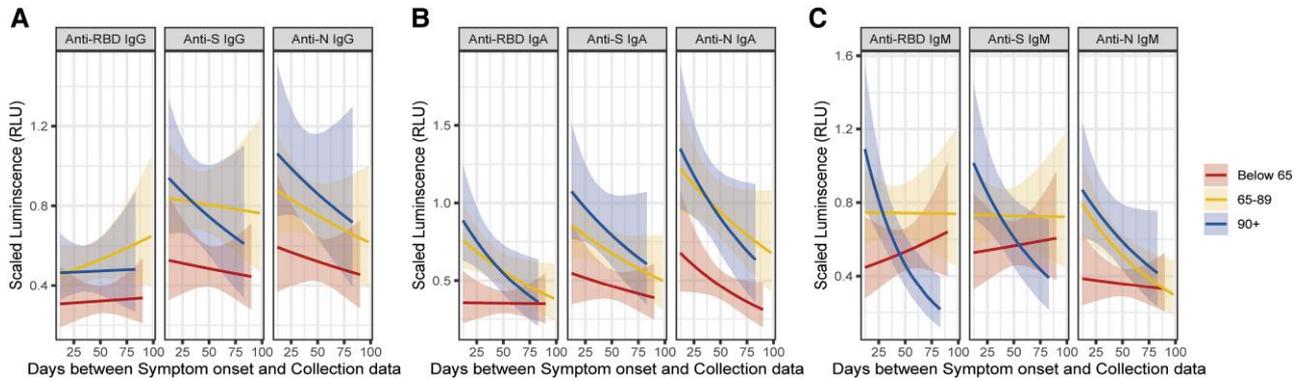
Figure 3. Age group–specific variation of antibody levels relative to the time of serum/plasma collection. *A–C*, Levels of anti-RBD, anti-S, and anti-N IgG, IgA, and IgM based on the number of days between onset of symptoms and serum/plasma collection per age group (<65, 65–89, and ≥90 years). The relationship was modelized with a generalized linear model of the gamma family per the following formula: scaled luminescence ∼ number of days × IgG antigen × age group (colored line). The SE of the models is displayed in the lighter section around the regression (n = 185). RBD, receptor-binding domain; RLU, relative light units.

Table 2. **Seroprevalence by Cohorts**. Individuals were segregated in 3 distinct cohorts based on age distribution. Demographic information is reported, as well as isotype and antigen-specific seroprevalence. Samples were considered positive for distinct antibodies based on a threshold set at a 3% false discovery rate of the density distribution from prepandemic serum/plasma samples.

	Total (n = 688)	<65 y (n = 174)	65–89 y (n = 342)	≥90 y (n = 172)
Age, y				
Mean (SD)	75.15 (18.8)	47.6 (12.4)	80 (7.3)	93.4 (3.1)
Median	82	50	82	93
Min–max	21–105	21–64	65–89	90–105
Sex, No. (%)				
Female	461 (67)	122 (70.1)	202 (59.1)	137 (79.7)
Male	227 (33)	52 (29.9)	140 (40.9)	35 (20.3)
Days between symptom onset and collection date				
Mean (SD)	48.60 (35.09)	60.36 (35.60)	46.77 (35.06)	35.82 (29.46)
Median	43	57	37	25
Min–max	1–162	1–161	1–162	3–127
No. of patients with date of collection	228	75	98	55
IgG, No. (%)				
RBD	451 (65.6)	105 (60.3)	223 (65.2)	123 (71.5)
Spike	521 (75.7)	128 (73.6)	253 (74)	140 (81.4)
Nucleocapsid	460 (66.9)	105 (60.3)	230 (67.3)	125 (72.7)
IgA, No. (%)				
RBD	369 (53.6)	69 (39.7)	202 (59.1)	98 (53.5)
Spike	389 (56.5)	79 (45.4)	204 (59.7)	106 (61.6)
Nucleocapsid	379 (55.1)	65 (37.4)	202 (59.1)	112 (65.1)
IgM, No. (%)				
RBD	363 (52.8)	80 (46)	185 (54.1)	98 (57)
Spike	348 (50.6)	77 (44.5)	176 (51.5)	95 (55.2)
Nucleocapsid	197 (28.6)	32 (18.4)	107 (31.3)	58 (33.7)
Neutralization				
Mean (SD)	58.6 (40)	50.6 (37.9)	60.6 (40.6)	62.8 (40.1)
Median	76.7	44.3	85	90.1

Abbreviation: RBD, receptor-binding domain.

antibodies, likely indicating an infection prior to molecular testing. Seroprevalence thresholds were previously established by our group by setting these at a 3% false discovery rate from the density distribution of a large panel of serum and plasma collected prior to 2019 [41]. In addition, to limit false positives from cross-reactivity with seasonal coronaviruses [42], participants were classified as convalescent seropositive if they had IgG antibodies to the nucleocapsid and another antigen (RBD and/or spike). To track

early seroconversion events, patients were also identified as positive if they had IgA and IgM antibodies.

Principal component analysis on the serology and PCR results showed a good clustering of the population in populations that were SARS-CoV-2 positive and negative (PC1 and PC2 explained 72.4% and 11.4% of the variance, respectively), particularly when only IgG levels were taken into consideration (PC1 and PC2 explained 93.8% and 3.6% of the variance; Supplementary Figure 2). This suggests that samples classified as positive by PCR had detectable levels of IgG and that the serologic data could be used to distinguish SARS-CoV-2–positive samples from SARS-CoV-2–negative ones. Although the principal component analysis showed a correlation between IgG levels and PCR results, no correlation was observed between IgG levels and PCR Ct values (Supplementary Figure 3). This suggests that although a positive PCR diagnosis for SARS-CoV-2 correlated with detectable SARS-CoV-2–specific IgG, the PCR Ct was independent to the levels of antibody detected following infection in this data set. Decentralized community PCR testing that was performed over various sites, including LTC homes and collection centers, may confound the data. In addition, samples were collected at various time points during acute infection, further complicating any attempt to using Ct values as a proxy for the amount of viral RNA measured during infection.

When antibody levels from the 533 SARS-CoV-2–positive samples with data on sex were compared, no significant differences in serologic results for IgG were found between females and males (Supplementary Figure 4A). In the case of IgA, females had significantly lower levels of anti-RBD ($P = .045$) and anti-S ($P = .047$; Supplementary Figure 4B, Supplementary Table 1). Females also had significantly lower levels of anti-RBD IgM ($P = .001$) and anti-S IgM ($P = .004$; Supplementary Figure 4C). Days between collection time and symptom onset were similar between males and females (Supplementary Figure 8). Differences in antibody by sex could be explained by characteristics of SARS-CoV-2 infections (disease severity, viral load, etc) and immune responses in both groups. Whether these differences seen in IgA and IgM levels are biologically relevant remains unclear.

When categorizing antibody levels by age groups (Table 2, Figure 1), we observed an age-dependent correlation with antibody levels. In fact, the ≥90 group consistently had significantly higher levels of antibodies than the <65 group, regardless of antibody isotype or viral antigen target. Other groups have reported similar trends [32, 33, 35]. This is somewhat in opposition to what is observed following SARS-CoV-2 immunizations, where age is negatively correlated with vaccine responses [28, 43]. Given that sera were collected for up to 180 days following symptom onset, a later collection time for some of the age groups is a possible cofounding factor. Unfortunately, days between sample collection and symptom onset was available for

only 33.1% of the cohort with detectable levels of antibodies. A density distribution by age group, for which this information was available, highlighted some differences, with a higher proportion of sera collected earlier in the ≥90 group (Supplementary Figure 9). However, considering samples collected within 30 days after symptom onset (Supplementary Figure 10), the same trend was observed, with older individuals having higher levels of antibodies. This observation may be due to the positive correlation between SARS-CoV-2 antibody responses and disease severity in previous studies [44, 45]. Given that older age is itself a risk factor for severe infections, higher levels of antibodies are likely an indirect result of high and persisting levels of viral antigens associated with infection severity. Presumably, a higher level of proinflammatory markers associated with increased disease severity could also contribute to an increased production of antibodies. Unfortunately, disease severity scores were not collected as part of this study, and because most patients remained in LTC or unspecified locations, hospitalization status was not an appropriate estimate of disease severity in our data set. It is possible that immunologic recall from previous coronavirus infection contributed to some of the differences in antibody levels in this cohort.

Given the importance of neutralizing antibodies in viral clearance and subsequent protection, we used a high-throughput protein–based surrogate neutralization assay to measure ACE2-spike interactions, considering that the number of samples in this cohort was prohibitive for PRNT50. Our assay has been shown to correlate with PRNT50 and has been validated with the World Health Organization's international reference panel [41, 46], and it is now used to validate new methods [47]. In this cohort, we showed that neutralization followed similar trends as antibody levels, whereas neutralization efficiency was positively correlated with age, with those aged >65 years having a significant higher level of neutralization activity overall (Table 2, Figure 1). Although B cells in the elderly have been shown to exhibit impairments in somatic hypermutation and affinity maturation processes [11, 13], we demonstrated that elderly persons generated high levels of antibody capable of efficiently neutralizing spike interactions with its receptor ACE2. In fact, because neutralization stems from infection-acquired immunity, all antibodies measured in this work correlated with neutralization (Figure 2, Supplementary Figure 5). Cross-sectional analysis of neutralization in relation to time since symptom onset revealed that neutralizing antibodies remained stable for at least 100 days in this cohort.

As expected, IgM and IgA antibodies against all 3 antigens (S, N, RBD) showed the fastest decay (Supplementary Table 4, Figure 3B and C, Supplementary Figure 6). It is worth noting that while all age groups showed a rapid decline for IgA, the group aged <65 years displayed more stable IgM levels over time, most likely due to the influence of outliers, which skewed

an already slower decline in contrast to the other age groups. Interestingly, in the case of IgG, antibodies targeting the nucleocapsid and the spike protein decayed at various rates, but antibodies targeting the RBD remained stable and even demonstrated an increase over time (Figure 3A). Since RBD is the principal target of neutralizing antibodies, this relative stability over time could explain the absence of a neutralization decline in our cohort [48]. In addition, antibodies targeting the nucleocapsid, regardless of isotype, showed the fastest decay, which is in line with previous knowledge [49, 50]. While decay rates are highly dependent on the target antigen and antibody isotype, age appears to influence the decline rate. In all cases, individuals aged ≥90 years showed the fastest decay despite the initial higher levels of antibodies.

Given that serum/plasma samples were collected up to 180 days following PCR testing, it is reasonable to assume that most patients survived SARS-CoV-2 infection, although information on outcome or disease severity was unavailable. In addition, recruitment occurred in early spring to fall of 2020, suggesting that most infections were caused by the ancestral SARS-CoV-2 strain, prior to variants in Canada. Nonetheless, this characterization of antibody responses in the elderly highlights that despite age, most individuals developed robust levels of antibodies with an associated high neutralization potential that remained stable over 100 days. We also showed that antibody decay rates increased with age and were dependent on the isotype and antigen. These data, in combination with studies examining vaccine-induced immune responses, Fc-mediated function, and cellular immunity, will contribute to our knowledge of natural and hybrid immunity, particularly in the elderly population.

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.

Notes

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