The Journal of Infectious Diseases

MAJOR ARTICLE

Antibody Responses to Respiratory Syncytial Virus: A Cross-Sectional Serosurveillance Study in the Dutch Population Focusing on Infants Younger Than 2 Years

Guy Berbers, Liesbeth Mollema, Fiona van der Klis, Gerco den Hartog, and Rutger Schepp

Center for Infectious Disease control, National Institute of Public Health and the Environment, Bilthoven, the Netherlands

Background. Respiratory syncytial virus (RSV) generally causes mild disease but can cause severe infections in (premature) infants and elderly adults. Here, we studied RSV-specific antibody concentrations throughout life with emphasis on infants and chronic obstructive pulmonary disease (COPD) patients.

Methods. Sera (N = 2655) from 2 nationwide cross-sectional studies in the Netherlands including individuals aged 0–90 years were analyzed for IgG and IgA antibodies to RSV prefusion F, postfusion F, N, Ga, and Gb proteins and for antibody avidity in 42 COPD patients.

Results. Maternal IgG concentrations declined to age 10–12 months. After the first year of life, approximately 40% of children lacked infection-induced IgA antibodies and may therefore be uninfected. All Dutch children showed serological evidence of RSV infection by age 3 years. Antibody concentrations reached a plateau by age 5–9 years and remains constant throughout life. COPD patients had similar levels and avidity of RSV-specific IgG antibodies compared with age-matched healthy controls.

Conclusions. RSV-IgG antibody patterns throughout life can be used to estimate the degree of immunity acquisition to RSV and to identify groups at increased risk of infection. Seroprevalence of IgA could be a proxy to determine RSV infection in children younger than 1 year.

Keywords. RSV; IgG; IgA; infection; infants; COPD; age.

Respiratory syncytial virus (RSV) is the main cause of severe acute lower respiratory tract infections in children younger than 2 years [1, 2] and is responsible for an estimated 1.7–3.0 million yearly hospital admissions in infants younger than 1 year globally [3]. RSV infection in young infants is also associated with an increased risk of asthma development later in life [4, 5], although primary RSV infections during early childhood (0–2 years) are mostly associated with mild (or absent) disease. Reinfections with RSV occur throughout life but generally become less severe or subclinical [6]. However, in frail elderly adults and adults with cardiopulmonary disease or immune deficiency, RSV infections can cause hospital admissions and mortality [7, 8].

The presence of RSV-specific serum immunoglobulin G (IgG) and nasal immunoglobulin A (IgA), and especially neutralizing antibodies, is positively associated with protection against RSV reinfection in adults, including elderly adults [9, 10]. In infants, in the first months of life, the development of a mucosal IgA response appears to be correlated with convalescence and IgG production is delayed [11]. In adults, circulating IgG-producing, but not IgA-producing, RSV-specific memory B cells are observed during convalescence [12]. This observed shortfall in IgA memory may partly explain the ability of RSV to cause recurrent (symptomatic) infections.

For infants in their first months of life, protection against RSV infection relies on the presence of sufficient levels of maternal antibodies [13]. Because RSV vaccines are not available yet, the only strategy to prevent RSV infection is currently passive immunization with a monoclonal antibody (palivizumab) that targets the metastable prefusion (prefusion F) and the stable postfusion (postfusion F) conformation of the fusion glycoprotein [14]. However, due to its cost, this therapeutic intervention is only prescribed in high-risk infants in high-income countries. New interventions such as passive immunization with neutralizing monoclonal antibodies (MAbs) directed against antigenic site Ø of prefusion F [15], maternal vaccination, or active immunization through vaccines based on prefusion F are in development and may become available in the coming years [16, 17]. For the successful implementation of RSV vaccines, insight into the pattern of primary RSV infections and the decay of protective maternal antibodies is essential for defining the putative vaccination window in the first year of

Received 25 March 2020; editorial decision 27 July 2020; accepted 17 August 2020; published online September 23, 2020.

Correspondence: Guy Berbers, PhD, RIVM, Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, the Netherlands (guy.berbers@rivm.nl).

The Journal of Infectious Diseases® 2021;224:269–78

DOI: 10.1093/infdis/jiaa483

life. Serosurveillance is a valuable tool to monitor the seroprevalence of RSV-specific antibodies in a population and can therefore provide insight into the dynamics of RSV infections in the first years of life and the maintenance of antibody levels throughout life, thus also in (elderly) adults. Such seroprevalence studies have been scarce, with the study by Nyiro et al the only example so far [18].

In the Netherlands, 3 nationwide cross-sectional representative serosurveillance studies (N = 7621–9948) were performed with an interval of roughly 10 years, with the last one from 2016/2017 [19–21]. Recently, we developed a high-throughput pentaplex immunoassay enabling us to measure specific antibody levels against 5 RSV proteins, prefusion F, postfusion F, Ga, Gb, and nucleoprotein (N), simultaneously [22], and which is very suitable for use in large-scale serosurveillance studies and vaccine trials.

The primary aim of this study was to determine the IgG levels to 5 RSV proteins in the Dutch population with special emphasis on the dynamics of RSV infections in infants 0–24 months of age and in chronic obstructive pulmonary disease (COPD) patients. In addition, as a secondary aim, the RSV-specific serum IgA levels were assessed to attempt to identify a possible marker for infection.

METHODS

Study Population

For this study, serum samples were used from 2 nationwide, cross-sectional seroepidemiological studies, called Pienter2 and Pienter3, with N = 7904 and N = 7621 and performed in 2006/2007 and 2016/2017, respectively. The studies had a similar design as described in detail elsewhere [20, 21]. In both studies, participants were asked to donate a blood sample and to complete a questionnaire. These studies were designed and conducted in accordance with the Good Clinical Practice guidelines established by the International Conference on Harmonization and with the Declaration of Helsinki. Ethical approval was obtained from local medical ethics committees in Almere, the Netherlands (Pienter2 study, ISRCTN 20164309) and from Noord-Holland (Pienter3 study, NL5467 (NTR5611). Written informed consent was obtained from all participants and from parents or guardians of minors.

Sample Collection

We selected at random 1219 and 1436 samples from the Pienter2 and Pienter3 studies, respectively, across all age cohorts until at least 50 samples in all age cohorts from 2 years and older were obtained, including almost all available samples from infants 0–24 months of age (n = 391 and 630, respectively). From both selections, IgA measurements were performed in 850 samples, including 459 from children 0–24 months of age. In the Pienter3 study, 42 participants were doctor-confirmed COPD patients with a mean age of 63 years (range 34–84 years). Their samples were used for IgG, IgA, and avidity measurements and compared with a healthy control subset with corresponding age from the Pienter3 study. The healthy control subset included 187 samples for the IgG measurement (mean age 61 years, range 50–71 years), 83 samples for the IgA measurement (mean age 60 years, range 51–70 years), and 96 for the avidity measurement (mean age 41 years, range 3–83 years). In addition, a panel of cord blood sera was obtained from the Radboud University Nijmegen (n = 17).

Serum Analysis of IgG, IgA, and Avidity

The RSV pentaplex immunoassay was performed as described previously [22] with minor changes. In this study, prefusion F (DS-CAV1) kindly provided by Barney Graham (National Institutes of Health) was used and an in-house serum pool containing high RSV-specific antibody concentrations was used as reference standard [22]. The First International Standard for Antiserum to RSV (National Institute for Biological Standards and Control code 16/284) and a reference serum (AstraZeneca/Medimmune) were used as controls. Five RSV antigens were coupled to fluorescent microbeads and the beads were incubated with serum samples in 2 dilutions (200-fold and 8000-fold), the reference serum in a dilution series, and 2 control sera on each 96-well assay plate. IgG antibody levels in Pienter2 study samples were measured using Bioplex 200 and Bioplex Manager 6.1 (Bio-Rad Laboratories). For each analyte, mean fluorescence intensity (MFI) was converted to arbitrary units (AU/mL) by interpolation from a 5-parameter logistic standard curve. The IgG levels of the Pienter3 samples, including the COPD patients, IgA levels and antibody avidity, were measured on the FLexmap 3D (Luminex) with Xponent software and further processed with Bioplex Manager 6.1. Sample measurements below the lower limit of linearity of the standard curve were set to the lower limit of quantification (LLOQ) of 0.05 AU/mL for all RSV proteins.

RSV-specific IgA concentrations were determined in a 200-fold dilution using goat F(ab′)2 anti-human IgA-PE (Southern Biotech) as secondary antibody. The Institute of Public Health and the Environment (RIVM) in-house reference standard serum was arbitrarily assigned an IgA potency of 10, 15, 5, 1, 1 AU/mL for prefusion F, postfusion F, N, Ga, and Gb, respectively, based on the MFI signal ratio between the 5 RSV proteins.

The avidity of the RSV-specific antibodies was determined with the same RSV-pentaplex immunoassay [22] using a 200-fold serum dilution for Ga and Gb and a 4000-fold dilution for prefusion F, postfusion F, and N antibodies. All samples were incubated for 10 minutes at room temperature in triplicate in the presence of 1.5M ammonium thiocyanate (NH_4SCN), 3.0 M NH_4SCN, or phosphate-buffered saline (PBS) (pH 7.4). The avidity index was expressed as the percentage of residual MFI IgG signal in comparison with the PBS signal, which was set at 100%.

Statistical Methods

Geometric mean concentrations (GMC) with 95% confidence intervals (CIs) were calculated using the Proc means procedure in SAS 94. Differences in the GMCs between COPD patients and healthy controls were tested with the *t* test. A *P* value < .05 was considered statistically significant. To calculate the half-life of maternal RSV IgG antibodies, a cutoff for infection using IgA concentrations was determined. IgA antibody levels (0–24 months of age) were transformed with the natural logarithm function. A cutoff of 0.19 AU/ml was identified, where the difference between sensitivity (0.9) and specificity (0.9) was minimal, in a mixture model using 2 normal distributions where observations of levels lower than the LLOQ were treated as left censored. Samples (0–10 months of age) with an IgA value below the cutoff were selected. We then fitted a linear model (y = ax + b) on the log-transformed IgG levels. The half-life of IgG antibodies was estimated by ln(2)/a using R (version 3.6.0), gamlss.cens package (version 5.0-1) and gamlss.mx package (version 4.3–5). Differences in avidity were calculated in GraphPad Prism v8.2.1 using 1-way ANOVA.

RESULTS

Seroprevalence of RSV-Specific IgG Concentrations

A distinct decay of maternal antiprefusion F IgG antibodies can clearly be identified from birth as determined in cord blood up to the age of 11–12 months in both Pienter studies, as illustrated in Figure 1A and 1B. Around 5–6 months of age, samples with an increase in the prefusion F antibody level started appearing and their number increased up to 2–4 years of age. At the age of 11–12 months, around 60% of the samples showed elevated prefusion F IgG antibody levels suggestive of a recent primary RSV infection, whereas 40% showed strongly declined maternal antibody levels. The 5 samples in the 2–4 years age group with an antiprefusion

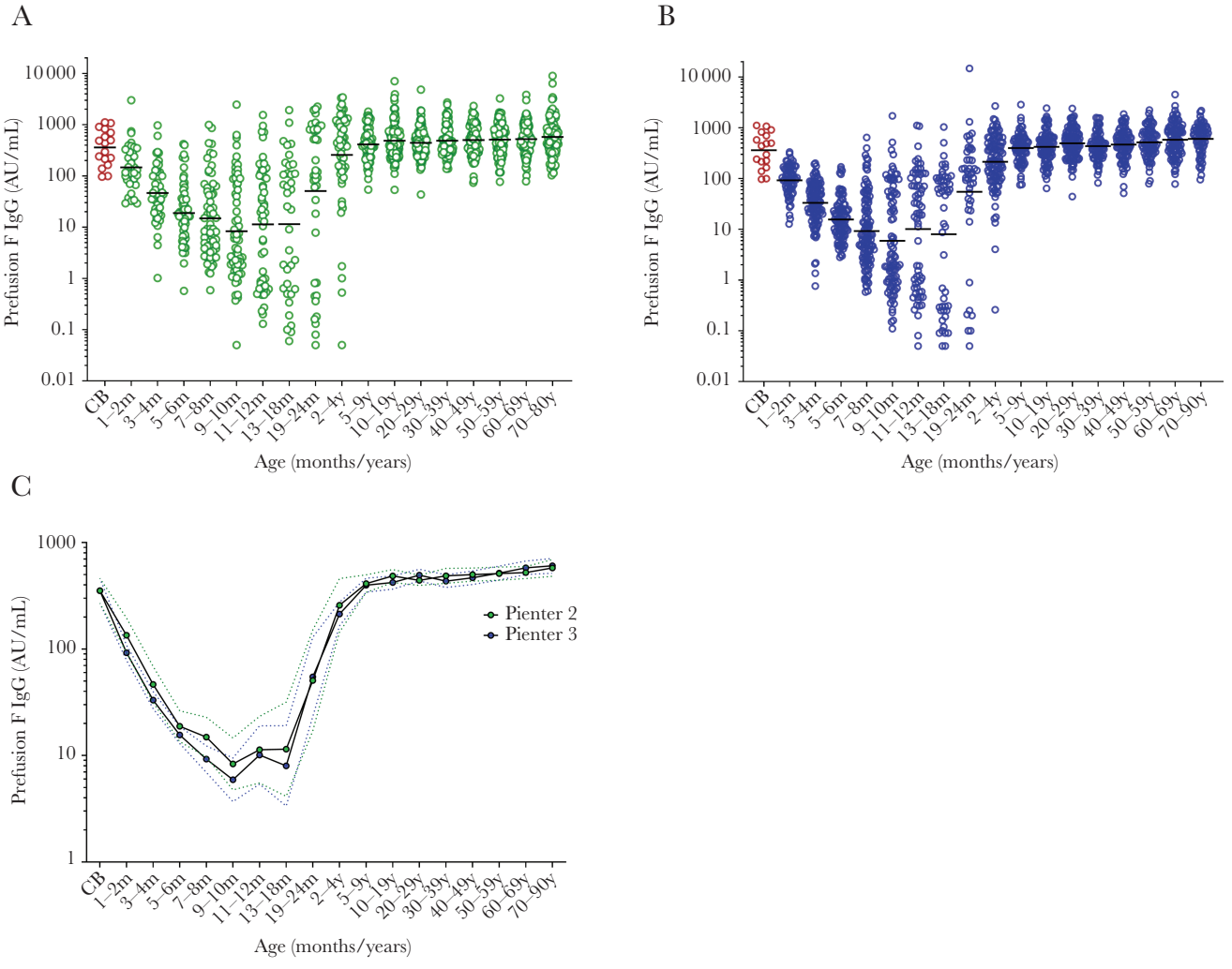

Figure 1. Scatterplots and geometric mean concentrations (GMCs; horizontal bars) of serum immunoglobulin G (IgG) concentrations (AU/mL) against prefusion F for different age groups. *A*, Green circles represent samples from the 2006–2007 Pienter2 study and (*B*) blue circles samples from the 2016–2017 Pienter3 study. Cord blood (CB) sera are represented by red circles. *C*, GMCs (continuous lines) and 95% confidence intervals (dotted lines) for both studies.

F IgG concentration below 3 AU/mL were all from children younger than 33 months. Subsequently, the prefusion F antibody GMCs reached a plateau similar to that of the cord blood samples, which remained stable throughout life with a slight increase in the eldest age cohorts (Table 1). Similar dynamics of the antibody levels were obtained for the other 4 RSV proteins tested with postfusion F- and N-specific antibody concentrations similar to prefusion F (Figure 2), whilst much lower concentrations were observed for Ga- and Gb-specific antibodies (Supplementary Figure 2 and Table 1). Antibody levels against all 5 RSV proteins were similar in the Pienter2 and Pienter3 studies for all age groups (Figure 1C and Figure 2).

Characteristics of 0 to 24 Month-Old Infants in Study Population

In Table 2 characteristics of the infants aged 0–24 months participating in the Pienter3 study are compared with this age cohort of the total Dutch population (N = 343 882). This study and the total Dutch cohorts have similar sex distribution, educational level of the mother, percentages of parents reporting a (very) good health condition for their child, percentages of cesarean delivery, and breast feeding. The percentage of

Table 1. IgG Geometric Mean Concentrations per Age Group in Pienter3 samples and Pienter2 Samples, and in COPD Patients Versus Healthy Controls

		Geometric Mean IgG, AU/mL (95% CI)				
Age Group	n	Prefusion F	Postfusion F	N	Ga	Gb
Pienter3 sample (n = 1436)						
1–2 mo	64	95.8 (80.8–113.6)	89.0 (70.5–112.4)	110.7 (93.3–131.3)	2.7 (2.1–3.5)	3.3 (2.7–4.1)
3–4 mo	102	40.1 (34.5–46.6)	36.1 (30.4–42.9)	50.4 (42.4–60.0)	1.0 (.8–1.2)	1.2 (.9–1.4)
5–6 mo	98	15.8 (13.2–18.8)	16.1 (12.9–20.2)	20.9 (16.8–26.1)	0.4 (.3–.5)	0.6 (.5–.8)
7–8 mo	110	9.3 (6.9–12.4)	10.3 (7.5–14.2)	15.8 (11.7–21.5)	0.4 (.3–.5)	0.3 (.2–.4)
9–10 mo	99	5.8 (3.9–8.8)	7.8 (5.0–12.3)	9.8 (6.6–14.4)	0.3 (.2–.4)	0.2 (.2–.3)
11–12 mo	68	8.9 (4.8–16.8)	9.7 (5.0–19.1)	15.7 (8.9–27.7)	0.5 (.3–.7)	0.3 (.2–.5)
13–18 mo	57	9.4 (4.2–21.0)	10.2 (4.3–24.1)	17.7 (9.2–34.1)	0.7 (.4–1.1)	0.4 (.2–.6)
19–24 mo	32	39.8 (12.6–126.0)	54.6 (17.7–168.7)	69.0 (26.5–179.9)	2.1 (.9–4.6)	1.2 (.5–2.7)
2–4 y	108	197.1 (147.4–263.5)	200.5 (156.1–257.5)	179.7 (138.0–234.1)	2.2 (1.7–3.0)	2.4 (1.8–3.3)
5–9 y	80	374.9 (319.3–440.1)	345.4 (290.7–410.2)	389.1 (331.1–457.3)	6.6 (5.3–8.2)	6.1 (4.7–7.9)
10–19 y	93	421.5 (365.5–486.1)	406.0 (344.1–479.1)	459.8 (393.4–537.4)	9.1 (7.6–11.0)	8.7 (7.0–10.7)
20–29 y	108	487.2 (428.3–554.1)	440.6 (377.7–513.9)	516.8 (449.2–594.5)	12.9 (10.9–15.4)	13.8 (11.5–16.6)
30–39 y	78	438.0 (381.4–503.1)	368.6 (311.8–435.7)	482.7 (414.5–562.1)	13.6 (10.9–16.8)	17.4 (14.3–21.1)
40–49 y	86	465.6 (401.4–540.2)	377.0 (321.1–442.7)	508.1 (427.9–603.3)	13.6 (11.6–15.9)	18.1 (14.8–22.0)
50–59 y	81	516.4 (445.1–599.1)	494.3 (410.2–595.8)	594.8 (502.9–703.5)	16.3 (13.4–19.8)	19.6 (16.2–23.7)
60–69 y	100	588.4 (509.2–680)	479.6 (409.0–562.4)	695.5 (592.9–816.0)	21.9 (18.5–26.0)	22.2 (18.4–26.8)
70–90 y	72	590.9 (502.9–694.4)	522.6 (432.3–631.8)	647.9 (533.2–787.3)	20.2 (15.8–25.9)	18.9 (15.0–23.8)
Pienter2 sample (n = 1219)						
1–2 mo	37	146.7 (104.7–205.7)	117.5 (78.6–175.5)	160.9 (112.7–229.7)	2.4 (1.7–3.4)	4.6 (3.0–7.1)
3–4 mo	45	46.5 (31.5–68.6)	42.8 (28.0–65.5)	59.8 (42.4–84.4)	0.8 (.6–1.2)	1.6 (1.1–2.4)
5–6 mo	60	19.6 (14.0–27.4)	19.3 (13.2–28.4)	27.9 (19.6–39.7)	0.5 (.3–0.7)	0.9 (.6–1.5)
7–8 mo	63	15.6 (10.0–24.3)	18.4 (11.6–29.3)	22.8 (14.8–35.3)	0.5 (.4–.7)	0.6 (.4–.9)
9–10 mo	61	9.0 (5.1–15.7)	10.1 (5.5–18.8)	16.7 (9.5–29.4)	0.4 (.3–.5)	0.5 (.3–.9)
11–12 mo	51	11.4 (5.3–24.5)	14.3 (6.6–31.1)	22.0 (11.1–43.8)	0.7 (.4–1.1)	0.5 (.2–.8)
13–18 mo	36	12.3 (4.4–34.8)	14.6 (5.3–40.1)	23.7 (10.2–55.0)	1.0 (.5–1.8)	0.6 (.3–1.3)
19–24 mo	38	56.0 (18.2–171.7)	56.6 (19.9–161.3)	85.2 (36.3–199.7)	1.4 (.8–2.4)	2.8 (1.4–5.7)
2–4 y	58	254.1 (144.0–448.6)	270.7 (161.9–452.7)	251.3 (163.1–387.0)	3.9 (2.6–6.0)	3.8 (2.4–6.1)
5–9 y	64	411.2 (340.2–497.1)	351.5 (279.8–441.8)	320.8 (254.9–403.9)	5.1 (3.7–7.0)	5.3 (3.9–7.2)
10–19 y	116	484.9 (421.7–557.5)	436.7 (372.2–512.3)	439.7 (372.4–519.2)	8.9 (7.4–10.8)	9.6 (7.9–11.7)
20–29 y	125	441.1 (395.1–492.6)	392.0 (342.9–448.1)	460.9 (401.2–529.5)	11.3 (9.6–13.4)	12.7 (10.6–15.2)
30–39 y	73	486.8 (414.5–571.6)	355.8 (295.5–428.3)	517.4 (435.4–614.9)	11.1 (8.6–14.4)	18.1 (14.4–22.6)
40–49 y	96	500.2 (434.3–576.2)	383.5 (331.4–443.8)	558.9 (486.9–641.5)	15.2 (12.8–18.1)	16.7 (14.0–19.8)
50–59 y	96	510.6 (442.8–588.7)	434.3 (370.1–509.5)	539.8 (455.2–640.3)	14.6 (12.0–17.6)	15.4 (12.5–19.0)
60–69 y	106	521.9 (458.8–593.6)	430.6 (372.4–497.9)	565.8 (489.0–654.8)	15.4 (12.8–18.4)	16.1 (13.5–19.3)
70–90 y	94	577.0 (482.0–690.6)	533.3 (437. 6–649.8)	697.6 (584.8–832.1)	17.0 (14.1–20.4)	18.1 (14.9–22.1)
COPD patients versus healthy controls						
COPD patients	42	491.0 (390.7–617.0)	532.7 (382.1–742.6)	636.5 (515.0–786.8)	13.8 (10.6–17.8)	14.3 (11.1–18.4)
Healthy controls	187	545.9 (493.3–604.2)	489.4 (435.2–550.5)	643.7 (576.0–719.4)	18.9 (16.6–21.5)	20.8 (18.2–23.7)

Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; IgG, immunoglobulin G.

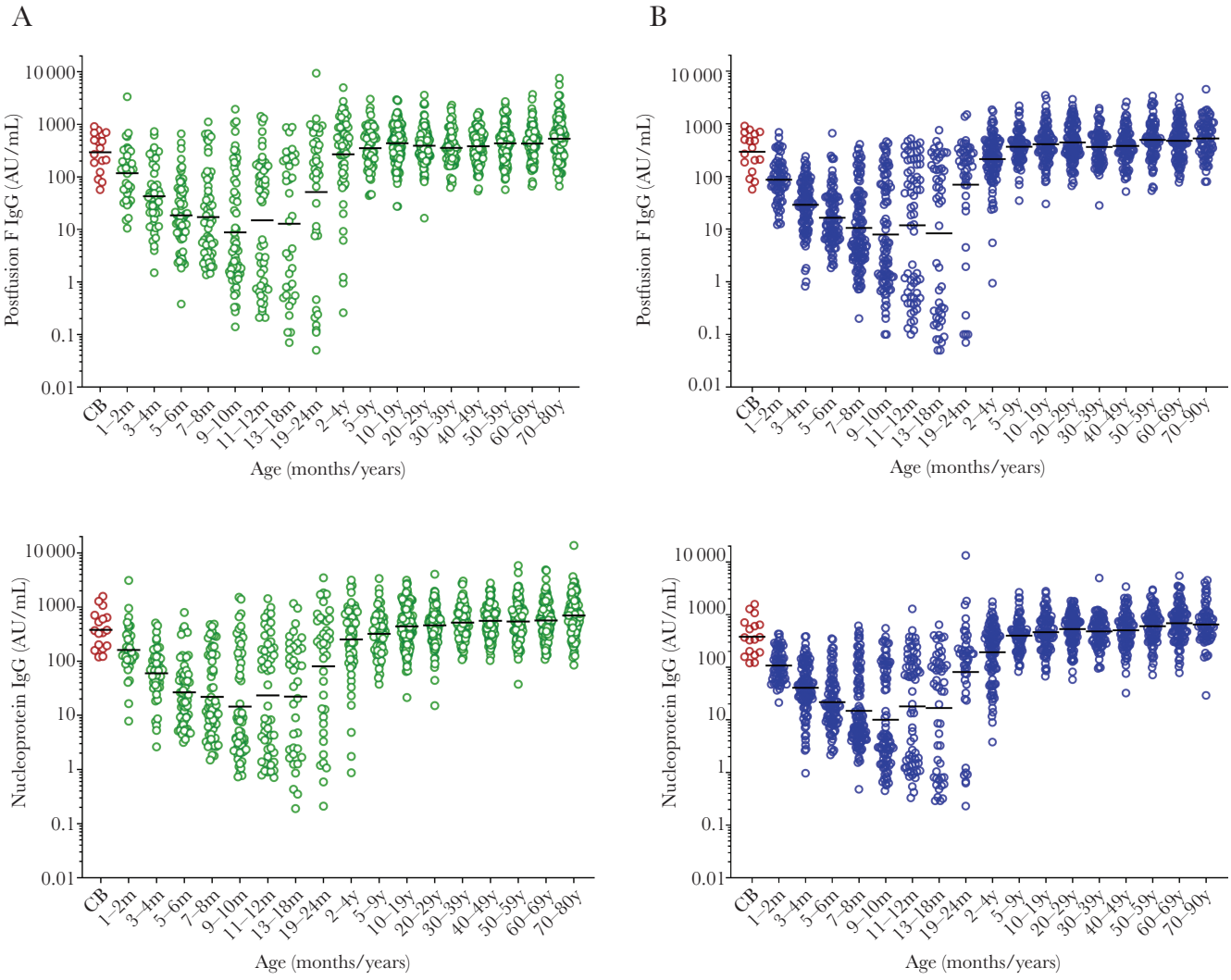

Figure 2. Scatterplots and geometric mean concentrations (horizontal bars) of serum immunoglobulin G (IgG) concentrations (AU/mL) against postfusion F protein and nucleoprotein from the Pienter2 study (*A*) and from the Pienter3 study (*B*). Cord blood (CB) sera are represented by red circles in all graphs.

premature infants and infants with low birth weight is somewhat lower in the Pienter3 subset compared with the national cohort, while the monthly income of the household is higher in the Pienter3 subset.

RSV-Specific IgA Concentrations

IgA levels were measured in a selected sample set from both serosurveillance studies focusing on children 0–24 months of age. In this age group, IgG can be maternally derived and produced endogenously, whereas IgA is not transferred across the placenta and therefore reflects exposure to RSV [23]. Up to 3–4 months, antiprefusion F IgA levels were very low (Figure 3A). From 5–6 months up to 5 years, IgA levels showed a gradual increase after which they remained constant throughout life. The patterns of IgA concentrations for the other 4 RSV proteins were similar, although the levels for Ga, Gb, and N were somewhat lower, while postfusion F IgA levels were very similar to prefusion F (Supplementary Figure 1). With the calculated cutoff for seroconversion of 0.19 AU/mL, we were able to discriminate between the antiprefusion F IgG levels in samples mainly composed of maternal antibodies (IgA ≤ 0.19 AU/mL) and samples showing evidence of a recent RSV infection (IgA > 0.19 AU/mL) (Figure 3B). Using the values from infants up to 10 months of age, we calculated a half-life decay time for the maternal anti-prefusion F IgG antibodies of 42 days (95% CI, 38–47).

COPD Patients and Avidity

Comparison of the IgG and IgA GMCs against 5 RSV proteins between the COPD cohort and the age-matched healthy control cohorts revealed no differences (Figure 4 and Table 1). Antibody concentrations in individuals with doctor-confirmed asthma also did not show differences in IgG concentrations compared with age-matched healthy controls (data not shown).

Table 2. Characteristics of Children Aged 0–2 Years With RSV IgG Antibodies Measured in the Pienter2 (n = 391) and the Pienter3 Samples (n = 630) in Comparison to the Dutch Population

Variable	Pienter2 Sample, No. (%)	Pienter3 Sample, No. (%)	Dutch Population, %
Age			
0 y	298 (76.2)	519 (82.4)	50.1[a]
1 y	93 (23.8)	111 (17.6)	49.9
Sex			
Female	214 (54.7)	328 (52.1)	48.7[a]
Male	177 (45.3)	302 (47.9)	51.3
Educational level of mother			
High	150 (38.4)	302 (47.9)	48.0[b]
Middle	147 (37.6)	209 (33.2)	37.0
Low	91 (23.3)	79 (12.5)	13.6
Missing	3 (0.8)	40 (6.4)	1.5
Monthly household income			
High	88 (22.5)	255 (40.5)	15.9[c]
Middle	193 (49.4)	246 (39.0)	53.4
Low	27 (6.9)	30 (4.8)	30.6
Did not answer/missing	83 (21.2)	99 (15.7)	NA
Reported health condition of child			
Very good/good	267 (68.3)	600 (95.2)	96.1[d]
Other category/missing	124 (31.7)	30 (4.8)	NA
Premature birth (<37 weeks) (missing = 78)	NA	18 (3.3)	7.1[e]
Breastfeeding (missing = 68)	NA	394 (70.1)	80[f]
Cesarean delivery (missing = 67)	NA	81 (14.4)	15.2[g]
Low birth weight, <2500 g (missing = 88)	NA	22 (4.1)	6.1[e]

All websites accessed on 20 December 2019.

Abbreviations: IgG, immunoglobulin G; NA, not available; RSV, respiratory syncytial virus.

[a]Statline: Bevolking; geslacht, leeftijd, generatie en migratieachtergrond, 1 januari. Statistics Netherlands (CBS). 2017. https://opendata.cbs.nl/statline/#/CBS/nl/dataset/37325/table?ts=1590656612401.

[b]Statline: Bevolking; hoogstbehaald onderwijsniveau en onderwijsrichting. Statistics Netherlands (CBS). 1e kwartaal 2017, vrouwen, 25–45 jaar. https://opendata.cbs.nl/statline/#/CBS/nl/dataset/82816NED/table?ts=1590657867536.

[c]Statline: Inkomen van huishoudens; inkomensklassen, huishoudenskenmerken. Statistics Netherlands (CBS), 2017. https://opendata.cbs.nl/statline/#/CBS/nl/dataset/83932NED/table?ts=1579769402920.

[d]Statline: Ervaren gezondheid, zorggebruik en leefstijl bij kinderen tot 12 jaar. Statistics Netherlands (CBS), 2017. https://opendata.cbs.nl/statline/#/CBS/nl/dataset/83716NED/table?ts=1590659073088.

[e]Volksgezondheidenzorg.info. National Institute for public Health and the Environment (RIVM). 2018. https://www.volksgezondheidenzorg.info/onderwerp/vroeggeboorte-en-laag-geboortegewicht/cijfers-context/huidige-situatie#node-vroeggeboorten-naar-zwangerschapsduur.

[f]Volksgezondheidenzorg.info. National Institute for public Health and the Environment (RIVM). 2015. https://www.volksgezondheidenzorg.info/onderwerp/borstvoeding/cijfers-context/huidige-situatie#node-aantal-vrouwen-dat-borstvoeding-geeft.

[g]Volksgezondheidenzorg.info. National Institute for public Health and the Environment (RIVM). 2017. https://www.volksgezondheidenzorg.info/onderwerp/zorg-rond-de-geboorte/cijfers-context/gebruik#node-bevallingen-naar-wijze-van-bevallen.

To explore the quality of these antibodies in the COPD patients, in addition to the quantity, we performed avidity measurements using NH_4SCN as a denaturing condition. The healthy controls in the avidity measurement were divided into age < 40 years and ≥ 40 years subsets. Differences in geomean avidity index (GMAI) between the 5 RSV proteins could be observed, with the IgG antibodies against postfusion F showing the highest GMAI (91%) in 1.5 M NH_4SCN denaturing conditions, followed by prefusion F (83%–86%), Gb (52%–62%), N (53%–58%), and Ga (37%–40%) (Figure 5A). In stringent denaturing conditions (3.0 M NH_4SCN) the GMAI for postfusion F remained high at 75%–78% whereas the GMAI for prefusion F dropped to 50%–51%. A sharp drop in GMAI was also observed for N, resulting in the lowest GMAI (12%) followed by Ga (16%–19%) and Gb (26%–32%) (Figure 5B). The only significant difference in GMAI between the COPD patients and 2 healthy control subsets was in GMAI of Gb for the COPD patients versus the healthy control < 40 years of age group ($P = .03$) at 1.5 M NH_4SCN.

DISCUSSION

Here we analyzed the seroprevalence of antibody concentrations to 5 RSV proteins in 2 nationwide cross-sectional studies covering all age groups from 0 to 90 years of age in the Netherlands. We showed declining maternal-derived IgG antibodies in the first year of life and initiation of production of endogenous IgG in infants around the age of 3–6 months. Using IgA seroconversion, we were able to distinguish infants with mostly maternally derived IgG from those with endogenous IgG, showing that by the age of 3 years virtually

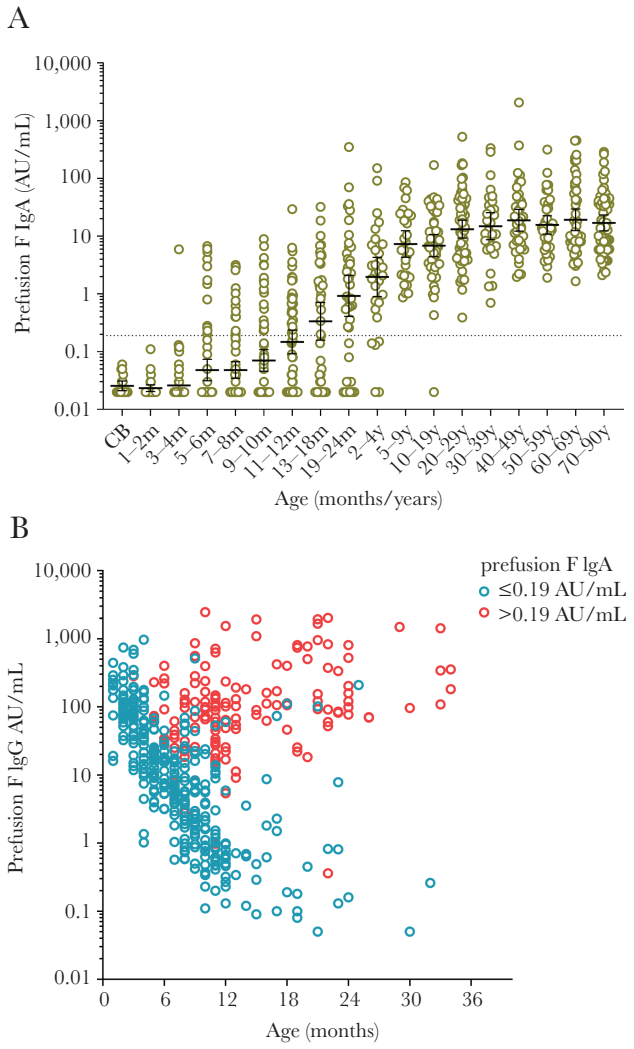

Figure 3. *A,* Scatterplots and geometric mean concentrations with 95% confidence intervals (horizontal bars) of serum immunoglobulin A (IgA) concentrations (AU/mL) against prefusion F are shown for all age groups. An estimated arbitrary IgA cutoff for seropositivity of 0.19 AU/mL is represented by the dotted line. *B,* Serum IgG concentrations (AU/mL) against prefusion F from children up to 3 years of age where red circles represent IgG concentrations of sera with an IgA concentration > 0.19 AU/mL and blue circles represent sera with an IgA concentration ≤ 0.19 AU/mL.

all Dutch children had encountered RSV. The specific antibody levels against the 4 RSV glycoproteins and the nucleoprotein showed a similar pattern and the GMCs remained constant from the age of 5 onwards. Pre- and postfusion F antibodies showed the highest avidity. No differences between the 2 Pienter studies could be observed, indicating that the epidemiological RSV situation has not changed in these years. COPD patients had similar antibody levels and avidity indices for the 5 RSV proteins compared with age-matched healthy controls.

Antibody concentrations are an effective readout to assess the immune status of the population. We show elaborate analyses of antibody concentrations to multiple RSV proteins throughout life in 2 serosurveillance studies. Because IgA is not transferred across the placenta, the presence of IgA could provide a useful tool to determine RSV infection in infants younger than 1 year when maternal IgG can still be present. This also allows a cutoff for exposure to RSV to be established. However, some samples with antiprefusion F IgA concentrations below the cutoff showed positive concentrations of IgG antibodies likely due to infection. This can be explained by the relatively slow induction of specific IgA antibodies (compare prefusion F IgG in Figure 1 with prefusion F IgA in Figure 3) combined with a shorter half-life resulting in prefusion F IgA concentrations below the proposed cutoff. These serological data showing seroconversion through RSV exposure before the age of 3 years are in line with epidemiological data that show increased incidence of RSV infection in the first 2 years and protection against RSV disease thereafter [1]. Although severe disease is rare in healthy adults, symptomatic reinfection occurs and challenge studies suggest that humoral immunity is not sufficient as even subjects with the highest serum anti-F, anti-Ga, and neutralizing antibody levels have a significant reinfection risk [12, 24, 25]. Maternal antibodies probably protect infants from RSV disease to some extent. However, it is not uncommon for premature infants up to 3–6 months of age to be hospitalized with severe RSV disease, with a negative correlation to preexisting maternal antibody concentrations to prefusion F [26, 27]. Deciphering the precise role of humoral immunity in this group is challenging as multiple other factors are likely to contribute to the increased risk of disease, although preterm infants do have lower levels of maternal IgG [27]. The decrease of maternal IgG RSV-specific antibodies in the first year of life in combination with IgA levels in these samples enabled us to calculate the half-life value of 42 days for these IgG antibodies. This is considerably lower than the 79 days found in the study of Nyiro et al [18] in the Kilifi cohort, but they did not take into account the possibility of recent infections via an IgA antibody cutoff, which evidently overestimates the half-life value by the generation of endogenous IgG antibodies after infection. Moreover, their setting is quite different than in our study but that might not be such an influential factor for an immunological parameter as IgG antibody half-life. Our value of 42 days is still longer than values found in immunology textbooks (25–30 days) but despite the use of our IgA cutoff we cannot exclude the possibility of endogenous IgG production by infants especially during at age of 5–10 months of life.

Because RSV infects the epithelial cells that form the wall of the respiratory tract [28], antibodies need to be present locally to protect against infection. How the levels of IgG and IgA detected in serum contribute to protection of the epithelium against RSV infection still needs to be established [29]. Although local RSV-specific IgA does not completely prevent infection, it can limit viral infectivity and replication [12, 30]. In contrast to influenza virus, RSV nasal IgA levels wane to preinfection levels within

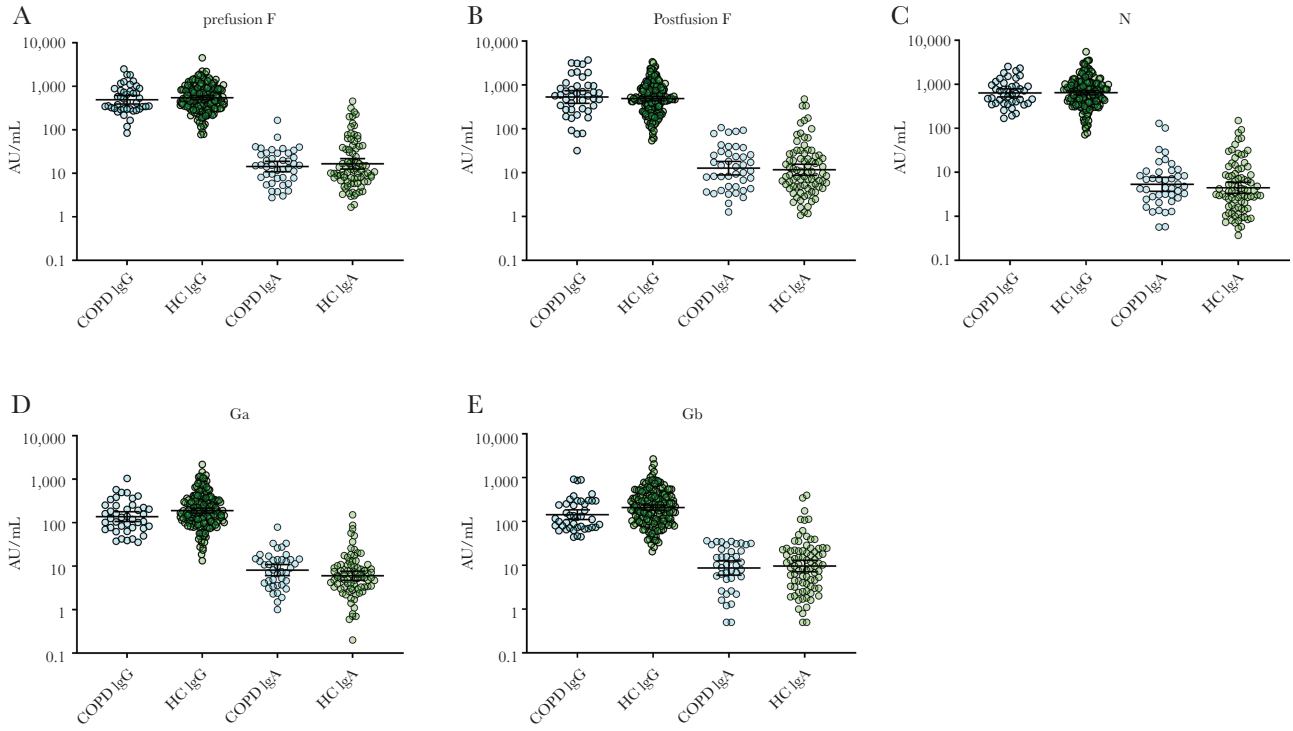

Figure 4. Comparison of the IgG and IgA concentrations (AU/mL) for the 5 RSV proteins between the COPD and the age-matched healthy control cohorts, for respectively (*A*) prefusion F, (*B*) postfusion F, (*C*) N, (*D*) Ga, and (*E*) Gb. Geometric mean concentrations and 95% confidence intervals are illustrated by horizontal bars. Abbreviations: COPD, chronic obstructive pulmonary disease; HC, healthy control; IgA, immunoglobulin A; IgG, immunoglobulin G; N, nucleoprotein; RSV, respiratory syncytial virus.

6–12 months [12], which could account for the frequent reinfections observed in challenge studies of healthy adults with identical RSV strains in as little as 2-month intervals [24].

RSV infections have been implicated in COPD exacerbations [31]. Analysis of the COPD patient samples showed no differences in quantities and avidity of RSV-specific antibodies with age-matched healthy controls. The binding capacity of the anti-Gb IgG antibodies might perhaps be lower in COPD patients, but this is not likely to cause an increased risk of infection. Serum antibody levels may not reflect local antibody

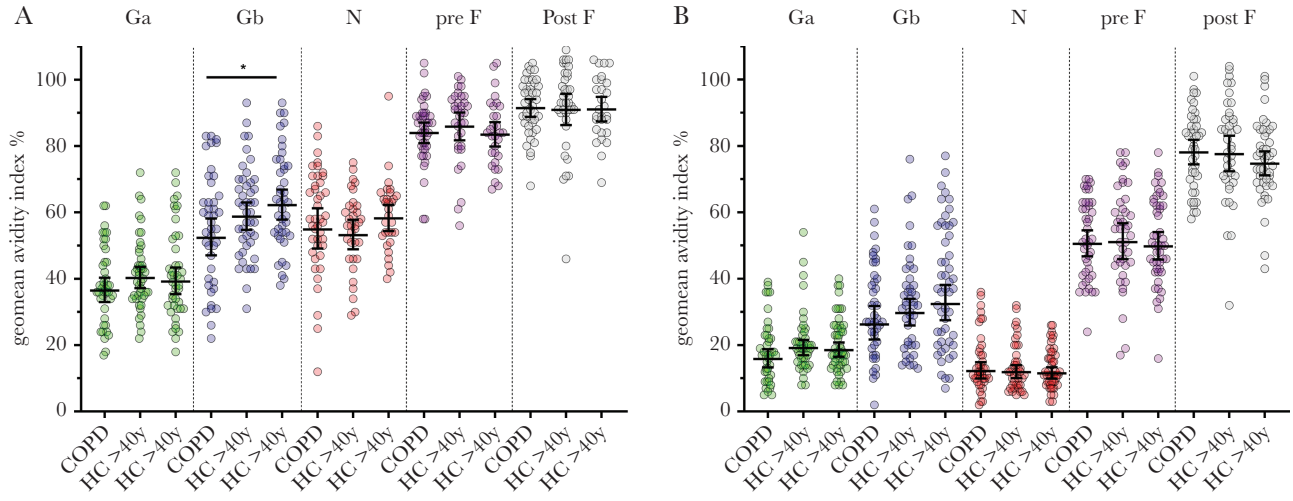

Figure 5. Avidity index in percentages of the IgG concentrations of the RSV proteins in sera from COPD patients and healthy control subsets aged < 40 years and ≥ 40 years, under (*A*) 1.5 M NH_4SCN denaturing conditions and (*B*) 3.0 M NH_4SCN denaturing conditions. Geomean avidity index and 95% confidence intervals are illustrated by horizontal bars. *Significant difference between COPD and HC >40 years ($P = .03$). Abbreviations: COPD, chronic obstructive pulmonary disease; HC, healthy control; IgG, immunoglobulin G; N, nucleoprotein; preF, prefusion F; postF, postfusion F; RSV, respiratory syncytial virus.

concentrations at the site of infection in COPD patients and therefore this should be studied further in detail. Although concentrations cannot be compared directly between antigens, an opposite trend between the IgG and IgA levels of the N protein on the one hand and of the pre/postfusion F proteins on the other hand could be observed. Relative changes in ratio could indeed indicate different concentrations of the N protein or the fusion proteins for IgA and IgG due to the underlying exposure and immune biology.

The avidity measurements revealed large differences in the binding strength of the antibodies directed against the 5 RSV proteins with the highest binding strength for prefusion F and postfusion F, although at more stringent denaturing conditions the postfusion F antibodies showed better avidity compared to the prefusion F antibodies, which is remarkable considering the virus neutralizing capacities of the latter. The N, Ga, and Gb antibodies showed limited avidity, supporting previous data that these might be less important for neutralizing RSV [22]. The Ga antibodies showed lower avidity than the Gb antibodies and might be cross-reacting Gb antibodies because RSV-B was predominantly circulating in the Netherlands during the sampling years of the 2 studies (2006/7 and 2016/17). In the age-matched healthy control panel, which did not include children younger than 10 years for this analysis, we additionally attempted to explore the relationship between avidity index and age. By dividing this panel into 4 groups with increasing age we could not statistically detect different avidities for the 5 RSV proteins, indicating that the avidity did not increase after the age of 10 years. To pinpoint possible effects of age on the avidity of the RSV-specific antibodies it must be mentioned that larger samples sizes and longitudinal studies are more appropriate instead of cross-sectional studies.

To our knowledge this is the first RSV serosurveillance study on this scale measuring IgG and IgA antibody levels against 5 specific RSV proteins and this is the strength of the current study. The only other serosurveillance study, by Nyiro [18] in Kenya, used an IgG enzyme-linked immunosorbent assay (ELISA) against the whole virus and different mathematical models to analyze the pattern of RSV seropositivity in children up to 12 years of age, which closely resemble the antibody concentration patterns we observed against the 5 RSV proteins. Another strength of our study is that the Pienter3 cohort of infants 0–24 months of age is a good representative of the whole Dutch population of that age. The main limitation of our study is that we measured the seroprevalence of RSV antibodies, which does not necessarily reflect protection.

In conclusion, we were able to determine the kinetics of RSV-specific antibodies throughout life using a sensitive multiplex immunoassay. These unique data provide insight into the status of the immune response against RSV at different ages and might help design intervention strategies.

Supplementary Data

Supplementary materials are available at *The Journal of 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

Acknowledgments. We thank Gerben Ferwerda, Radboud University, Nijmegen for the supply of cord blood samples; Barney Graham, National Institutes of Health for the donation of prefusion F; Mark Esser and Deirdre Wilkin, AstraZeneca for the donation of postfusion F, N, Ga, and Gb; and RESCEU investigators Elizabeth Clutterbuck, Simon Drysdale, and Charlotte Vernhes for critically reviewing the manuscript.

Financial support. This work was supported by the National Institute of Public Health and the Environment (RIVM), of the Dutch Ministry of Health, Welfare and Sport, the Netherlands; and by the Respiratory Syncytial Virus Consortium in Europe (RESCEU). RESCEU has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (grant number 116019), which receives support from the European Union Horizon 2020 Research and Innovation Program and the European Federation of Pharmaceutical Industries and Associations.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med **2009**; 360:588–98.
2. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet **2010**; 375:1545–55.
3. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet **2017**; 390:946–58.
4. Bønnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H. Association between respiratory infections in early life and later asthma is independent of virus type. J Allergy Clin Immunol **2015**; 136:81–6.e4.
5. Smyth RL, Openshaw PJ. Bronchiolitis. Lancet **2006**; 368:312–22.
6. Hall CB, Simões EA, Anderson LJ. Clinical and epidemiologic features of respiratory syncytial virus. Curr Top Microbiol Immunol **2013**; 372:39–57.

7. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med **2005**; 352:1749–59.
8. Hardelid P, Pebody R, Andrews N. Mortality caused by influenza and respiratory syncytial virus by age group in England and Wales 1999–2010. Influenza Other Respir Viruses **2013**; 7:35–45.
9. Walsh EE, Falsey AR. Humoral and mucosal immunity in protection from natural respiratory syncytial virus infection in adults. J Infect Dis **2004**; 190:373–8.
10. Falsey AR, Walsh EE. Relationship of serum antibody to risk of respiratory syncytial virus infection in elderly adults. J Infect Dis **1998**; 177:463–6.
11. Tsutsumi H, Matsuda K, Yamazaki H, Ogra PL, Chiba S. Different kinetics of antibody responses between IgA and IgG classes in nasopharyngeal secretion in infants and children during primary respiratory syncytial virus infection. Acta Paediatr Jpn **1995**; 37:464–8.
12. Habibi MS, Jozwik A, Makris S, et al; Mechanisms of Severe Acute Influenza Consortium Investigators. Impaired antibody-mediated protection and defective IgA B-cell memory in experimental infection of adults with respiratory syncytial virus. Am J Respir Crit Care Med **2015**; 191:1040–9.
13. Ogilvie MM, Vathenen AS, Radford M, Codd J, Key S. Maternal antibody and respiratory syncytial virus infection in infancy. J Med Virol **1981**; 7:263–71.
14. Impact-RSV Study Group. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics **1998**; 102:531–7.
15. Graham BS. Vaccine development for respiratory syncytial virus. Curr Opin Virol **2017**; 23:107–12.
16. Graham BS. Vaccines against respiratory syncytial virus: The time has finally come. Vaccine **2016**; 34:3535–41.
17. Kurzweil V, Tang R, Galinski M, et al. Translational sciences approach to RSV vaccine development. Expert Rev Vaccines **2013**; 12:1047–60.
18. Nyiro JU, Kombe IK, Sande CJ, et al. Defining the vaccination window for respiratory syncytial virus (RSV) using age-seroprevalence data for children in Kilifi, Kenya. PLoS One **2017**; 12:e0177803.
19. Mollema L, De Melker H, Hahné S, Van Weert J, Berbers G, Van Der Klis F. PIENTER 2-project: second research project on the protection against infectious diseases offered by the national immunization programme in the Netherlands, **2010**. https://www.rivm.nl/publicaties/pienter-2-project-second-research-project-on-protection-against-infectious-diseases. Accessed 22 August 2020.
20. Verberk JDM, Vos RA, Mollema L, et al. Third national biobank for population-based seroprevalence studies in the Netherlands, including the Caribbean Netherlands. BMC Infect Dis **2019**; 19:470.
21. van der Klis FR, Mollema L, Berbers GA, de Melker HE, Coutinho RA. Second national serum bank for population-based seroprevalence studies in the Netherlands. Neth J Med **2009**; 67:301–8.
22. Schepp RM, de Haan CAM, Wilkins D, et al. Development and standardization of a high-throughput multiplex immunoassay for the simultaneous quantification of specific antibodies to five respiratory syncytial virus proteins. mSphere **2019**; 4:e00236-19.
23. Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H. Evolution of maternofetal transport of immunoglobulins during human pregnancy. Am J Reprod Immunol **1996**; 36:248–55.
24. Hall CB, Walsh EE, Long CE, Schnabel KC. Immunity to and frequency of reinfection with respiratory syncytial virus. J Infect Dis **1991**; 163:693–8.
25. Hall CB, Long CE, Schnabel KC. Respiratory syncytial virus infections in previously healthy working adults. Clin Infect Dis **2001**; 33:792–6.
26. Trento A, Rodríguez-Fernández R, González-Sánchez MI, et al. The complexity of antibody responses elicited against the respiratory syncytial virus glycoproteins in hospitalized children younger than 2 years. Front Microbiol **2017**; 8:2301.
27. de Sierra TM, Kumar ML, Wasser TE, Murphy BR, Subbarao EK. Respiratory syncytial virus-specific immunoglobulins in preterm infants. J Pediatr **1993**; 122:787–91.
28. Zhang L, Peeples ME, Boucher RC, Collins PL, Pickles RJ. Respiratory syncytial virus infection of human airway epithelial cells is polarized, specific to ciliated cells, and without obvious cytopathology. J Virol **2002**; 76:5654–66.
29. Piedra PA, Jewell AM, Cron SG, Atmar RL, Glezen WP. Correlates of immunity to respiratory syncytial virus (RSV) associated-hospitalization: establishment of minimum protective threshold levels of serum neutralizing antibodies. Vaccine **2003**; 21:3479–82.
30. Bagga B, Cehelsky JE, Vaishnaw A, et al. Effect of preexisting serum and mucosal antibody on experimental respiratory syncytial virus (RSV) challenge and infection of adults. J Infect Dis **2015**; 212:1719–25.
31. Mohan A, Chandra S, Agarwal D, et al. Prevalence of viral infection detected by PCR and RT-PCR in patients with acute exacerbation of COPD: a systematic review. Respirology **2010**; 15:536–42.