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Stability of cytokine and immunoglobulin concentrations in the general population: prepandemic basal concentrations and intraindividual changes until the COVID-19 pandemic

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Background: While there is wide evidence on concentrations of cytokines in patients attending health care facilities, evidence is scant on physiological, basal concentrations of cytokines in the general population and across sociodemographic groups, as well as on their potential stability over time. Furthermore, from a public health perspective it is remarkable that no studies have analyzed intraindividual changes in such concentrations from before the COVID-19 pandemic until its outbreak.

Objectives: To investigate: (a) prepandemic concentrations of cytokines and immunoglobulins to viral exposures in a general, non-institutionalized population, and their associated sociodemographic variables; (b) the intraindividual change in such concentrations between a prepandemic period (2016–17) and the initial pandemic period (2020–21); and (c) whether such change was similar in participants who in 2020–21 were SARS-CoV-2 seronegative and seropositive, and between participants who did and did not develop COVID-19.

Methods: We conducted a prospective cohort study in 240 individuals from the general population of Barcelona, Spain. Thirty cytokines and 31 immunoglobulins were measured in paired serum samples collected in 2016–17 and 2020–21 in the same individuals.

Results: The median value of the relative intraindividual change in cytokine concentrations between 2016 and 2020 was <15% for 29 of the 30 cytokines. A substantial number of participants had an intraindividual increase or decrease

≥15% in some cytokines. No major differences in intraindividual changes of cytokine and immunoglobulin levels between 2016 and 2020 were observed between participants who did and did not develop COVID-19.

Conclusion: We provide novel information on physiological, basal ex-vivo concentrations of cytokines and immunoglobulins in a general population, which should be relevant for clinical practice and public health. Intraindividual changes in cytokines and immunoglobulins during the 4 years from 2016–17 to 2020–21 were moderate, and they did not differ between participants who in 2020–21 were SARS-CoV-2 seropositive and seronegative, nor between participants who did and did not develop COVID-19 disease. These findings are also novel and relevant for medicine and public health. In particular, the stability in the biomarkers is relevant to assess the role of the immunological and inflammatory state (measured through baseline levels of cytokines and immunoglobulins) in the development of SARS-CoV-2 seropositivity and COVID-19 disease, as well as in the susceptibility to other infections and pathologies.

KEYWORDS

cytokines, immunoglobulins, SARS-CoV-2, COVID-19, population-based

1 Introduction

While there is wide evidence on concentrations of cytokines in patients attending health care facilities, evidence is scant on physiological, basal ex-vivo concentrations of cytokines in the general population and across sociodemographic groups, as well as on their potential stability over time. Variation within individuals of cytokine concentrations is not well characterized either (1–11). Furthermore, from a public health perspective it is remarkable that no studies have analyzed intraindividual changes in concentrations of cytokines and immunoglobulins from the period before the COVID-19 pandemic until its outbreak (12, 13).

It is certainly well known that individual levels of cytokines fluctuate considerably during the clinical course of many diseases (1–4, 9, 11), as was –and is– also the case in patients with COVID-19 (14–21). While concentrations of cytokines and immunoglobulins return to baseline levels at convalescence or recovery, others seem to persist altered for longer periods of time, reflecting more persistent alterations of the cellular immune system (22–27). Assessing the influence of basal physiological ex-vivo levels of cytokines and immunoglobulins on the risk of SARS-CoV-2 infection and COVID-19 disease (28) requires that such biomarkers be measured before the pandemic, and that they remain relatively stable over time. Additional biomarkers of interest related to baseline immune state, which could also predict infection and disease susceptibility are total antibody isotypes and subclasses, and antibodies against chronic viruses such as cytomegalovirus (CMV) and Epstein–Barr virus (EBV), known to affect the immune system (3, 4, 29). EBV infects B cells and alters the development of regulatory NKT subsets (30). CMV activates many arms of the immune system, and together with its modulatory strategies results in a major impact on immune system homeostasis (31). Other viral exposures may shape as well the immune system and influence responses to further challenges; particularly, pre-existing immunity to common cold human coronaviruses (HCoV) may affect the risk of SARS-CoV-2 infection and COVID-19 susceptibility through antibody crossreactivity (12, 13). It has been reported that pre-existing humoral and cellular immunity to HCoV impacts the outcomes of SARS-CoV-2 infection and COVID-19 disease (32–34).

Assessing the variation over time of cytokine and immunoglobulin concentrations, and the influence on such levels of sociodemographic and lifestyle factors in a general Western population can provide novel information with potential uses in clinical practice and research on biomarkers of disease susceptibility. The present study stands out for its unique value of having paired pre-pandemic and pandemic immune profiles from the same individuals. Thus, the longitudinal approach advances current understanding compared to previous cross-sectional or disease-specific studies.

The objectives of the present study were to investigate (a) prepandemic concentrations of cytokines, total immunoglobulins and immunoglobulins to viral exposures in a general, non-institutionalized population, and their associated sociodemographic variables; (b) the intraindividual change in such concentrations between a prepandemic period (2016–17) and the initial pandemic period (2020–21); and (c) whether such change was similar or different in participants who in 2020–21 were SARS-CoV-2 seronegative and seropositive, and between participants who did and did not develop COVID-19.

2 Methods

2.1 Study population

The present prospective cohort study was based on the Barcelona Health Survey (BHS) of 2016, whose methods have been described in detail (35–37). The BHS generated a sample representative of the general, adult, non-institutionalized population of the city of Barcelona (Spain).

Abbreviations: BHS, Barcelona Health Survey; BMI, body mass index; CI, confidence interval; CMV, cytomegalovirus; COVID-19, coronavirus disease 2019; EBV, Epstein–Barr virus; EGF, epidermal growth factor; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HCoV, human common cold coronavirus; HGF, hepatocyte growth factor; IFN, interferon; Igs, immunoglobulins; IL, interleukin; IP-10, IFN-γ induced protein; LOQ, limit of quantification; MCP-1, monocyte chemoattractant protein; MFI, median fluorescent intensity; MIG, monokine induced by IFN-γ; MIP, macrophage inflammatory protein; OR, odds ratio; RANTES, regulated on activation normal T cell expressed and secreted; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Through face-to-face interviews, the survey collected information about sociodemographic factors, chronic disorders, life styles, uses of healthcare services and preventive practices. At the end of the 2016 BHS interview, participants were offered to take part in a health examination, and 240 individuals accepted. Subsequently, between 15 July 2016 and 4 May 2017, a nurse interviewed again face-to-face such individuals, measured body parameters, and collected blood and urine samples (35, 37). Participants had been asked to fast for at least 8 h before blood extraction. Blood was collected in a vacuum system tube and centrifuged for 15 min × 3,000 rpm at 4°C to obtain serum, which was divided in 1–3 mL aliquots and stored at −80°C (35, 37). The prepandemic levels of the cytokines and immunoglobulins assessed in the present report were analyzed in such serum samples (see sections 2.3., 2.4., and 2.5. below).

After scientific, financial and logistic preparations, on October 2020, in a severe phase of the pandemic, the 240 participants began to be invited to a follow-up visit, which 174 (72.5%) attended between 18 November 2020 and 7 June 2021 (35). Thus, for the present analyses our study spans from 2016 to 17, when the baseline interviews and collection of biological samples first took place, to 2020–21, when the follow-up visit and collection of biological samples took place again. During the follow-up visit a nurse measured their weight, height. She also collected a nasopharyngeal swap, and new blood and urine samples, which constitute a crucial scientific resource of the present cohort study to analyze immunological components of the SARS-CoV-2 infection. The median time between the extraction of biological samples in 2016–17 and 2020–21 was 4.1 years. Compared to the 66 subjects who did not attend the follow-up visit, the 174 participants were more commonly women, younger, born in Catalonia, with a lower body mass index (BMI), more affluent, and with better self-perceived health (35). While some analyses reported in the present paper are based on the 174 individuals, analyses of the intraindividual change (from 2016–17 to 2020–21) of cytokines and immunoglobulins are based on the 154 participants who had not received any COVID-19 vaccine at the time of the follow-up visit (35). Characteristics of the 154 participants have been published in Table 1 of Porta et al. (35).

The Ethics Committee of the Parc de Salut Mar reviewed and approved the study protocols, and all participants signed an informed consent before sample collection and completing questionnaires (37). All methods were performed in accordance with the relevant guidelines and regulations.

2.2 Socioeconomic and living conditions

Shortly before the follow-up visit in 2020–21, the participants completed an online survey concerning signs and symptoms of COVID-19, diagnostic tests performed and their results, use of healthcare services, and vaccination, all during the previous months of the pandemic. This information was ascertained as well with the data bases of the System of Diseases of Mandatory Reporting of the Agency of Public Health of Barcelona, and of the Public Data Analysis for Health Research and Innovation Program of Catalonia (PADRIS) of the Healthcare Quality and Evaluation Agency of Catalonia (AQUAS) (38). The PADRIS databases contain detailed records on demographics, laboratory results, medications dispensed by pharmacies, Primary Care physician visits, procedures, and medical admissions from public hospitals across Catalonia; therefore, PADRIS allows the retrieval of diagnoses of all diseases and health disorders and conditions recorded in primary care and public hospitals, including chronic diseases such as hypertension, dyslipidemia, osteoarthritis, among others (see section 2.7). This data was used to complement information collected during the study. During follow-up the study also collected information on participants' lifestyle and living conditions during the pandemic (35). During the visit, the nurse clarified answers to the online survey and asked further questions on vaccination, weight changes, and pregnancies. A household outdoor index was computed taking into account the number of individuals living in the same household, the availability and use of an outdoor space; the score of the index increased as the number of individuals increased and the availability and frequency of use of the outdoor space decreased. Other factors included in the online survey were: work conditions, use of public and private transport, and individual measures taken to avoid infection (35).

2.3 Quantification of cytokines, chemokines and growth factors

The Cytokine Human Magnetic 30-Plex Panel from Invitrogen™ was used to measure concentrations (pg/mL) of the following 30 cytokines, chemokines and growth factors in the prepandemic (2016–17) and pandemic (2020–21) serum samples: (39, 40) epidermal growth factor (EGF), fibroblast growth factor (FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF), interferon (IFN)-α, IFN-γ, interleukin (IL)-1RA, IL-1β, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p40/p70), IL-13, IL-15, IL-17, IFN-γ induced protein (IP-10), monocyte chemoattractant protein (MCP-1), monokine induced by IFN-γ (MIG), macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated on activation normal T cell expressed and secreted (RANTES) and eotaxin. Individually paired prepandemic and pandemic samples were tested in the same assay plate. Each plate included 16 serial dilutions (2-fold) of a standard curve, and two blank controls. Samples were acquired on a Luminex 100/200 instrument and analyzed in xPONENT software 3.1. The concentration of each analyte was obtained by interpolating the median fluorescent intensity (MFI) to a 5-parameter logistic regression curve and reported as pg./mL using the drLumi R package. Methods to measure cytokines, chemokines and growth factors concentrations were based on a previous study in which the performance of several commercial kits was compared; we chose the method with the most accurate results. In addition, the technique was improved by making some changes to the experimental design of the controls and the standard curve (41). Limits of quantification (LOQ) were estimated based on cutoff values of the 30% coefficient of variation (CV) of the standard curve for each analyte. When the value of an analyte was below the lower LOQ, the mid-value of this limit for the corresponding plate was assigned; and when a sample value was above the corresponding upper LOQ, the assigned value was twice this LOQ. Lower LOQ ranged from 0.07 pg./mL for VEGF to 16.2 pg./mL for G-CSF; upper LOQ ranged from 981 pg./mL for IP-10 to 77,553 pg./mL for IL-1RA (Supplementary Table 1). For most cytokines, the percentage of quantification (i.e., the percentage of participants with concentrations between the lower and the upper LOQ) was > 70%, while for 9 cytokines the percentage of quantification ranged from 14% for IFN-γ to 68% for MIP-1α (Supplementary Table 1; Figure 1).

The intraindividual changes in the concentrations reported in this paper were not due to changes in the percentages of quantification of

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TABLE 1 Concentrations of 30 cytokines in 2016–17 and 2020–21.

Cytokine	Concentrations (pg/mL)		Change of concentrations 2016–2020	
	2016–2017	2020–2021	Δ absolute (pg/mL)	Δ relative[a] (%)
Growth factors				
G-CSF (median)	32.39	36.50	0.00	0.00
(P25, P75)	(<LOQ, 111.7)	(12.70, 129.4)	(−8.50, 41.88)	(−3.92, 23.80)
Geometric mean	31.48	39.37		
Δ ≥ 15%				33.1
∇ ≥ 15%				16.2
EGF (median)	85.28	119.4[b]	28.36	7.15
(P25, P75)	(43.16, 142.4)	(78.74, 173.5)	(−14.75, 74.80)	(−3.06, 20.73)
Geometric mean	82.17	119.4		
Δ ≥ 15%				32.5
∇ ≥ 15%				7.1
FGF (median)	12.90	14.79	0.00	0.00
(P25, P75)	(4.85, 33.57)	(6.26, 31.31)	(−7.00, 7.52)	(−18.09, 22.68)
Geometric mean	14.40	13.81		
Δ ≥ 15%				29.9
∇ ≥ 15%				32.5
GM-CSF (median)	6.98	9.81	0.00	0.00
(P25, P75)	(1.09, 38.19)	(1.09, 38.35)	(−5.32, 10.30)	(−14.44, 32.47)
Geometric mean	6.78	8.41		
Δ ≥ 15%				31.2
∇ ≥ 15%				24.0
HGF (median)	367.4	490.3[b]	103.7	5.42
(P25, P75)	(262.6, 550.1)	(333.3, 734.5)	(15.15, 291.4)	(0.88, 10.21)
Geometric mean	377.7	489.5		
Δ ≥ 15%				9.1
∇ ≥ 15%				3.2
VEGF (median)	5.46	5.97	0.70	6.75
(P25, P75)	(2.57, 8.73)	(3.41, 10.20)	(−0.40, 2.22)	(−8.18, 36.68)
Geometric mean	4.54	5.50		
Δ ≥ 15%				42.9
∇ ≥ 15%				17.5
Chemokines				
IL-8 (median)	16.42	21.22[b]	3.39	8.44
(P25, P75)	(11.58, 24.10)	(14.31, 28.52)	(0.00, 9.46)	(0.00, 16.32)
Geometric mean	16.03	21.14		
Δ ≥ 15%				28.6
∇ ≥ 15%				1.3
IP-10 (median)	6.51	1.06[b]	−5.01	−96.40
(P25, P75)	(4.17, 9.71)	(0.46, 2.45)	(−7.77, −2.73)	(−153.2, −60.50)
Geometric mean	6.46	1.01		
Δ ≥ 15%				4.5
∇ ≥ 15%				90.9
RANTES (median)	3,313	3,264	−1.26	0.00
(P25, P75)	(2,618, 5,970)	(2,521, 4,643)	(−443.0, 132.0)	(−1.88, 0.63)

(Continued)

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TABLE 1 (Continued)

Cytokine	Concentrations (pg/mL)		Change of concentrations 2016–2020	
	2016–2017	2020–2021	Δ absolute (pg/mL)	Δ relative[a] (%)
Geometric mean	3,626	3,400		
Δ ≥ 15%				0.0
∇ ≥ 15%				0.0
EOTAXIN (median)	74.52	75.04	−1.59	−0.51
(P25, P75)	(58.23, 100.8)	(52.46, 101.3)	(−17.50, 13.57)	(−4.89, 4.74)
Geometric mean	74.76	73.73		
Δ ≥ 15%				4.5
∇ ≥ 15%				3.9
MIP-1α (median)	36.29	50.81	0.00	0.00
(P25, P75)	(<LOQ, 109.0)	(<LOQ, 121.7)	(−18.36, 45.41)	(−8.20, 21.71)
Geometric mean	34.34	40.33		
Δ ≥ 15%				27.9
∇ ≥ 15%				19.5
MIP-1β (median)	166.1	205.0	13.08	2.23
(P25, P75)	(90.91, 348.0)	(116.7, 466.3)	(−42.55, 93.55)	(−3.60, 11.15)
Geometric mean	212.5	238.1		
Δ ≥ 15%				15.6
∇ ≥ 15%				6.5
MCP-1 (median)	639.4	654.5	43.13	0.99
(P25, P75)	(466.1, 870.7)	(526.9, 874.8)	(−93.31, 159.1)	(−2.42, 4.01)
Geometric mean	678.9	699.8		
Δ ≥ 15%				0.6
∇ ≥ 15%				1.3
MIG (median)	<LOQ	<LOQ	0.00	0.00
(P25, P75)	(<LOQ, 20.63)	(<LOQ, 20.63)	(0.00, 0.00)	(0.00, 0.00)
Geometric mean	<LOQ	<LOQ		
Δ ≥ 15%				15.6
∇ ≥ 15%				17.5
TH1				
IL-2 (median)	9.45	19.94	1.16	7.12
(P25, P75)	(2.48, 51.20)	(3.96, 61.05)	(−3.90, 23.73)	(−16.77, 54.71)
Geometric mean	13.65	18.04		
Δ ≥ 15%				46.1
∇ ≥ 15%				26.6
IL-12 (median)	56.31	58.08	1.99	0.95
(P25, P75)	(41.20, 87.28)	(37.04, 87.84)	(−13.44, 13.04)	(−5.63, 5.75)
Geometric mean	58.80	56.87		
Δ ≥ 15%				6.5
∇ ≥ 15%				7.8
IFN-γ (median)	<LOQ	<LOQ	0.00	0.00
(P25, P75)	(<LOQ, 0.22)	(<LOQ, <LOQ)	(0.00, 0.00)	(0.00, 0.00)
Geometric mean	0.22	0.22		
Δ ≥ 15%				9.7

(Continued)

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TABLE 1 (Continued)

Cytokine	Concentrations (pg/mL)		Change of concentrations 2016–2020	
	2016–2017	2020–2021	Δ absolute (pg/mL)	Δ relative[a] (%)
∇ ≥ 15%				8.4
TH2				
IL-4 (median)	1.54	<LOQ	0.00	0.00
(P25, P75)	(1.47, 4.87)	(<LOQ, 7.14)	(0.00, 0.00)	(0.00, 0.00)
Geometric mean	3.35	3.60		
Δ ≥ 15%				17.5
∇ ≥ 15%				15.6
IL-5 (median)	<LOQ	<LOQ	0.00	0.00
(P25, P75)	(<LOQ, 1.11)	(<LOQ, 1.11)	(0.00, 0.41)	(0.00, 26.29)
Geometric mean	0.83	0.88		
Δ ≥ 15%				26.6
∇ ≥ 15%				21.4
IL-13 (median)	6.70	6.70	0.00	0.00
(P25, P75)	(<LOQ, 16.22)	(<LOQ, 20.66)	(−4.02, 4.81)	(−13.56, 25.51)
Geometric mean	6.10	6.47		
Δ ≥ 15%				28.6
∇ ≥ 15%				24.0
Pro-inflammatory				
IL-1β (median)	0.92	1.09	0.00	0.00
(P25, P75)	(<LOQ, 2.26)	(0.55, 1.83)	(−0.32, 0.40)	(−18.72, 40.19)
Geometric mean	1.05	1.10		
Δ ≥ 15%				34.4
∇ ≥ 15%				26.6
TNF-α (median)	3.12	4.97	0.93	13.77
(P25, P75)	(1.34, 14.20)	(1.34, 25.75)	(−0.19, 8.59)	(−4.00, 86.00)
Geometric mean	3.97	5.62		
Δ ≥ 15%				48.7
∇ ≥ 15%				20.1
IL-6 (median)	14.10	23.76	1.53	4.53
(P25, P75)	(4.52, 54.29)	(8.28, 67.14)	(−6.86, 14.99)	(−6.95, 35.91)
Geometric mean	16.27	22.64		
Δ ≥ 15%				39.0
∇ ≥ 15%				16.9
IFN-α (median)	17.89	22.05	1.21	0.10
(P25, P75)	(5.41, 52.65)	(7.94, 52.64)	(−7.20, 12.70)	(−8.66, 20.91)
Geometric mean	18.29	21.38		
Δ ≥ 15%				33.8
∇ ≥ 15%				15.6
IL-2R (median)	138.1	149.5	7.99	1.12
(P25, P75)	(50.46, 399.8)	(61.52, 447.7)	(−63.43, 85.15)	(−9.86, 13.41)
Geometric mean	158.6	162.7		
Δ ≥ 15%				22.7
∇ ≥ 15%				16.2

(Continued)

TABLE 1 (Continued)

Cytokine	Concentrations (pg/mL)		Change of concentrations 2016–2020	
	2016–2017	2020–2021	Δ absolute (pg/mL)	Δ relative[a] (%)
IL-17 (median)	<LOQ	<LOQ	0.00	0.00
(P25, P75)	(<LOQ, 1.56)	(<LOQ, 1.65)	(0.00, 0.92)	(0.00, 30.14)
Geometric mean	<LOQ	<LOQ		
Δ ≥ 15%				26.6
∇ ≥ 15%				18.2
Regulatory				
IL-7 (median)	15.40	17.50	0.00	0.00
(P25, P75)	(<LOQ, 32.51)	(<LOQ, 39.21)	(−5.98, 10.40)	(−6.44, 14.57)
Geometric mean	12.00	13.78		
Δ ≥ 15%				24.7
∇ ≥ 15%				18.2
Anti-inflammatory				
IL-10 (median)	3.02	3.28	0.00	0.00
(P25, P75)	(<LOQ, 19.45)	(<LOQ, 24.10)	(−2.62, 4.44)	(−15.30, 32.51)
Geometric mean	3.79	4.15		
Δ ≥ 15%				29.9
∇ ≥ 15%				25.3
IL-15 (median)	<LOQ	<LOQ	0.00	0.00
(P25, P75)	(<LOQ, 45.30)	(<LOQ, 54.65)	(0.00, 2.34)	(0.00, 0.70)
Geometric mean	9.47	11.50		
Δ ≥ 15%				19.5
∇ ≥ 15%				14.9
IL-1RA (median)	263.9	238.1	−29.94	−2.83
(P25, P75)	(154.3, 515.4)	(118.5, 521.9)	(−121.4, 77.22)	(−9.60, 4.84)
Geometric mean	290.3	255.2		
Δ ≥ 15%				7.8
∇ ≥ 15%				11.7

Absolute intraindividual change and relative intraindividual change ($N = 154$). *Δ absolute:* Difference between the individual concentrations in 2020–21 with respect to the individual concentrations in 2016–17, in absolute terms (pg/mL). Δ relative: Difference between the individual concentrations in 2020–21 with respect to the individual concentrations in 2016–17, in relative terms (percentage of change). P25: percentile 25th. P75: percentile 75th. <LOQ: value lower than the minimum concentration of the corresponding range of the limits of quantification. See Supplementary Table 1. Δ ≥ 15%: percentage of individuals with a relative increase in the concentrations between 2016 and 2020 equal to or greater than 15%. Includes individuals with concentrations unquantified in 2016 and quantified in 2020 (as explained in Methods, the concentration in 2016 was half the lower limit of quantification). ∇ ≥ 15%: percentage of individuals with a relative decrease in the concentrations between 2016 and 2020 equal to or greater than 15%. Includes individuals with concentrations quantified in 2016 and unquantified in 2020 (as explained in Methods, the concentration in 2020 was half the lower limit of quantification).

[a]Units used for computing the relative intraindividual change (Δ relative) were base 10 logtransformed pg/mL.

[b]*p* value <0.05, Mann–Whitney's U test (two-tail).

each cytokine or due to changes in the limits of quantification: the two samples of each individual (2016–17 and 2020–21) were analyzed in the same laboratory plate (thus, at the same time) with the same lower and upper limit of quantification (Supplementary Table 1; Figure 1).

2.4 Serology of viral exposures

The levels of IgM, IgA and IgG against the Nucleocapsid protein of the 4 human common cold coronaviruses (HCoV-229E, OC43, NL63, HKU1), two Epstein–Barr virus (EBV) antigens (EA-D, VCA p18), and two Cytomegalovirus (CMV) antigens (pp65, pp150), were assessed by high-throughput multiplex quantitative suspension array technology (qSAT) in a FlexMap3D instrument, as previously described (12, 13), and data QA/QC and preprocessing were performed with R. Briefly, antigen-coupled beads were added to a 384-well µClear® flat bottom plate in multiplex. A hyper-immune plasma pool at 3-fold 10 serial dilutions starting from 1:250 was used as positive control in each assay plate for QA/QC and calibration purposes. Final dilution of test samples was 1:500. To quantify IgA and IgM, samples and controls were pre-treated with anti-human IgG (Gullsorb) at 1:10 dilution, to avoid IgG interferences. Median fluorescence intensity (MFI) was reported for each isotype-antigen.

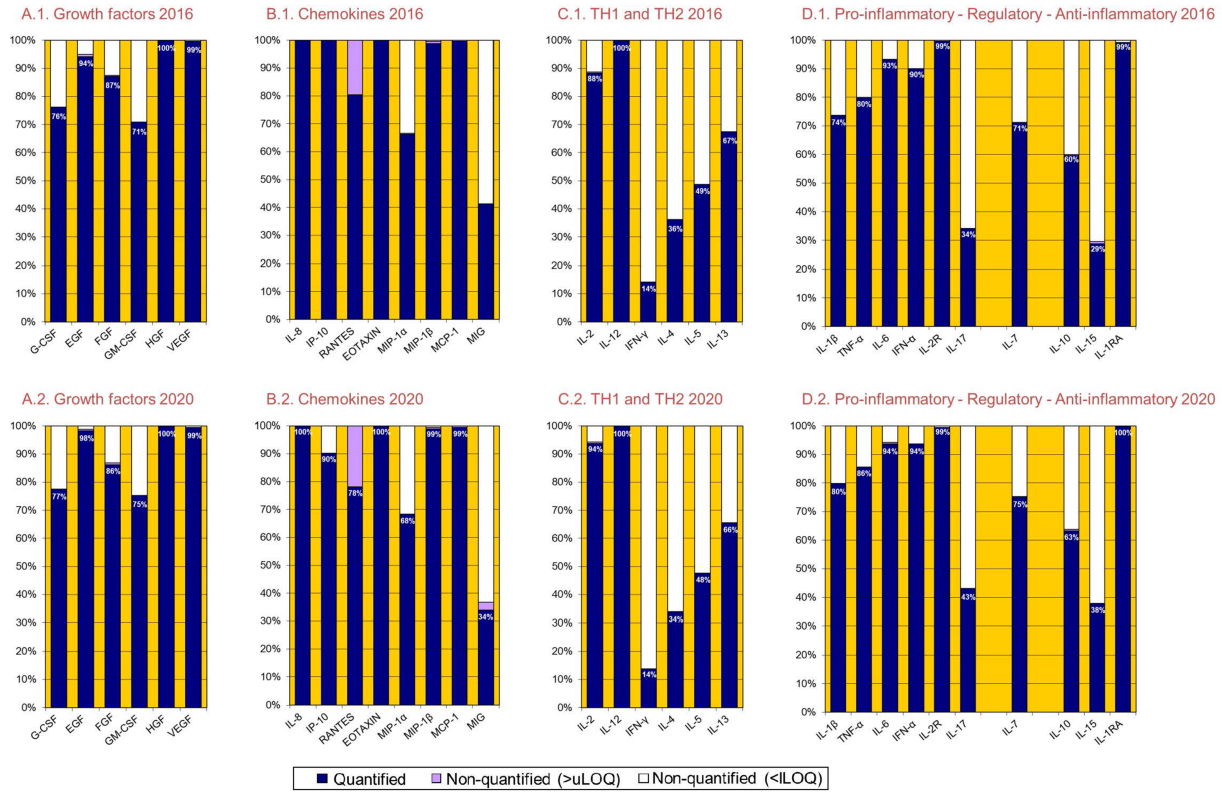

FIGURE 1
Percentage of quantification of 30 cytokines in 2016–17 and 2020–21.

2.5 Quantification of total immunoglobulins

The quantification of total immunoglobulins (IgE, IgA, IgM, IgG1, IgG2, IgG3, IgG4) was performed with the Antibody Isotyping 7-Plex Human ProcartaPlex™ panel (Thermo Fisher Scientific, Vienna, Austria) following the manufacturer's instructions. Samples were tested at 1/200000 and 1/500000 dilutions, acquired on a Luminex 100/200 instrument and analyzed in xPONENT software 3.1. The concentration of each isotype was obtained by interpolating the median fluorescent intensity (MFI) to a 5-parameter logistic regression curve and reported as µg/mL.

In the 240 prepandemic samples, no significant correlations among cytokines and IgA and IgG isotypes against HCoV, CMV and EBV were observed. By contrast, 12 cytokines showed positive, statistically significant correlations with all 8 IgMs against HCoV, CMV and EBV, whereas two chemokines showed inverse correlations (all $\rho \leq 0.35$; Supplementary Figure 1; Supplementary Table 2).

Some significant correlations were observed among pairs of cytokines; the highest correlation coefficients were observed between IL-2 and IFN-α, IL-2 and MIP-1β, and IFN-α and MIP-1β (all three $\rho > 0.84$ and $p < 0.001$; Supplementary Figure 2). A correlation coefficient of 0.73 between G-CSF and TNF-α was also observed ($p < 0.001$).

While most correlation coefficients among the 24 isotype-antigen combinations and the 8 total immunoglobulins were <0.43, some high coefficients were observed between total IgM and the IgMs against CMV, EBV and HCoV; e.g., among total IgM and IgM EBV EAD, IgM N HKU1, and IgM CMV pp65 (all three $\rho > 0.66$ and $p < 0.001$; Supplementary Figure 3).

2.6 Determination of SARS-CoV-2 infection and COVID-19 disease

2.6.1 SARS-CoV-2 infection

SARS-CoV-2 infection was determined at the Center for Genomic Regulation (CRG) in all 174 members of the cohort who attended the follow-up visit in 2020–21 by real time reverse-transcriptase polymerase chain reaction (rRT-PCR) in nasopharyngeal swabs. Briefly, samples were collected in 600 µL of lysis solution (DNA/RNA Shield, Zymo) to inactivate the virus, break membranes and stabilize the RNA. Samples were processed in a TECAN Dreamprep robot to isolate the RNA using the Quick-DNA/RNA Viral MagBead kit (Zymo; #R2140), and the purified RNA was analyzed by rRT-PCR in an ABI 7900 HT (384 wells) following the CDC standard procedure. Positive and negative controls were included in each assay plate. Among the 174 participants, there were 4 rRT-PCR-positives (35).

To detect previous SARS-CoV-2 infections, antibody serological status of each participant was assessed in serum samples analyzed at the ISGlobal Immunology Laboratory in Barcelona. The MFI levels of IgG, IgM and IgA were assessed by high-throughput multiplex quantitative suspension array technology, including 5 SARS-CoV-2

antigens (35), as described in section 2.4 for the other viral exposures (15, 42).

Of the 154 participants mentioned above, 41 were SARS-CoV-2 seropositive (26.6%) at the time of the follow-up visit in 2020–21 (including all 4 positives by the follow-up rRT-PCR), 9 indeterminate (5.8%), and 104 seronegative (67.5%). There were no major differences in the main characteristics of seropositive and seronegative participants (Supplementary Table 5 of 35).

2.6.2 COVID-19 disease

Cases of COVID-19 disease have been described in detail (35). In total there were 20 cases of COVID-19 disease at the time of the follow-up visit in 2020–21. All were seropositive for SARS-CoV-2 in our immunological assay, and all reported COVID-19 related symptoms. Specifically, 10 cases provided information of a positive diagnostic test for SARS-CoV-2 infection (including all 4 positives at the follow-up rRT-PCR), and 2 or more COVID-19 related signs or symptoms; 2 were diagnosed of COVID-19 by a physician; and 8 had COVID-19 related signs or symptoms (35, 43). There were no major differences in the main characteristics of participants with and without COVID-19 (Table 1 of 35).

2.7 Comorbidities

Detailed information on comorbidities was obtained from PADRIS (section 2.2) and the BHS (section 2.1). Specifically, comorbidities were identified based on diagnostic codes from PADRIS, using the International Classification of Diseases, 10th Revision (ICD-10). All available diagnoses recorded prior to the COVID-19 pandemic were reviewed and classified into major disease categories such as cardiovascular, respiratory, and musculoskeletal disorders, among others. This strategy aligns with previous research that has employed PADRIS data to assess chronic disease prevalence and multimorbidity patterns in the Catalan population (44–46).

No associations among prepandemic comorbidities, and cytokines or immunoglobulins, were observed: most correlation coefficients among comorbidities and the 30 cytokines, the 24 isotype-antigen combinations and the 8 total immunoglobulins were <0.25, and not statistically significant (Supplementary Figures 4a,b). Only some modest negative coefficients were observed between IgMs and BMI, and between IgMs, some total IgGs and dyslipidemia (Supplementary Figure 4b): the highest correlations were for IgMs against CMV pp65 and EBV VCA p18 with BMI ($\rho = -0.324$ and -0.280, respectively, $p < 0.001$), and for total IgG4 and total IgM with dyslipidemia ($\rho = -0.269$ and -0.256, respectively, $p = 0.001$).

2.8 Statistical analyses

Univariate statistics were computed as customary (47). Spearman's rank correlation coefficient (ρ) was used to evaluate correlations between pairs of cytokines, total immunoglobulins and isotype-antigen combinations. Scatterplots of concentrations of cytokines and immunoglobulins in 2020–21 against concentrations in 2016–17 were used to compare individual concentrations between both periods. The paired correlation test was used to compare paired continuous data.

To analyze the intraindividual change of concentrations of cytokines and immunoglobulins between the baseline/prepandemic period (2016–17) and the pandemic period (2020–21), absolute intraindividual change and relative intraindividual change were obtained by computing the difference between the individual concentrations in 2020–21 with respect to the individual concentrations in 2016–17, in absolute terms (pg/mL, μg/mL or MFIs) and in relative terms (percentage of change of concentrations base 10 log-transformed), respectively. The percentage of individuals with a relative increase and with a relative decrease equal to or greater than 15% was also computed using the relative changes of concentrations (base 10 log-transformed) of each cytokine and immunoglobulin.

We computed the number of cytokines in each person with a relative intraindividual change ≥15% as follows: for each subject we added the number of cytokines whose relative change (increase or decrease) in levels from 2016–17 to 2020–201 was equal to or greater than 15%. Similarly, we calculated the number of immunoglobulins in each person with a relative intraindividual change ≥15%.

The relative intraindividual change of cytokine and immunoglobulin concentrations from 2016–17 to 2020–21 was compared between participants who were SARS-CoV-2 seronegative and seropositive, between participants who did and did not develop COVID-19 disease, by sociodemographic variables (sex, age, BMI, tobacco smoking, and educational level), and by comorbidities. Thus, to avoid biases, analyses considered the whole population of 154 persons who were at risk for infection, rather than only the seropositives at risk for COVID-19 (48). Mann–Whitney's *U* test was used to assess differences in concentrations and differences in relative change.

Cytokine levels measured in samples collected in 2020–21 might be altered by COVID-19 in participants who had developed the disease when their sample was collected. Therefore, for participants who developed COVID-19, we assessed whether the individual concentration and the intraindividual change was related to the interval of time elapsed between onset of COVID-19 and the blood draw in which levels of cytokines (and immunoglobulins) were analyzed. The median of such time interval was 8.4 months (range: 0.6 to 13 months).

All tests were two-tailed. Statistical analyses were conducted using R, version 4.3.3 (Boston, MA, 2024), and SPSS version 22.0.0.0 (IBM SPSS Statistics, Armonk, NY, 2013).

3 Results

3.1 Intraindividual change in concentrations of cytokines

The median value of the relative intraindividual change in cytokine levels between 2016 and 2020 was <15% for 29 of the 30 cytokines; specifically, between −2.83 and 13.77% (Table 1). However, a value of zero or near zero in such median was compatible, in some cytokines, with a substantial number of participants having an intraindividual increase or decrease ≥15% in the same cytokine. For instance, the mentioned median was 0 for G-CSF while 33% of individuals had an increase in G-CSF ≥ 15 and 16% a decrease in G-CSF ≥ 15%.

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TABLE 2 Relative intraindividual change (%) of cytokine concentrations from 2016–17 to 2020–21 in participants SARS-CoV-2 seronegative and seropositive, and in participants without COVID-19 disease and with COVID-19 disease.

Cytokine[a]	SARS-CoV-2 status		P[b]	COVID-19 disease		P[b]
	Seronegative (*N* = 104)	Seropositive (*N* = 41)		No COVID (*N* = 134)	COVID (*N* = 20)	
Growth factors						
G-CSF (median)	0.00	0.86	0.439	0.00	0.00	0.489
(P25, P75)	(−5.25, 23.32)	(0.00, 27.34)		(−5.30, 23.30)	(0.00, 34.99)	
Δ ≥ 15%	32.7	36.6		32.8	35.0	
∇ ≥ 15%	16.3	12.2		17.2	10.0	
EGF (median)	7.89	3.01	0.122	7.54	0.64	0.255
(P25, P75)	(−2.56, 20.90)	(−4.63, 13.78)		(−2.64, 20.73)	(−12.04, 24.00)	
Δ ≥ 15%	35.6	22.0		32.8	30.0	
∇ ≥ 15%	4.8	14.6		5.2	20.0	
FGF (median)	0.00	0.00	0.479	0.00	−2.88	0.186
(P25, P75)	(−17.21, 24.71)	(−23.36, 22.33)		(−17.79, 24.33)	(−24.90, 2.32)	
Δ ≥ 15%	30.8	29.3		31.3	20.0	
∇ ≥ 15%	27.9	31.7		29.9	35.0	
GM-CSF (median)	0.00	0.00	0.222	0.00	0.00	0.292
(P25, P75)	(−10.69, 45.56)	(−17.73, 15.64)		(−11.55, 35.13)	(−23.97, 15.38)	
Δ ≥ 15%	34.6	24.4		32.1	25.0	
∇ ≥ 15%	23.1	26.8		23.1	30.0	
HGF (median)	5.95	4.29	0.329	5.65	3.97	0.319
(P25, P75)	(1.83, 10.20)	(−1.86, 9.49)		(1.35, 10.23)	(−3.02, 8.08)	
Δ ≥ 15%	5.8	12.2		9.7	5.0	
∇ ≥ 15%	3.8	2.4		3.0	5.0	
VEGF (median)	8.71	2.30	0.246	7.73	2.05	0.191
(P25, P75)	(−6.51, 39.93)	(−8.66, 23.11)		(−7.97, 39.75)	(−17.63, 21.68)	
Δ ≥ 15%	45.2	41.5		44.8	30.0	
∇ ≥ 15%	13.5	22.0		16.4	25.0	
Chemokines						
IL-8 (median)	5.44	12.04	0.012	7.25	9.67	0.248
(P25, P75)	(−1.13, 16.46)	(4.26, 23.96)		(0.00, 16.32)	(2.49, 21.61)	
Δ ≥ 15%	28.8	34.1		28.4	30.0	
∇ ≥ 15%	1.9	0.0		1.5	0.0	
IP-10 (median)	−96.08	−108.8	0.582	−94.22	−113.7	0.503
(P25, P75)	(−157.6, −68.37)	(−149.7, −42.46)		(−153.2, −60.50)	(−177.5, −60.16)	
Δ ≥ 15%	2.9	9.8		3.7	10.0	
∇ ≥ 15%	93.3	82.9		91.0	90.0	
RANTES (median)	0.00	−0.02	0.829	0.00	−0.01	0.722
(P25, P75)	(−1.72, 0.66)	(−2.70, 0.61)		(−1.89, 0.63)	(−1.89, 1.64)	
Δ ≥ 15%	0.0	0.0		0.0	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
EOTAXIN (median)	−0.29	−1.60	0.779	−0.16	−1.99	0.378
(P25, P75)	(−4.89, 5.14)	(−4.78, 3.43)		(−4.93, 5.28)	(−4.37, 2.44)	
Δ ≥ 15%	2.9	9.8		5.2	0.0	
∇ ≥ 15%	5.8	0.0		4.5	0.0	

(Continued)

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TABLE 2 (Continued)

Cytokine[a]	SARS-CoV-2 status		P[b]	COVID-19 disease		P[b]
	Seronegative (*N* = 104)	Seropositive (*N* = 41)		No COVID (*N* = 134)	COVID (*N* = 20)	
MIP-1α (median)	0.00	0.00	0.571	0.00	0.00	0.199
(P25, P75)	(−6.97, 23.91)	(−9.41, 16.42)		(−4.03, 23.82)	(−20.69, 7.85)	
Δ ≥ 15%	27.9	26.8		29.1	20.0	
∇ ≥ 15%	19.2	19.5		18.7	25.0	
MIP-1β (median)	3.68	0.98	0.683	2.90	0.49	0.425
(P25, P75)	(−4.11, 11.19)	(−3.07, 10.17)		(−3.12, 11.37)	(−5.85, 6.59)	
Δ ≥ 15%	14.4	19.5		15.7	15.0	
∇ ≥ 15%	6.7	4.9		6.7	5.0	
MCP-1 (median)	0.55	2.32	0.424	1.14	−0.17	0.320
(P25, P75)	(−2.74, 3.60)	(−2.66, 4.14)		(−1.86, 4.08)	(−4.59, 3.74)	
Δ ≥ 15%	1.0	0.0		0.7	0.0	
∇ ≥ 15%	1.0	2.4		0.7	5.0	
MIG (median)	0.00	0.00	0.724	0.00	0.00	0.344
(P25, P75)	(0.00, 0.00)	(−11.90, 0.00)		(0.00, 0.00)	(−36.97, 0.00)	
Δ ≥ 15%	17.3	12.2		15.7	15.0	
∇ ≥ 15%	16.3	19.5		16.4	25.0	
TH1						
IL-2 (median)	6.24	7.58	0.887	7.12	4.83	0.604
(P25, P75)	(−16.89, 57.47)	(−13.94, 39.53)		(−16.28, 58.64)	(−32.22, 42.01)	
Δ ≥ 15%	46.2	46.3		46.3	45.0	
∇ ≥ 15%	26.9	24.4		25.4	35.0	
IL-12 (median)	1.37	0.00	0.968	0.60	1.52	0.656
(P25, P75)	(−5.43, 5.64)	(−6.13, 7.28)		(−5.71, 5.51)	(−5.37, 7.36)	
Δ ≥ 15%	5.8	7.3		6.7	5.0	
∇ ≥ 15%	9.6	4.9		8.2	5.0	
IFN-γ (median)	0.00	0.00	0.260	0.00	0.00	0.347
(P25, P75)	(0.00, 0.00)	(0.00, 0.00)		(0.00, 0.00)	(0.00, 0.00)	
Δ ≥ 15%	8.7	14.6		10.4	5.0	
∇ ≥ 15%	9.6	7.3		8.2	10.0	
TH2						
IL-4 (median)	0.00	0.00	0.038	0.00	0.00	0.482
(P25, P75)	(0.00, 0.00)	(0.00, 15.24)		(0.00, 0.00)	(0.00, 0.00)	
Δ ≥ 15%	16.3	24.4		17.2	20.0	
∇ ≥ 15%	16.3	9.8		17.2	5.0	
IL-5 (median)	0.00	0.00	0.847	0.00	0.00	0.898
(P25, P75)	(0.00, 13.80)	(−15.42, 45.86)		(0.00, 20.69)	(−18.57, 50.66)	
Δ ≥ 15%	24.0	34.1		26.1	30.0	
∇ ≥ 15%	19.2	24.4		20.9	25.0	
IL-13 (median)	0.00	0.00	0.878	0.00	0.00	0.793
(P25, P75)	(−11.36, 31.84)	(−12.87, 20.51)		(−13.90, 25.51)	(−13.70, 41.03)	
Δ ≥ 15%	29.8	29.3		29.1	25.0	
∇ ≥ 15%	24.0	22.0		24.6	20.0	

(Continued)

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TABLE 2 (Continued)

Cytokine[a]	SARS-CoV-2 status		P[b]	COVID-19 disease		P[b]
	Seronegative (*N* = 104)	Seropositive (*N* = 41)		No COVID (*N* = 134)	COVID (*N* = 20)	
Pro-inflammatory						
IL-1β (median)	0.00	0.00	0.145	0.00	0.00	0.512
(P25, P75)	(−6.67, 42.57)	(−61.24, 0.00)		(−15.98, 40.19)	(−75.74, 62.24)	
Δ ≥ 15%	39.4	22.0		35.8	25.0	
∇ ≥ 15%	23.1	31.7		25.4	35.0	
TNF-α (median)	17.48	12.93	0.975	14.75	0.00	0.698
(P25, P75)	(−6.95, 87.01)	(0.00, 57.90)		(−4.82, 87.12)	(0.00, 57.77)	
Δ ≥ 15%	50.0	48.8		49.3	45.0	
∇ ≥ 15%	21.2	17.1		20.1	20.0	
IL-6 (median)	4.78	1.91	0.806	6.67	0.00	0.182
(P25, P75)	(−7.41, 31.49)	(−2.69, 38.44)		(−6.76, 39.18)	(−13.71, 14.36)	
Δ ≥ 15%	38.5	36.6		41.8	20.0	
∇ ≥ 15%	15.4	17.1		15.7	25.0	
IFN-α (median)	0.10	4.22	0.518	0.10	2.11	0.565
(P25, P75)	(−8.46, 25.19)	(−10.43, 18.87)		(−8.26, 21.80)	(−16.40, 20.70)	
Δ ≥ 15%	36.5	26.8		33.6	35.0	
∇ ≥ 15%	15.4	17.1		14.2	25.0	
IL-2R (median)	1.51	0.00	0.533	1.71	−4.16	0.177
(P25, P75)	(−7.80, 13.30)	(−11.75, 15.05)		(−9.19, 13.79)	(−19.78, 7.83)	
Δ ≥ 15%	22.1	24.4		23.1	20.0	
∇ ≥ 15%	13.5	19.5		14.2	30.0	
IL-17 (median)	0.00	0.00	0.327	0.00	0.00	0.387
(P25, P75)	(0.00, 42.16)	(−0.19, 13.93)		(0.00, 38.42)	(0.00, 0.00)	
Δ ≥ 15%	28.8	24.4		28.4	15.0	
∇ ≥ 15%	17.3	19.5		17.9	20.0	
Regulatory						
IL-7 (median)	0.00	0.00	0.510	0.00	0.00	0.259
(P25, P75)	(−6.29, 13.76)	(−3.95, 27.48)		(−6.06, 14.98)	(−11.82, 12.01)	
Δ ≥ 15%	23.1	29.3		24.6	25.0	
∇ ≥ 15%	18.3	12.2		17.9	20.0	
Anti-inflammatory						
IL-10 (median)	0.00	0.00	0.106	0.00	0.00	0.140
(P25, P75)	(−11.39, 55.37)	(−25.45, 8.32)		(−11.48, 35.17)	(−26.85, 0.00)	
Δ ≥ 15%	32.7	22.0		32.1	15.0	
∇ ≥ 15%	22.1	31.7		23.1	40.0	
IL-15 (median)	0.00	0.00	0.816	0.00	0.00	0.772
(P25, P75)	(0.00, 0.58)	(−1.69, 6.23)		(0.00, 0.57)	(0.00, 18.43)	
Δ ≥ 15%	19.2	22.0		18.7	25.0	
∇ ≥ 15%	13.5	14.6		11.9	20.0	
IL-1RA (median)	−2.47	−3.44	0.458	−2.04	−7.36	0.061
(P25, P75)	(−9.62, 6.01)	(−9.32, 3.10)		(−9.30, 5.93)	(−12.62, −0.33)	
Δ ≥ 15%	7.7	4.9		8.2	5.0	
∇ ≥ 15%	12.5	12.2		10.4	20.0	

[a]Units used for computing the relative intraindividual change were base 10 logtransformed pg/mL.
[b]Mann–Whitney's U test (two-tail).

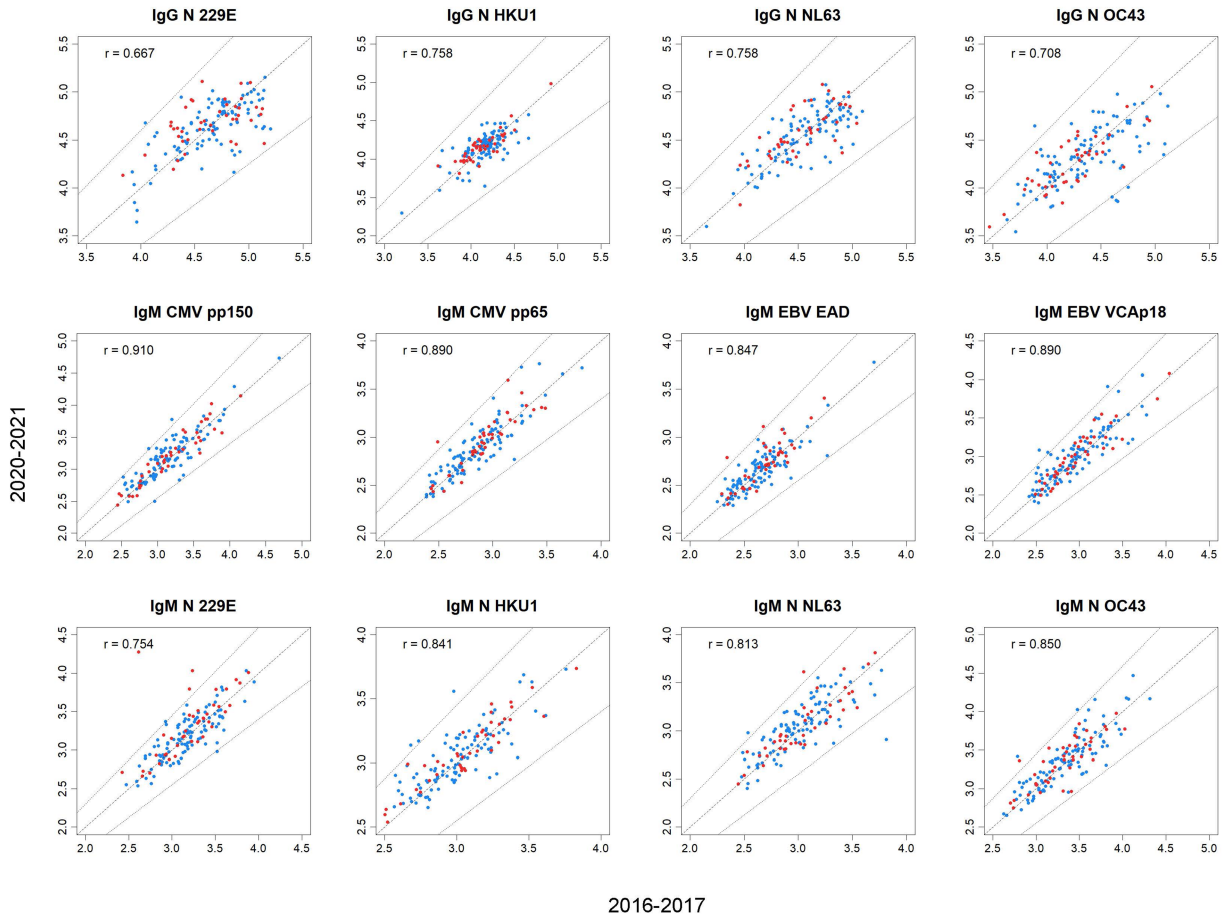

FIGURE 2
Scatterplots of concentrations (log10, pg./mL) of cytokines in 2020–21 against concentrations in 2016–17 by SARS-CoV-2 seropositivity. Blue dots, seronegative; red dots, seropositive. A 15% change is delimited by the two lines at both sides of the diagonal line indicating no change. All *p*-values <0.001.

More specifically, 15 of the 30 cytokines had a null *median* relative intraindividual change. Twelve other cytokines had a modest positive *median* relative intraindividual change, ranging from 0.10 to 13.77%. Finally, 2 other cytokines had a modest negative *median* relative intraindividual change (EOTAXIN and IL-1RA; Table 1). For 11 cytokines, the *percentage of participants* having a relative intraindividual increase ≥15% ranged from 29.9 to 48.7%. For these 11 cytokines, the percentage of participants with an intraindividual decrease ≥15% ranged from 7.1 to 32.5%. For IP-10, the percentage of participants having a relative intraindividual decrease ≥15% was 90.9%. Thus, for the vast majority of cytokines, either (a) the percentage of participants with an increase in concentrations was similar to the percentage of participants with a decrease in concentrations, or (b) the percentage of participants with an increase was slightly larger than the percentage with a decrease (Table 1). Neither of these two predominant patterns was specific of one type of cytokines. Concentrations in 2016–17 are presented as possible reference values.

No major differences in cytokine intraindividual changes between 2016 and 2020 were observed between participants who were SARS-CoV-2 seronegative and seropositive, nor between participants who did and did not develop COVID-19 disease (Table 2; Figure 2). The difference in the median change was statistically significant in only two instances (IL-8 and IL-4); even then, the difference was null or only slightly higher in SARS-CoV-2 seropositives than in seronegatives. The change tended to be similar (in seropositives and seronegatives, and in participants with and without COVID-19 disease) when using the median of the change and the 4 percentages shown in Table 2: increase (Δ) ≥ 15% and decrease (∇) ≥ 15% by outcome. IP-10, the only cytokine that showed a substantial decrease in the overall population (Table 1), showed a highly similar decrease in participants who were SARS-CoV-2 seronegative and seropositive, and in participants who did and did not develop COVID-19.

Among the 20 participants who developed COVID-19, no associations were observed between cytokine concentrations measured in 2020–21 and the time from disease onset to the blood draw in which cytokine concentrations were analyzed (Supplementary Figure 4). Remarkably, the 4 participants with the shortest intervals from disease onset to blood draw (<2 months) did not have higher concentrations of cytokines (Supplementary Figure 5a), neither higher relative intraindividual increases (Supplementary Figure 5b), than participants with longer intervals. Similarly, participants with the longest intervals from disease onset to

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TABLE 3 Relative intraindividual change (%) from 2016–17 to 2020–21 of concentrations of cytokines, by sex (*N* = 154).

Cytokine[a]	Men (*N* = 72)	Women (*N* = 82)	P[b]
Growth factors			
G-CSF (median)	0.00	0.00	0.170
(P25, P75)	(0.00, 26.70)	(−13.53, 20.14)	
Δ ≥ 15%	34.7	31.7	
∇ ≥ 15%	6.9	24.4	
EGF (median)	7.21	6.35	0.244
(P25, P75)	(−1.86, 22.35)	(−3.82, 16.81)	
Δ ≥ 15%	37.5	28.0	
∇ ≥ 15%	8.3	6.1	
FGF (median)	0.00	0.00	0.205
(P25, P75)	(−15.24, 25.49)	(−20.19, 20.02)	
Δ ≥ 15%	33.3	26.8	
∇ ≥ 15%	25.0	35.4	
GM-CSF (median)	0.00	0.00	0.771
(P25, P75)	(−13.11, 35.30)	(−16.33, 31.51)	
Δ ≥ 15%	33.3	29.3	
∇ ≥ 15%	22.2	25.6	
HGF (median)	5.32	6.10	0.559
(P25, P75)	(0.86, 9.78)	(1.53, 10.38)	
Δ ≥ 15%	8.3	9.8	
∇ ≥ 15%	2.8	3.7	
VEGF (median)	9.92	4.09	0.523
(P25, P75)	(−8.27, 47.50)	(−7.98, 28.51)	
Δ ≥ 15%	45.8	40.2	
∇ ≥ 15%	19.4	15.9	
Chemokines			
IL-8 (median)	10.35	5.68	0.365
(P25, P75)	(0.08, 16.81)	(−0.26, 14.85)	
Δ ≥ 15%	33.3	24.4	
∇ ≥ 15%	1.4	1.2	
IP-10 (median)	−98.79	−93.31	0.497
(P25, P75)	(−158.9, −63.92)	(−137.1, −60.19)	
Δ ≥ 15%	6.9	2.4	
∇ ≥ 15%	88.9	92.7	
RANTES (median)	0.00	−0.45	0.066
(P25, P75)	(−1.40, 0.62)	(−2.73, 0.64)	
Δ ≥ 15%	0.0	0.0	
∇ ≥ 15%	0.0	0.0	
EOTAXIN (median)	−0.57	−0.51	0.891
(P25, P75)	(−5.10, 5.14)	(−4.60, 4.27)	
Δ ≥ 15%	4.2	4.9	
∇ ≥ 15%	2.8	4.9	
MIP-1β (median)	3.77	0.61	0.115
(P25, P75)	(−2.97, 13.74)	(−3.91, 7.42)	
Δ ≥ 15%	18.1	13.4	

(Continued)

TABLE 3 (Continued)

Cytokine[a]	Men (*N* = 72)	Women (*N* = 82)	P[b]
∇ ≥ 15%	4.2	8.5	
MCP-1 (median)	1.41	0.76	0.643
(P25, P75)	(−1.73, 4.33)	(−2.6, 3.43)	
Δ ≥ 15%	1.4	0.0	
∇ ≥ 15%	1.4	1.2	
TH1			
IL-2 (median)	21.49	0.00	0.029
(P25, P75)	(−10.80, 66.76)	(−21.33, 36.26)	
Δ ≥ 15%	55.6	37.8	
∇ ≥ 15%	20.8	31.7	
IL-12 (median)	0.97	0.69	0.937
(P25, P75)	(−5.88, 5.41)	(−5.50, 6.01)	
Δ ≥ 15%	8.3	4.9	
∇ ≥ 15%	9.7	6.1	
Pro-inflammatory			
IL-1β (median)	0.00	0.00	0.079
(P25, P75)	(−3.90, 60.81)	(−32.29, 29.28)	
Δ ≥ 15%	41.7	28.0	
∇ ≥ 15%	23.6	29.3	
TNF-α (median)	22.21	6.41	0.262
(P25, P75)	(0.00, 82.08)	(−16.5, 86.98)	
Δ ≥ 15%	52.8	45.1	
∇ ≥ 15%	12.5	26.8	
IL-6 (median)	11.98	3.76	0.150
(P25, P75)	(−5.92, 71.89)	(−7.6, 21.98)	
Δ ≥ 15%	43.1	35.4	
∇ ≥ 15%	16.7	17.1	
IFN-α (median)	5.00	0.00	0.280
(P25, P75)	(−3.59, 23.50)	(−11.83, 20.74)	
Δ ≥ 15%	37.5	30.5	
∇ ≥ 15%	15.3	15.9	
IL-2R (median)	2.70	−0.95	0.026
(P25, P75)	(−4.45, 17.10)	(−13.23, 11.12)	
Δ ≥ 15%	29.2	17.1	
∇ ≥ 15%	11.1	20.7	
Regulatory			
IL-7 (median)	0.00	0.00	0.222
(P25, P75)	(−6.15, 30.31)	(−8.94, 12.49)	
Δ ≥ 15%	31.9	18.3	
∇ ≥ 15%	18.1	18.3	
Anti-inflammatory			
IL-1RA (median)	−2.83	−2.38	0.696
(P25, P75)	(−8.89, 5.97)	(−10.40, 4.33)	
Δ ≥ 15%	11.1	4.9	
∇ ≥ 15%	12.5	11.0	

Cytokines quantified in more than 70% of participants. P25: percentile 25th. P75: percentile 75th.
[a]Units used for computing the relative intraindividual change were base 10 logtransformed pg/mL.
[b]Mann–Whitney's U test (two-tail).

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TABLE 4 Concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein–Barr and common cold infections, and of total Igs from 2016–17 to 2020–21.

Immunoglobulin	Concentrations (MFI)		Change of concentrations 2016–2020	
	2016–2017	2020–2021	Δ absolute (MFI)	Δ relative[a] (%)
IgA				
IgA CMV pp150 (median)	504.0	540.0	11.25	0.37
(P25, P75)	(357.9, 832.5)	(380.8, 876.5)	(−89.63, 104.1)	(−2.36, 3.38)
Geometric mean	622.4	665.5		
Δ ≥ 15%				4.5
∇ ≥ 15%				0.6
IgA CMV pp65 (median)	599.5	650.3	28.00	0.96
(P25, P75)	(422.2, 867.4)	(447.9, 876.1)	(−92.13, 152.9)	(−2.30, 3.65)
Geometric mean	692.2	732.3		
Δ ≥ 15%				1.9
∇ ≥ 15%				0.6
IgA EBV EAD (median)	442.5	463.5	7.00	0.34
(P25, P75)	(304.8, 637.8)	(331.8, 717.5)	(−94.38, 90.13)	(−2.29, 3.69)
Geometric mean	500.7	516.1		
Δ ≥ 15%				1.3
∇ ≥ 15%				1.3
IgA VCAp18 (median)	492.8	512.5	17.50	0.62
(P25, P75)	(349.8, 759.8)	(373.8, 844.8)	(−63.13, 121.4)	(−2.08, 3.48)
Geometric mean	584.4	615.9		
Δ ≥ 15%				1.9
∇ ≥ 15%				0.6
IgA N 229E (median)	1,366	1,316	−20.00	−0.32
(P25, P75)	(746.1, 3,534)	(813.6, 3,102)	(−614.8, 359.4)	(−3.80, 3.45)
Geometric mean	1813	1,693		
Δ ≥ 15%				2.6
∇ ≥ 15%				5.2
IgA N HKU1 (median)	728.8	767.5	29.25	0.91
(P25, P75)	(476.4, 1,186)	(501.8, 1,343)	(−120.5, 159.5)	(−2.51, 3.11)
Geometric mean	854.5	900.8		
Δ ≥ 15%				2.6
∇ ≥ 15%				0.0
IgA NL 63 (median)	1,470	1,610	−39.25	−0.46
(P25, P75)	(780.1, 4,399)	(842.8, 4,143)	(−738.0, 343.6)	(−3.76, 4.54)
Geometric mean	2008	1986		
Δ ≥ 15%				6.5
∇ ≥ 15%				4.5
IgA N OC43 (median)	1,492	1,541	34.25	0.39
(P25, P75)	(916.5, 2,900)	(998.8, 2,785)	(−271.1, 399.4)	(−2.74, 3.18)
Geometric mean	1758	1818		
Δ ≥ 15%				2.6
∇ ≥ 15%				0.6
IgG				
IgG CMV pp150 (median)	15,503	18,515	1,201	1.40

(Continued)

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TABLE 4 (Continued)

Immunoglobulin	Concentrations (MFI)		Change of concentrations 2016–2020	
	2016–2017	2020–2021	Δ absolute (MFI)	Δ relative[a] (%)
(P25, P75)	(5,506, 29,463)	(5,842, 38,776)	(−1,066, 9,371)	(−1.36, 3.99)
Geometric mean	13,823	15,842		
Δ ≥ 15%				1.9
∇ ≥ 15%				0.0
IgG CMV pp65 (median)	6,902	7,422	472.5	0.81
(P25, P75)	(4,665, 10,348)	(4,770, 10,334)	(−1,393, 2013)	(−1.92, 3.13)
Geometric mean	6,811	7,237		
Δ ≥ 15%				0.6
∇ ≥ 15%				0.0
IgG EBV EAD (median)	7,021	6,891	279.0	0.49
(P25, P75)	(4,939, 9,931)	(5,372, 9,830)	(−1790, 1800)	(−2.25, 3.06)
Geometric mean	7,041	7,116		
Δ ≥ 15%				0.0
∇ ≥ 15%				0.0
IgG VCAp18 (median)	8,049	8,612	654.3	0.69
(P25, P75)	(4,280, 14,355)	(4,842, 16,546)	(−1,011, 2,890)	(−1.93, 3.39)
Geometric mean	8,936	9,703		
Δ ≥ 15%				1.9
∇ ≥ 15%				0.6
IgG N 229E (median)	46,792	46,290	766.3	0.15
(P25, P75)	(26,485, 75,860)	(29,671, 68,957)	(−11,162, 12,147)	(−2.56, 2.70)
Geometric mean	43,851	43,610		
Δ ≥ 15%				0.6
∇ ≥ 15%				0.0
IgG N HKU1 (median)	14,316	14,930	727.8	0.63
(P25, P75)	(10,244, 18,907)	(11,510, 18,319)	(−2,581, 2,936)	(−1.57, 2.40)
Geometric mean	13,970	14,393		
Δ ≥ 15%				0.0
∇ ≥ 15%				0.0
IgG NL 63 (median)	38,033	38,897	−68.75	−0.04
(P25, P75)	(22,612, 61,267)	(23,036, 56,672)	(−8,797, 8,325)	(−2.45, 2.58)
Geometric mean	35,810	36,023		
Δ ≥ 15%				0.0
∇ ≥ 15%				0.0
IgG N OC43 (median)	19,501	20,370	167.8	0.15
(P25, P75)	(11,251, 36,874)	(11,784, 34,252)	(−6,438, 5,787)	(−3.02, 3.03)
Geometric mean	20,837	20,054		
Δ ≥ 15%				0.6
∇ ≥ 15%				2.6
IgM				
IgM CMV pp150 (median)	1,303	1,451	76.00	1.01
(P25, P75)	(788.9, 2,301)	(778.0, 2,600)	(−149.9, 404.1)	(−2.06, 3.72)
Geometric mean	1,406	1,497		
Δ ≥ 15%				1.3

(Continued)

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TABLE 4 (Continued)

Immunoglobulin	Concentrations (MFI)		Change of concentrations 2016–2020	
	2016–2017	2020–2021	Δ absolute (MFI)	Δ relative[a] (%)
∇ ≥ 15%				0.6
IgM CMV pp65 (median)	690.3	705.8	22.50	0.60
(P25, P75)	(466.8, 1,039)	(482.9, 1,090)	(−79.50, 155.5)	(−1.73, 3.63)
Geometric mean	718.5	764.8		
Δ ≥ 15%				0.6
∇ ≥ 15%				0.0
IgM EBV EAD (median)	429.5	456.3	12.00	0.51
(P25, P75)	(296.5, 590.2)	(311.6, 621.9)	(−52.50, 81.25)	(−2.25, 3.66)
Geometric mean	445.0	464.5		
Δ ≥ 15%				1.3
∇ ≥ 15%				0.0
IgM VCAp18 (median)	853.3	947.0	51.75	1.11
(P25, P75)	(495.5, 1,482)	(561.6, 1,565)	(−90.00, 282.6)	(−2.33, 4.08)
Geometric mean	901.4	977.5		
Δ ≥ 15%				1.9
∇ ≥ 15%				0.0
IgM N 229E (median)	1,500	1,641	73.00	0.87
(P25, P75)	(866.9, 2,419)	(880.3, 2,618)	(−322.1, 490.6)	(−2.96, 4.52)
Geometric mean	1,483	1,636		
Δ ≥ 15%				2.6
∇ ≥ 15%				0.6
IgM N HKU1 (median)	1,032	1,103	48.50	0.85
(P25, P75)	(700.8, 1,598)	(781.8, 1,648)	(−117.3, 271.9)	(−1.42, 3.86)
Geometric mean	1,061	1,147		
Δ ≥ 15%				1.9
∇ ≥ 15%				0.0
IgM NL 63 (median)	1,023	1,067	72.25	1.12
(P25, P75)	(668.0, 1,597)	(724.4, 1,661)	(−160.4, 339.5)	(−2.54, 4.37)
Geometric mean	1,053	1,120		
Δ ≥ 15%				1.3
∇ ≥ 15%				0.6
IgM N OC43 (median)	2,134	2,279	53.75	0.67
(P25, P75)	(1,188, 3,516)	(1,184, 3,685)	(−342.9, 657.5)	(−2.63, 4.27)
Geometric mean	2,140	2,257		
Δ ≥ 15%				1.9
∇ ≥ 15%				0.0
Total immunoglobulin				
IgG1 (median)	6,175	6,867	588.6	1.05
(P25, P75)	(5,050, 8,691)	(5,455, 9,168)	(−1,101, 1860)	(−1.55, 3.21)
Geometric mean	6,518	7,020		
Δ ≥ 15%				0.0
∇ ≥ 15%				0.0
IgG2 (median)	3,147	3,196	179.6	0.71

(Continued)

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TABLE 4 (Continued)

Immunoglobulin	Concentrations (MFI)		Change of concentrations 2016–2020	
	2016–2017	2020–2021	Δ absolute (MFI)	Δ relative[a] (%)
(P25, P75)	(2,480, 3,719)	(2,679, 3,905)	(−154.2, 489.3)	(−0.64, 2.12)
Geometric mean	3,072	3,248		
Δ ≥ 15%				0.0
∇ ≥ 15%				0.0
IgG3 (median)	1,289	1,225	−23.11	−0.28
(P25, P75)	(1,038, 1,537)	(1,022, 1,444)	(−228.6, 135.7)	(−2.65, 1.65)
Geometric mean	1,242	1,187		
Δ ≥ 15%				0.0
∇ ≥ 15%				0.0
IgG4 (median)	221.9	231.4	11.83	1.00
(P25, P75)	(123.9, 405.6)	(139.0, 424.0)	(−20.63, 47.45)	(−1.72, 4.46)
Geometric mean	229.2	245.3		
Δ ≥ 15%				1.3
∇ ≥ 15%				0.6
Sum of IgGs (median)	11,361	11,946	670.3	0.64
(P25, P75)	(9,364, 14,489)	(9,920, 14,370)	(−1,122, 2,488)	(−1.01, 2.35)
Geometric mean	11,396	12,091		
Δ ≥ 15%				0.0
∇ ≥ 15%				0.0
IgE (median)	0.23	0.24	0.01	2.61
(P25, P75)	(0.15, 0.35)	(0.16, 0.37)	(−0.01, 0.04)	(−3.91, 11.04)
Geometric mean	0.23	0.25		
Δ ≥ 15%				16.9
∇ ≥ 15%				9.1
IgA (median)	249.8	265.8	11.37	0.92
(P25, P75)	(195.9, 300.6)	(206.8, 319.9)	(−22.74, 47.55)	(−1.74, 3.71)
Geometric mean	243.9	257.4		
Δ ≥ 15%				0.6
∇ ≥ 15%				0.0
IgM (median)	935.5	972.8	26.02	0.32
(P25, P75)	(727.7, 1,387)	(752.2, 1,433)	(−105.8, 186.0)	(−1.51, 3.34)
Geometric mean	985.6	1,038		
Δ ≥ 15%				0.0
∇ ≥ 15%				0.0

Absolute intraindividual change and relative intraindividual change ($N = 154$). Δ absolute: Difference between the individual concentrations in 2020–21 with respect to the individual concentrations in 2016–17, in absolute terms (MFIs for 24 isotype-antigen combinations, μg/mL for total immunoglobulins). Δ relative: Difference between the individual concentrations in 2020–21 with respect to the individual concentrations in 2016–17, in relative terms (percentage of change). P25: percentile 25th. P75: percentile 75th. <LOQ: value lower than the minimum concentration of the corresponding range of the limits of quantification. See Supplementary Table 1. Δ ≥ 15%: percentage of individuals with a relative increase in the concentrations between 2016 and 2020 equal to or greater than 15%. ∇ ≥ 15%: percentage of individuals with a relative decrease in the concentrations between 2016 and 2020 equal to or greater than 15%.
[a]Units used for computing the relative intraindividual change were base 10 logtransformed MFI for levels of isotype-antigen combinations, and base 10 logtransformed μg/mL for levels of total Igs.

blood draw did not show lower cytokine concentrations or higher relative decreases.

98% of participants had 4 or more cytokines with a relative intraindividual change (increase or decrease) of concentrations from 2016–17 to 2020–21 ≥ 15%. 21% of participants had between 15 to 27 cytokines with a relative change ≥15%, while no participants had all 30 cytokines with a relative change lower than 15%. The percentage of changes was not different by SARS-CoV-2 infection seropositivity or by COVID-19 disease (Supplementary Table 3).

The intraindividual change in cytokine concentrations from 2016–17 to 2020–21 was similar by sex (Table 3). Only IL-2 increased more in men, although women had higher IL-2 concentrations in 2016–17 than men (Supplementary Table 4). Prepandemic concentrations and intraindividual change in cytokines were also similar across age groups (Supplementary Tables 5a,b), although concentrations of some cytokines were higher in younger participants (e.g., G-CSF, TNF-α), and concentrations of others were lower in younger participants (e.g., IL-8, EOTAXIN, MCP-1). Prepandemic concentrations and change were also similar by BMI (Supplementary Tables 6a,b), by tobacco smoking (Supplementary Tables 7a,b), and by educational level (Supplementary Tables 8a,b). Although differences for a few intraindividual changes by age or by smoking were statistically significant, the magnitude of such differences was small. Again, concentrations in 2016–17 are presented as possible reference values in different sociodemographic groups.

3.2 Intraindividual change in concentrations of immunoglobulins against viral exposures and of total immunoglobulins

The *median* value of the relative intraindividual change in immunoglobulin levels between 2016 and 2020 was virtually null for the 24 isotype-antigen combinations for CMV, EBV, and human common cold coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63 i HCoV-HKU1) and for the seven total immunoglobulins (as well as for the sum of IgG 1–4 subclasses). *Median* values of the relative change ranged from −0.46% for IgA NL63 to 2.61% for total IgE (Table 4). Similarly, the *percentage of participants* having a relative intraindividual change (increase or decrease) ≥ 15% was virtually null for all immunoglobulins (total and virus-specific), ranging from 0.0 to 6.5%, except for total IgE, for which 17% of participants had a relative intraindividual increase ≥15 and 9% of participants had a relative decrease ≥15%. The highest median absolute intraindividual changes were observed for IgGs, the immunoglobulin isotype with the highest concentrations.

No major differences in intraindividual changes in immunoglobulin levels (in isotype-antigen combinations and in total immunoglobulins) were observed between participants who were SARS-CoV-2 seronegative and seropositive, nor between participants who did and did not develop COVID-19 disease (Table 5; Figure 3). The change tended to be similar (in seropositives and seronegatives, in participants who did and did not develop COVID-19 disease) when using the median of the change and the 4 percentages ($\Delta \geq 15\%$ and $\nabla \geq 15\%$).

Differences in prepandemic levels of total IgM and of IgMs against viral exposures were observed between men and women and across age groups: levels of total IgM and all eight IgMs against CMV, EBV, and HCoV were statistically significantly higher in women than men, and in younger groups than in older participants (Supplementary Tables 9a and 10a). Differences were also observed in prepandemic levels of IgMs by BMI and tobacco smoking: higher concentrations of IgMs were observed in normal weight participants than in overweight or obese participants (Supplementary Table 11a; Supplementary Figure 4b), while lower concentrations were found in former smokers (Supplementary Table 12a). Nevertheless, no differences by sex, age, BMI or smoking were found in intraindividual changes from 2016–17 to 2020–21 in levels of IgMs (Supplementary Tables 9b, 10b, 11b, 12b).

Higher prepandemic levels of total IgGs were also observed in women, and in younger participants. Younger participants also had higher levels of IgAs against CMV and EBV (Supplementary Table 10a); while higher levels of IgA CMV pp65 and IgA EBV EAD were observed in obese participants (Supplementary Table 11a). Again, virtually no significant differences in the intraindividual change of levels of the mentioned immunoglobulins were found by sex, age or BMI (Supplementary Tables 9b, 10b, 11b). Higher intraindividual changes of total immunoglobulins were observed in participants with dyslipidemia (Supplementary Table 13). Lower levels of IgG CMV pp150 were found in participants with higher education. No other significant differences in prepandemic levels or in their change were observed by educational level (Supplementary Tables 14a,b).

4 Discussion

Intraindividual changes in concentrations of cytokines and immunoglobulins during the period from 2016–17 to 2020–21 were moderate –perhaps surprisingly so, given the epidemiological context. But maybe less surprisingly, since the long-term stability of an individual's immune system in the absence of immunological challenge does not require that stability is maintained during infection (4). The 4-year stability suggests that in spite of brief changes due to infections o reactivations of viruses such as EBV, or vaccinations, concentrations return fairly quickly to basal levels. Results thus indicate a rather stable basal state of the immune system (3, 4). This study appears to be the first to document this relative stability of cytokine blood biomarkers in a general, non-institutionalized population, during a period that precedes and includes the pandemic, and with repeated blood samples from the same individuals.

Immune biomarker stability could have implications for clinical and public health practice: it might contribute to personalized preventive strategies, informing on patient vaccine or treatment responsiveness, or serve for patient monitoring in infectious disease contexts.

Previous studies have shown that many individuals have stable immune systems. This was reported by Brodin et al., for instance, in 99 healthy adults and 210 healthy twins (2, 3). Certain functional units of immunity, involving cytokines, vary across individuals primarily as a consequence of non-heritable factors, suggesting that the immune system of healthy individuals is much shaped by the environment, including chronic viral infection as well as socioeconomic factors as cohabitation and housing conditions (2–5).

Cytokine concentrations measured in 2020–21 were not associated with the time interval from disease onset to blood draw among the 20 participants who developed COVID-19 (Supplementary Figure 4; the interval was less than 2 months in only 4 of the 20 individuals, and in such 4 participants concentrations were not particularly increased). The observation suggests that factors other than that interval, such as the basal status of the immune system, might play a more significant role in determining cytokine

concentrations post-infection. The present report did not aim at analyzing the long-term consequences of COVID-19 on the immunological system.

In addition to the immunological interest, the result mentioned in the previous paragraph has methodological relevance as well, because intraindividual changes in cytokines and immunoglobulins during the 4 years were similar between participants who in 2020–21 were SARS-CoV-2 seropositive and seronegative, and between participants who did and did not develop COVID-19 disease. The similarity indicates that it is valid to use prepandemic levels of cytokines and immunoglobulins to assess their risk relationship (protective or harmful) with the development of SARS-CoV-2 seropositivity and COVID-19 disease (28). The longitudinal time sequence is unequivocal (2016–17 vs. 2020–21). By contrast, the cross-sectional analysis of the possible association between levels of cytokines and immunoglobulins in 2020–21 and SARS-CoV-2 seropositivity and COVID-19 disease (obviously, also in 2020–21) would not allow to analyze that relationship, because the putative cause and the effect were measured very near in time.

We did not observe many differences in cytokine concentrations by main sociodemographic groups; essentially, higher concentrations of some cytokines (G-CSF, TNF-α) in younger participants, and higher concentrations of Il-2 in women.

More differences were apparent for immunoglobulins: higher levels in younger participants of total IgG, total IgM, and IgMs and IgAs against CMV, EBV, and HCoV, and higher levels in women than men of total IgG, total IgM and all eight IgMs against CMV, EBV, and HCoV. Levels of IgMs were higher in normal weight participants than in overweight or obese participants, and lower in former smokers. Levels of IgG CMV pp150 were lower in participants with higher education, as previously observed (8, 29). Differences in immunological markers are expected by age, sex, lifestyle, and living conditions (3–7, 10, 29, 49, 50). One possible explanation for the decrease in the concentration of IgMs with age can be found in the Tagonski's work, which states that there is a reduction of new antibodies in the aging process of the immune system due to a decreased proliferative capacity of the B cells (51). In relation to women having higher IgMs than men (6, 7), evidence suggests that women have stronger humoral and T-cell immune responses than men (52). Our results on concentrations of cytokines and immunoglobulins by sociodemographic factors in a general Western population provide novel information, with potential uses in clinical practice and research; e.g., as reference values for population subgroups defined by sex, age, education, BMI, and smoking (1, 6–8).

Changes observed in the concentrations of cytokines during the study period were of higher magnitude than for immunoglobulins. This was in part expected due to the increase of cytokines when infectious / inflammatory processes occur (1, 3, 9), whereas total antibody isotypes and subclasses are comprised by polyclonal antibodies against numerous different antigens / pathogens and specific exposures are diluted. Also, antibodies against CMV or EBV would increase only in case of first infections that may have happened during childhood or early adulthood, or reactivations, which should not occur often in healthy populations. Of particular interest is the stability of antibodies against coronaviruses of common cold, despite regular exposure; the stability suggests that they induce short-lived antibody responses (53).

The choice to Log10 transform cytokine concentrations before calculating the percent relative change underlies the primary finding that cytokine concentrations are stable. Small perturbations in cytokine levels of less than 10-fold (e.g., 2–5 times) may have a substantial effect on the immune response during disease and vaccination. Some previous studies took the Log10 transformation after assessing the fold-change, not before (20, 21). The two main reasons why results on the relative change are based on log-transformed data are: First, the relative changes with original concentrations could sometimes be large; e.g., in the interquartile ranges, six times or more with respect to the baseline concentration. As the Mann–Whitney's *U* test, also known as the Wilcoxon Rank Sum test, was practically identical when we used the original concentrations and when we used log-transformed concentrations, we preferred to give tables with more homogeneous values. And second, having a more homogeneous scale of relative change in the percentages, with a same cut-off point (i.e., at less the 15% of increase or decrease in the relative change) for all cytokines and all immunoglobulins, the reader can get a quick idea of whether a specific cytokine or immunoglobulin has varied more over time compared to the 2016–17 values. The log-transformed concentrations are only for presentation purposes. The relevant results are that intra-individual variations in cytokine and immunoglobulin levels in *unvaccinated* citizens are similar between those who were infected with SARS and those who were not, and between those who developed COVID-19 and those who did not.

Some limitations and strengths of the study have been previously discussed (35) and are just summarized here. Because the amount of information and results that we report is already quite high, given the size of the study population, we did not report other possible predictors of the change in cytokines and immunoglobulins, such as seasonality, temperature, other infections, or other immunological and genetic parameters. In section 2.7 above we inform that no associations among prepandemic comorbidities, and cytokines or immunoglobulins, were observed; only some modest negative relationships were observed between IgMs and BMI, and between IgMs, some total IgGs and dyslipidemia (Supplementary Figure 4b).

The generalizability of the findings may be somewhat limited by some characteristics of the cohort, even if this is a cohort from the general population of Barcelona (i.e., different from cohorts based on hospitals or other health care facilities); naturally, a Western population is not representative of other populations, which have other environmental and social pressures, from endemic infectious diseases to pollution to poverty. Future replication in larger and diverse populations is necessary. Also, further studies focused on how environmental exposures (e.g., nutritional status, other contaminants) may influence longitudinal immune biomarker dynamics will be necessary, stimulated by the present findings. Some of the strengths of the present study are assay reproducibility, the use of optimized standard assay protocols, and timepoint consistency; they support the robustness and validity of our conclusions.

The study size, statistical power and precision were often low; yet, numerous estimates were precise. On the other hand, the imputation of concentrations in the less detected and quantified cytokines (e.g., MIG, IFN-γ, and IL-17) may overestimate their intraindividual change. We analyzed 30 cytokines, 24 isotype-antigen combination immunoglobulins, and 7 total immunoglobulins, a relatively large amount in itself, although common in the clinical literature. We could thus perform a considerable number of comparisons and, since ours

TABLE 5 Relative intraindividual change (%) of 24 isotype-antigen combinations for cytomegalovirus, Epstein–Barr and common cold infections, and of total Igs concentrations from 2016–17 to 2020–21 in participants SARS-CoV-2 seronegative and seropositive, and in participants without COVID-19 disease and with COVID-19 disease.

Immunoglobulin[a]	SARS-CoV-2 status		P[b]	COVID-19 disease		P[b]
	Seronegative (*N* = 104)	Seropositive (*N* = 41)		No COVID (*N* = 134)	COVID (*N* = 20)	
IgA						
IgA CMV pp150 (median)	0.25	0.41	0.777	0.37	0.18	0.809
(P25, P75)	(−2.86, 3.38)	(−2.18, 3.06)		(−2.66, 3.43)	(−2.04, 3.09)	
Δ ≥ 15%	4.8	4.9		4.5	5.0	
∇ ≥ 15%	1.0	0.0		0.7	0.0	
IgA CMV pp65 (median)	0.50	1.55	0.139	0.77	1.60	0.275
(P25, P75)	(−2.86, 3.31)	(−0.90, 4.08)		(−2.49, 3.82)	(−0.91, 3.63)	
Δ ≥ 15%	2.9	0.0		2.2	0.0	
∇ ≥ 15%	1.0	0.0		0.7	0.0	
IgA EBV EAD (median)	0.08	0.16	0.450	0.48	0.00	0.522
(P25, P75)	(−2.63, 3.85)	(−0.90, 3.75)		(−2.34, 3.55)	(−1.45, 4.00)	
Δ ≥ 15%	1.9	0.0		1.5	0.0	
∇ ≥ 15%	1.9	0.0		1.5	0.0	
IgA VCAp18 (median)	0.13	1.35	0.254	0.50	1.08	0.220
(P25, P75)	(−2.48, 3.13)	(−1.63, 4.34)		(−2.50, 3.46)	(0.18, 5.27)	
Δ ≥ 15%	1.9	2.4		2.2	0.0	
∇ ≥ 15%	1.0	0.0		0.7	0.0	
IgA N 229E (median)	−0.58	−0.02	0.409	−0.12	−0.93	0.830
(P25, P75)	(−4.40, 3.21)	(−3.03, 4.95)		(−3.70, 3.36)	(−4.40, 5.16)	
Δ ≥ 15%	2.9	2.4		3.0	0.0	
∇ ≥ 15%	5.8	4.9		6.0	0.0	
IgA N HKU1 (median)	0.64	1.21	0.871	0.92	0.56	0.367
(P25, P75)	(−2.65, 3.39)	(−1.98, 2.22)		(−2.36, 3.32)	(−2.94, 1.64)	
Δ ≥ 15%	2.9	2.4		2.2	5.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgA NL 63 (median)	−0.19	−0.78	0.617	−0.28	−1.05	0.855
(P25, P75)	(−4.62, 4.84)	(−3.44, 4.33)		(−3.76, 4.74)	(−4.35, 4.09)	
Δ ≥ 15%	5.8	9.8		6.0	10.0	

(Continued)

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TABLE 5 (Continued)

Immunoglobulin[a]	SARS-CoV-2 status		P[b]	COVID-19 disease		P[b]
	Seronegative (*N* = 104)	Seropositive (*N* = 41)		No COVID (*N* = 134)	COVID (*N* = 20)	
∇ ≥ 15%	4.8	4.9		5.2	0.0	
IgA N OC43 (median)	−0.44	0.85	0.139	0.39	0.51	0.408
(P25, P75)	(−3.78, 3.23)	(−1.04, 3.73)		(−2.86, 2.89)	(−2.00, 4.44)	
Δ ≥ 15%	2.9	2.4		2.2	5.0	
∇ ≥ 15%	1.0	0.0		0.7	0.0	
IgG						
IgG CMV pp150 (median)	1.40	2.11	0.499	1.17	2.12	0.169
(P25, P75)	(−1.38, 4.00)	(−1.28, 4.66)		(−1.55, 3.94)	(0.04, 5.26)	
Δ ≥ 15%	0.0	4.9		1.5	5.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgG CMV pp65 (median)	0.33	2.08	0.039	0.67	1.98	0.384
(P25, P75)	(−2.48, 2.78)	(−1.33, 5.71)		(−2.03, 3.50)	(−1.75, 2.73)	
Δ ≥ 15%	1.0	0.0		0.7	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgG EBV EAD (median)	0.30	1.51	0.102	0.37	1.38	0.405
(P25, P75)	(−3.06, 2.81)	(−1.38, 4.09)		(−2.32, 2.88)	(−1.21, 3.72)	
Δ ≥ 15%	0.0	0.0		0.0	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgG VCAp18 (median)	0.62	1.32	0.422	0.62	1.37	0.573
(P25, P75)	(−2.25, 3.41)	(−1.08, 4.21)		(−1.93, 3.23)	(−3.14, 4.27)	
Δ ≥ 15%	2.9	0.0		2.2	0.0	
∇ ≥ 15%	0.0	2.4		0.7	0.0	
IgG N 229E (median)	0.06	1.43	0.189	0.15	0.30	0.957
(P25, P75)	(−2.49, 2.26)	(−2.62, 5.62)		(−2.56, 2.63)	(−2.90, 3.01)	
Δ ≥ 15%	1.0	0.0		0.7	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgG N HKU1 (median)	0.34	1.02	0.319	0.41	0.94	0.302
(P25, P75)	(−2.28, 2.50)	(−1.10, 2.48)		(−1.63, 2.37)	(−0.70, 2.78)	
Δ ≥ 15%	0.0	0.0		0.0	0.0	

(Continued)

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TABLE 5 (Continued)

Immunoglobulin[a]	SARS-CoV-2 status		P[b]	COVID-19 disease		P[b]
	Seronegative (*N* = 104)	Seropositive (*N* = 41)		No COVID (*N* = 134)	COVID (*N* = 20)	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgG NL 63 (median)	−0.18	1.31	0.084	−0.04	0.55	0.648
(P25, P75)	(−2.88, 2.42)	(−1.51, 4.28)		(−2.48, 2.68)	(−2.33, 2.50)	
Δ ≥ 15%	0.0	2.4		0.0	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgG N OC43 (median)	0.04	0.30	0.453	−0.06	1.01	0.369
(P25, P75)	(−3.93, 3.02)	(−2.39, 3.38)		(−3.56, 3.03)	(−0.94, 3.16)	
Δ ≥ 15%	1.0	0.0		0.7	0.0	
∇ ≥ 15%	3.8	0.0		3.0	0.0	
IgM						
IgM CMV pp150 (median)	1.14	0.80	0.645	1.25	−0.19	0.305
(P25, P75)	(−2.00, 4.69)	(−2.13, 3.26)		(−1.85, 4.01)	(−2.99, 3.62)	
Δ ≥ 15%	1.9	0.0		1.5	0.0	
∇ ≥ 15%	1.0	0.0		0.7	0.0	
IgM CMV pp65 (median)	0.34	0.75	0.881	0.55	0.85	0.961
(P25, P75)	(−2.07, 3.92)	(−1.32, 2.85)		(−1.87, 3.81)	(−0.99, 2.09)	
Δ ≥ 15%	0.0	2.4		0.7	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgM EBV EAD (median)	0.63	0.09	0.607	0.53	−0.06	0.801
(P25, P75)	(−2.88, 3.50)	(−1.20, 3.85)		(−2.50, 3.73)	(−1.52, 2.52)	
Δ ≥ 15%	0.0	4.9		1.5	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgM VCAp18 (median)	0.98	1.38	0.944	1.11	1.15	0.813
(P25, P75)	(−2.26, 4.05)	(−2.35, 4.23)		(−2.33, 4.02)	(−2.10, 5.07)	
Δ ≥ 15%	2.9	0.0		2.2	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgM N 229E (median)	0.19	1.66	0.080	0.66	1.57	0.644
(P25, P75)	(−3.43, 4.17)	(−1.23, 4.97)		(−3.28, 4.60)	(−0.13, 2.80)	
Δ ≥ 15%	1.0	7.3		3.0	0.0	

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TABLE 5 (Continued)

Immunoglobulin[a]	SARS-CoV-2 status		P[b]	COVID-19 disease		P[b]
	Seronegative (*N* = 104)	Seropositive (*N* = 41)		No COVID (*N* = 134)	COVID (*N* = 20)	
∇ ≥ 15%	1.0	0.0		0.7	0.0	
IgM N HKU1 (median)	0.71	1.58	0.843	0.69	1.60	0.847
(P25, P75)	(−1.82, 4.33)	(−1.35, 2.63)		(−1.63, 4.18)	(−1.15, 2.81)	
Δ ≥ 15%	2.9	0.0		2.2	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgM NL 63 (median)	1.12	0.48	0.399	1.27	0.29	0.384
(P25, P75)	(−2.78, 5.40)	(−2.58, 3.71)		(−2.57, 5.08)	(−2.29, 3.46)	
Δ ≥ 15%	1.0	2.4		1.5	0.0	
∇ ≥ 15%	1.0	0.0		0.7	0.0	
IgM N OC43 (median)	0.43	1.19	0.440	0.53	1.27	0.719
(P25, P75)	(−2.68, 4.20)	(−1.43, 4.32)		(−2.63, 4.37)	(−2.67, 3.48)	
Δ ≥ 15%	1.9	2.4		1.5	5.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
Total Immunoglobulin						
IgG1 (median)	1.12	1.07	0.696	0.79	1.93	0.026
(P25, P75)	(−1.66, 3.23)	(−1.01, 3.97)		(−1.88, 3.00)	(0.79, 5.09)	
Δ ≥ 15%	0.0	0.0		0.0	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgG2 (median)	0.79	0.52	0.775	0.61	0.82	0.468
(P25, P75)	(−0.80, 2.47)	(−0.52, 1.61)		(−0.68, 2.12)	(−0.43, 2.47)	
Δ ≥ 15%	0.0	0.0		0.0	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgG3 (median)	−0.62	−0.01	0.920	−0.62	0.33	0.171
(P25, P75)	(−2.31, 1.51)	(−3.51, 1.77)		(−2.65, 1.53)	(−2.10, 1.81)	
Δ ≥ 15%	0.0	0.0		0.0	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgG4 (median)	0.73	1.05	0.745	1.08	0.71	0.772
(P25, P75)	(−1.82, 4.52)	(−1.76, 4.43)		(−1.72, 4.42)	(−2.63, 6.09)	
Δ ≥ 15%	1.0	2.4		1.5	0.0	

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TABLE 5 (Continued)

Immunoglobulin[a]	SARS-CoV-2 status		P[b]	COVID-19 disease		P[b]
	Seronegative (*N* = 104)	Seropositive (*N* = 41)		No COVID (*N* = 134)	COVID (*N* = 20)	
∇ ≥ 15%	1.0	0.0		0.7	0.0	
Sum of IgGs (median)	0.79	0.55	0.864	0.59	1.93	0.038
(P25, P75)	(−1.34, 2.32)	(−0.83, 2.97)		(−1.40, 2.19)	(−0.49, 3.68)	
Δ ≥ 15%	0.0	0.0		0.0	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgE (median)	2.57	2.64	0.414	2.80	2.13	0.679
(P25, P75)	(−3.79, 9.26)	(−4.83, 16.56)		(−3.65, 10.64)	(−4.62, 20.48)	
Δ ≥ 15%	14.4	26.8		14.9	30	
∇ ≥ 15%	10.6	4.9		10.4	0.0	
IgA (median)	0.90	0.98	0.375	0.90	1.16	0.458
(P25, P75)	(−2.54, 3.62)	(−1.61, 5.32)		(−2.50, 3.71)	(−1.49, 4.72)	
Δ ≥ 15%	1.0	0.0		0.7	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	
IgM (median)	0.16	0.89	0.083	0.24	1.21	0.072
(P25, P75)	(−1.98, 2.95)	(−0.56, 4.58)		(−1.78, 3.22)	(−0.41, 5.20)	
Δ ≥ 15%	0.0	0.0		0.0	0.0	
∇ ≥ 15%	0.0	0.0		0.0	0.0	

[a]Units used for computing the relative intraindividual change were base 10 logtransformed MFI for levels of isotype-antigen combinations, and base 10 logtransformed μg/mL for levels of total Igs.
[b]Mann–Whitney's U test (two-tail).

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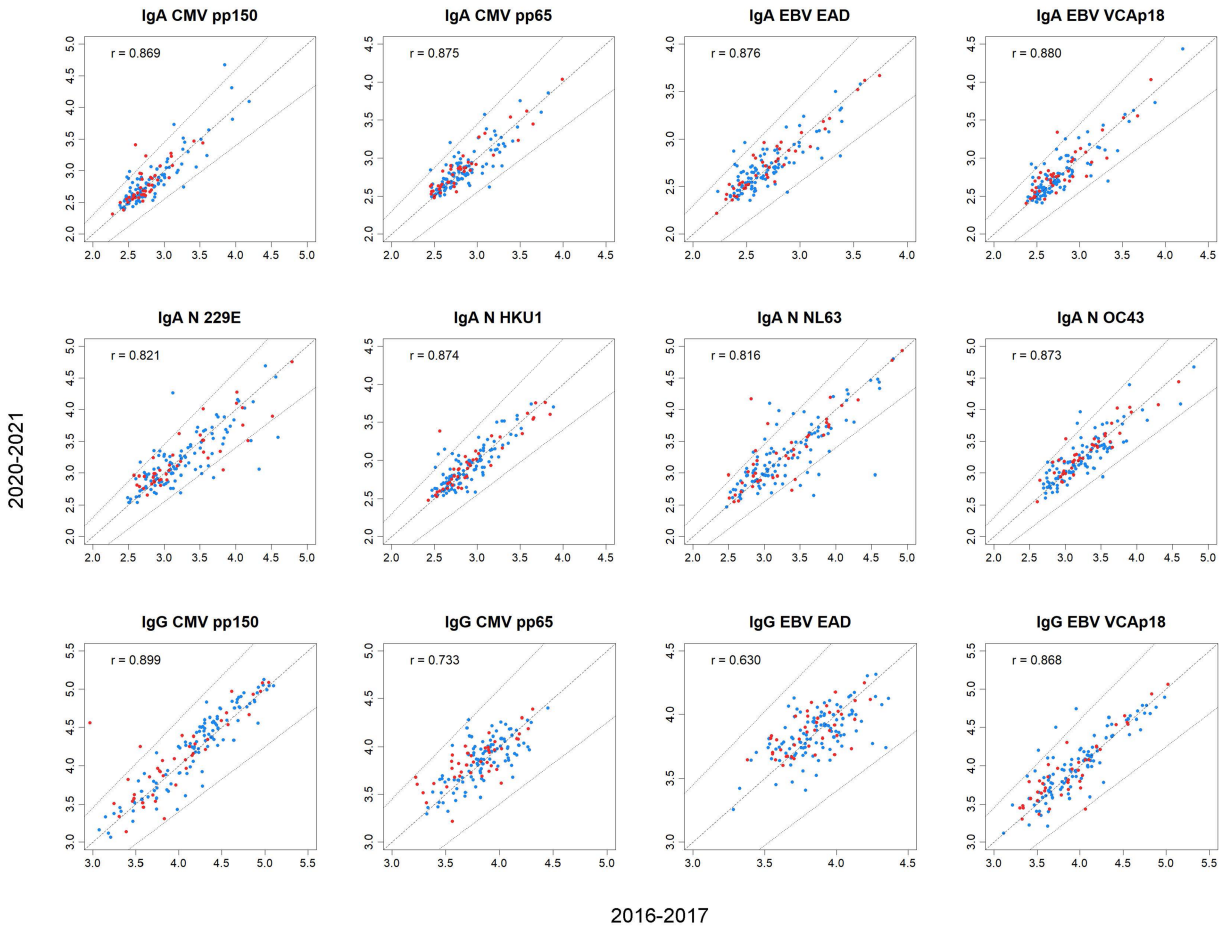

FIGURE 3
Scatterplots of concentrations (log10, MFI, μg/mL) of immunoglobulins in 2020–21 against concentrations in 2016–17 by SARS-CoV-2 seropositivity. Blue dots, seronegative; red dots, seropositive. A 15% change is delimited by the two lines at both sides of the diagonal line indicating no change. All *p*-values <0.001.

is the first study of its kind (assessing intraindividual change in a general, non-institutionalized population), it is only logical that we assessed comprehensively such change and the influence of SARS-CoV-2 infection, COVID-19, and sociodemographic factors. These features of the study may generate false positives, and replication of our findings in larger population-based, longitudinal studies is required; but they have also strengths, since the number of potentially relevant cytokines and immunoglobulins is high. The comparisons (e.g., in intraindividual changes in concentrations across cytokines by COVID-19 disease status) could not generally be based on *a priori* clinical knowledge, because virtually none exists for such changes.

5 Conclusion

We provide novel information on physiological, basal ex-vivo concentrations of cytokines and immunoglobulins in a general population, which should be relevant for clinical practice and public health. Intraindividual changes in cytokines and immunoglobulins during the 4 years from 2016–17 to 2020–21 were moderate, and they did not differ between participants who in 2020–21 were SARS-CoV-2 seropositive and seronegative, nor between participants who did and did not develop COVID-19 disease. These findings are also novel and relevant for medicine and public health. In particular, the stability in the biomarkers is relevant to assess the role of the immunological and inflammatory state (measured through baseline levels of cytokines and immunoglobulins) in the development of SARS-CoV-2 seropositivity and COVID-19 disease, as well as in the susceptibility to other infections and pathologies. Immune biomarker stability might contribute to personalized preventive strategies, informing on patient vaccine or treatment responsiveness, or serve for patient monitoring in infectious disease contexts.

Data availability statement

The raw data supporting the conclusions of this article may be made available by the authors upon reasonable request, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Parc de Salut Mar Barcelona. The studies were conducted in

accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MG: Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision. JP: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing, Software, Validation. RA: Data curation, Investigation, Methodology, Resources, Writing – review & editing. LC: Data curation, Writing – review & editing, Formal analysis, Software. DP-M: Data curation, Formal analysis, Software, Writing – review & editing, Resources, Validation. JV-G: Resources, Validation, Writing – review & editing, Investigation. CR: Investigation, Resources, Writing – review & editing, Data curation. FB: Investigation, Resources, Writing – review & editing, Supervision. LT: Investigation, Resources, Writing – review & editing, Funding acquisition, Methodology. CD: Investigation, Methodology, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Supervision. GM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. MP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2025.1548379/full#supplementary-material

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Supplemental Table 1. Limits of quantification, and percentage of participants with concentrations between the lower and the upper limits of quantification.

	Period 2016-17 (N = 240)			Period 2020-21 (N = 174)		
Cytokine	**Lower LOQ** (range)	**Upper LOQ** (range)	**Quantification (%)**	**Lower LOQ** (range)	**Upper LOQ** (range)	**Quantification (%)**
Growth factors						
G-CSF	5.30 – 16.18	26117 – 40458	76.3	5.30 – 12.57	26117 – 40458	77.6
EGF	3.01 – 9.48	4120 – 5465	94.2	3.95 – 9.48	4120 – 5465	98.3
FGF	2.10 – 3.27	3155[a]	87.1	2.10 – 3.27	3155[a]	86.2
GM-CSF	0.43 – 2.19	6740 – 7103	70.8	0.43 – 2.19	6740 – 7103	75.3
HGF	3.35 – 11.18	13001 – 13550	100.0	5.04 – 11.18	13001 – 13550	100.0
VEGF	0.07 – 0.10	1103 – 1634	99.6	0.07 – 0.10	1103 – 1634	99.4
Chemokines						
IL-8	0.26 – 0.82	5205 – 5790	100.0	0.34 – 0.82	5205 – 5790	100.0
IP-10	0.14 – 0.46	981 – 1829	100.0	0.14 – 0.25	981 – 1829	90.2
RANTES	5.80 – 13.38	2985 – 7633	80.4	6.96 – 13.38	2985 – 7633	78.2
EOTAXIN	0.45 – 1.39	1237 – 1721	100.0	0.45 – 1.12	1237 – 1721	100.0
MIP-1α	9.37 – 15.29	13529 – 14115	66.3	9.37 – 15.29	13529 – 14115	68.4
MIP-1β	1.60 – 4.15	12184 – 13057	98.8	1.87 – 4.15	12184 – 12672	98.9
MCP-1	4.04 – 11.49	16148 – 18550	99.6	4.04 – 11.35	16783 – 18550	99.4
MIG	6.26 – 12.54	3285 – 3592	41.3	6.26 – 9.70	3285 – 3592	36.8
TH1						
IL-2	0.53 – 0.98	12958 – 13778	88.3	0.53 – 0.98	13024 – 13778	93.7
IL-12	0.66 – 2.34	5649 – 6880	100.0	0.66 – 2.34	5649 – 6880	100.0
IFN-γ	0.22 – 0.45	1638 – 1764	14.2	0.28 – 0.45	1638 – 1764	13.8
TH2						
IL-4	1.35 – 3.74	29732 – 34733	36.3	1.99 – 3.74	29732 – 32423	33.9
IL-5	0.60 – 1.11	2802 – 3937	48.8	0.60 – 1.11	2802 – 3937	47.7
IL-13	1.40 – 4.30	7930 – 9986	67.5	1.40 – 3.54	7930 – 9986	65.5
Pro-inflammatory						
IL-1β	0.32 – 0.55	1909 – 2676	73.8	0.32 – 0.55	1909 – 2676	79.9
TNF-α	0.47 – 0.89	4313 – 4615	80.0	0.47 – 0.89	4313 – 4615	85.6
IL-6	0.70 – 1.87	7800 – 9425	93.3	0.70 – 1.87	7800 – 9425	93.7
IFN-α	2.27 – 5.51	7350 – 8270	90.0	2.27 – 3.30	7390 – 8270	93.7
IL-2R	2.07 – 5.03	16856 – 17210	99.6	2.07 – 5.03	16856 – 17210	99.4
IL-17	0.89 – 1.22	16006 – 17321	34.2	0.89 – 1.22	16006 – 17321	43.1
Regulatory						
IL-7	2.80 – 5.69	9585 – 12205	71.3	2.80 – 5.69	9585 – 12205	75.3
Anti-inflammatory						
IL-10	0.55 – 1.06	11047 – 11810	59.6	0.55 – 1.06	11047 – 11689	63.2
IL-15	3.45 – 6.68	17496 – 18539	28.7	3.45 – 6.68	17496 – 18539	37.9
IL-1RA	3.94 – 10.77	68762 – 77553	99.2	3.94 – 10.77	68871 – 77553	100.0

LOQ: limit of quantification (pg/mL), see Methods section *2.3. Quantification of cytokines, chemokines and growth factors*
[a] Same upper limit of quantification in all plates in the laboratory analyses.
Participants of the 2020-21 period were the 174 participants (out of the 240 participants of the baseline period), who attended the follow-up visit (i.e. all 174 participants are part of the group of 240), see Methods section *2.1. Study population*

Supplemental Table 2. Correlations between cytokines and IgM antibodies against CMV, EBV and HCoV in 2016-17.

		G-CSF	EGF	FGF	GM-CSF	IL-8	EOTAXIN	MIP-1α	MIP-1β	IL-2	IL-12	IL-4	IL-13	TNF-α	IL-6	IFN-α	IL-2R	IL-17	IL-7	IL-10	IL-15	IL-1RA
IgM_CMV_pp150	ρ	**0.249**	**0.300**	**0.239**	**0.259**	**-0.199**	**-0.245**	**0.286**	**0.323**	**0.374**	**0.174**	0.036	0.112	**0.215**	**0.186**	**0.341**	**0.230**	**0.133**	0.113	**0.309**	**0.150**	**0.243**
	p value	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	0.007	0.582	0.085	0.001	0.004	<0.001	<0.001	0.039	0.080	<0.001	0.020	<0.001
IgM_CMV_pp65	ρ	**0.181**	**0.250**	**0.149**	**0.177**	**-0.248**	**-0.226**	0.107	**0.276**	**0.298**	-0.018	0.050	0.071	**0.236**	0.069	**0.228**	**0.183**	0.087	-0.012	**0.189**	0.110	**0.195**
	p value	0.005	<0.001	0.021	0.006	<0.001	<0.001	0.097	<0.001	<0.001	0.776	0.441	0.274	<0.001	0.288	<0.001	0.004	0.180	0.853	0.003	0.090	0.002
IgM_EBV_EAD	ρ	**0.210**	**0.329**	**0.213**	**0.220**	**-0.235**	**-0.161**	**0.147**	**0.340**	**0.355**	0.101	0.047	**0.147**	**0.254**	**0.166**	**0.335**	**0.235**	**0.197**	0.062	**0.233**	**0.146**	**0.231**
	p value	0.001	<0.001	0.001	0.001	<0.001	0.013	0.023	<0.001	<0.001	0.120	0.468	0.023	<0.001	0.010	<0.001	<0.001	0.002	0.337	<0.001	0.023	<0.001
IgM_EBV_VCAp18	ρ	**0.142**	**0.247**	**0.135**	**0.150**	**-0.239**	**-0.200**	**0.128**	**0.258**	**0.282**	0.119	0.095	0.076	**0.217**	0.061	**0.221**	**0.152**	0.096	-0.015	**0.158**	0.106	**0.249**
	p value	0.028	<0.001	0.037	0.020	<0.001	0.002	0.048	<0.001	<0.001	0.066	0.143	0.241	0.001	0.347	0.001	0.019	0.137	0.817	0.014	0.101	<0.001
IgM_N_229E	ρ	**0.314**	**0.319**	**0.234**	**0.292**	**-0.181**	**-0.160**	**0.189**	**0.311**	**0.353**	**0.135**	0.044	**0.229**	**0.341**	**0.239**	**0.310**	**0.247**	**0.166**	**0.141**	**0.247**	0.113	**0.298**
	p value	<0.001	<0.001	<0.001	<0.001	0.005	0.013	0.003	<0.001	<0.001	0.037	0.500	<0.001	<0.001	<0.001	<0.001	<0.001	0.010	0.029	<0.001	0.079	<0.001
IgM_N_HKU1	ρ	**0.240**	**0.301**	**0.186**	**0.266**	**-0.184**	**-0.198**	**0.173**	**0.286**	**0.340**	0.112	0.108	**0.155**	**0.246**	**0.158**	**0.285**	**0.222**	**0.141**	0.082	**0.289**	**0.131**	**0.216**
	p value	<0.001	<0.001	0.004	<0.001	0.004	0.002	0.007	<0.001	<0.001	0.084	0.096	0.017	<0.001	0.014	<0.001	0.001	0.029	0.204	<0.001	0.043	0.001
IgM_N_NL63	ρ	**0.207**	**0.241**	**0.151**	**0.159**	**-0.196**	**-0.226**	**0.127**	**0.242**	**0.251**	0.110	0.068	0.073	**0.248**	**0.133**	**0.222**	**0.197**	0.089	0.034	**0.178**	0.109	**0.155**
	p value	0.001	<0.001	0.019	0.014	0.002	<0.001	0.050	<0.001	<0.001	0.088	0.296	0.259	<0.001	0.040	0.001	0.002	0.169	0.600	0.006	0.093	0.016
IgM_N_OC43	ρ	**0.226**	**0.325**	**0.148**	**0.230**	**-0.139**	**-0.106**	**0.137**	**0.313**	**0.318**	**0.198**	**0.167**	0.120	**0.287**	**0.195**	**0.294**	**0.248**	**0.178**	0.045	**0.253**	**0.180**	**0.296**
	p value	<0.001	<0.001	0.022	<0.001	0.032	0.102	0.034	<0.001	<0.001	0.002	0.009	0.064	<0.001	0.002	<0.001	<0.001	0.006	0.487	<0.001	0.005	<0.001

ρ: Spearman's rho. Bold ρ: p<0.05.
Marked in orange: cytokines positively associated with all or virtually all IgM.
Marked in green: cytokines inversely associated with all or virtually all IgM.
Cytokines HGF, VEGF, IP-10, RANTES, MCP-1, MIG, IFN-γ, IL-5, and IL-1β are not shown because no ρ had a p<0.05.

Supplemental Table 3. Percentage of participants with different numbers of cytokines with a relative intraindividual change of concentrations from 2016-17 to 2020-21 equal or greater than 15%, by SARS-CoV-2 seropositivity, and by COVID-19 disease.

Change in cytokine concentrations[a]	Total (N = 154) (%)	SARS-CoV-2 status		P	COVID-19 disease		P
		Seronegative (N = 104) (%)	Seropositive (N = 41) (%)		No COVID (N = 134) (%)	COVID (N = 20) (%)	
Change ≥15% (Δ ≥15% or ∇ ≥15%) (median)[b]	12.0	12.0	12.0	0.940[c]	12.0	12.5	0.985[c]
No change ≥15%	0.0	–	–		–	–	
Change ≥15% in 1 to 10 CKs	40.3	38.5	43.9	0.576[d]	39.6	45.0	0.635[d]
Change ≥15% in >10 CKs	59.7	61.5	56.1		60.4	55.0	
Δ ≥15% (median)[b]	6.0	6.0	6.0	0.682[c]	6.0	3.0	0.238[c]
No Δ ≥15%	6.5	6.7	4.9	>0.999[d]	6.7	5.0	>0.999[d]
Δ ≥15% in 1 to 10 CKs	68.2	67.3	68.3		67.9	70.0	
Δ ≥15% in >10 CKs	25.3	26.0	26.8		25.4	25.0	
∇ ≥15% (median)[b]	4.0	3.0	5.0	0.529[c]	3.5	6.5	0.104[c]
No ∇≥15%	3.2	1.0	9.8	0.031[d]	3.0	5.0	0.739[d]
∇ ≥15% in 1 to 10 CKs	84.4	85.5	82.9		84.3	85.0	
∇ ≥15% in >10 CKs	12.3	13.5	7.3		12.7	10.0	

[a] Units used for computing the relative intraindividual change were base 10 logtransformed pg/mL.
[b] Median number of cytokines per participant that meet the corresponding intraindividual change concentration.
[c] Mann-Whitney's *U* test (two-tail).
[d] Fisher's exact test (two-tail).

68% of participants had between 1 and 10 cytokines with a relative intraindividual increase of concentrations ≥15%. The maximum number of cytokines with an increase ≥15% in one person was 24, while 6.5% of participants had no cytokine with an increase ≥15%. The number of cytokines with an increase ≥15% was similar for seropositive and seronegative SARS-CoV-2 infection, and for participants with and without COVID-19 disease.

63% of participants had between 1 and 5 cytokines with a relative intraindividual decrease ≥15%. The maximum number of cytokines with a decrease ≥15% in one person was 27 (90% of 30 cytokines), and while 3.2% of participants had no cytokine with a decrease ≥15%. The number of cytokines with a decrease ≥15% for participant was higher for SARS-CoV-2 seropositives participants (vs. seronegatives), and for participants who developed COVID-19 disease (vs. participants without COVID-19).

Supplemental Table 4. Concentrations of 30 cytokines in 2016-17 by sex (N=240).

Cytokine (pg/mL)	**Total** (N = 240)	**Men** (N = 113)	**Women** (N = 127)	**P**[a]
Growth factors				
G-CSF (median)	33.35	32.39	49.48	0.180
(P25, P75)	(8.09, 108.0)	(6.29, 98.33)	(11.30, 125.5)	
Geometric mean	35.72	31.65	39.78	
EGF (median)	85.75	72.05	88.83	0.062
(P25, P75)	(41.3, 154.9)	(38.03, 119.7)	(47.89, 211.6)	
Geometric mean	80.02	64.91	96.41	
FGF (median)	12.90	12.34	13.36	0.634
(P25, P75)	(4.35, 33.03)	(4.60, 30.96)	(4.19, 33.42)	
Geometric mean	14.61	14.13	15.05	
GM-CSF (median)	8.83	8.53	10.49	0.607
(P25, P75)	(1.09, 46.36)	(1.09, 46.17)	(1.09, 47.64)	
Geometric mean	8.04	7.24	8.82	
HGF (median)	367.4	371.3	363.6	0.249
(P25, P75)	(262.6, 525.0)	(286.1, 582.6)	(246.0, 511.9)	
Geometric mean	381.4	404.7	361.7	
VEGF (median)	5.37	4.54	5.46	0.978
(P25, P75)	(2.36, 9.53)	(2.55, 9.87)	(2.13, 8.75)	
Geometric mean	4.53	4.66	4.42	
Chemokines				
IL-8 (median)	16.25	17.44	15.25	0.109
(P25, P75)	(12.16, 23.47)	(12.77, 24.68)	(11.17, 22.66)	
Geometric mean	16.13	17.15	15.28	
IP-10 (median)	6.68	6.71	6.52	0.810
(P25, P75)	(4.18, 10.13)	(4.43, 9.80)	(3.98, 10.18)	
Geometric mean	6.73	6.84	6.63	
RANTES (median)	3414	3455	3339	0.899
(P25, P75)	(2808, 4212)	(2829, 3980)	(2733, 4760)	
Geometric mean	3603	3519	3679	
EOTAXIN (median)	77.07	87.08	70.98	0.001
(P25, P75)	(58.44, 100.9)	(63.62, 110.9)	(54.96, 95.66)	
Geometric mean	76.92	85.94	69.70	
MIP-1α (median)	36.29	28.91	38.02	0.183
(P25, P75)	(<LOQ, 113.6)	(<LOQ, 109.3)	(<LOQ, 118.1)	
Geometric mean	37.09	33.05	41.10	
MIP-1β (median)	172.9	147.3	200.5	0.042
(P25, P75)	(93.08, 446.9)	(85.39, 316.3)	(100.2, 578.1)	
Geometric mean	216.2	176.5	259.0	
MCP-1 (median)	654.4	672.0	624.5	0.159
(P25, P75)	(493.9, 894.9)	(506.6, 969.8)	(488.1, 834.2)	
Geometric mean	707.8	736.6	683.1	
MIG (median)	<LOQ	<LOQ	<LOQ	–
(P25, P75)	(<LOQ, 26.85)	(<LOQ, 30.51)	<LOQ, 20.63)	
Geometric mean	10.52	11.03	10.09	

[continued]

Supplemental Table 4. Continued.

Cytokine (pg/mL)	**Total**	**Men**	**Women**	**P**[a]
TH1				
IL-2 (median)	12.60	9.13	18.87	0.028
(P25, P75)	(2.46, 61.21)	(1.9, 39.10)	(3.63, 101.4)	
Geometric mean	14.13	9.79	19.60	
IL-12 (median)	57.61	51.66	61.49	0.078
(P25, P75)	(40.98, 89.25)	(32.02, 82.86)	(45.36, 92.09)	
Geometric mean	60.24	55.81	64.47	
IFN-γ (median)	<LOQ	<LOQ	<LOQ	–
(P25, P75)	(<LOQ, 0.22)	(<LOQ, 0.22)	(<LOQ, 0.22)	
Geometric mean	<LOQ	0.22	<LOQ	
TH2				
IL-4 (median)	1.54	1.54	1.54	0.461
(P25, P75)	(1.42, 4.57)	(1.42, 4.70)	(1.42, 4.55)	
Geometric mean	3.46	3.57	3.37	
IL-5 (median)	<LOQ	0.77	<LOQ	–
(P25, P75)	(<LOQ, 1.11)	(<LOQ, 1.11)	(<LOQ, 1.11)	
Geometric mean	0.84	0.93	0.78	
IL-13 (median)	6.90	5.48	8.35	0.227
(P25, P75)	(<LOQ, 17.34)	(<LOQ, 17.83)	(1.77, 16.25)	
Geometric mean	7.12	6.38	7.84	
Pro-inflammatory				
IL-1β (median)	0.91	0.93	0.85	0.600
(P25, P75)	(<LOQ, 2.00)	(<LOQ, 2.22)	(<LOQ, 1.80)	
Geometric mean	1.07	1.14	1.01	
TNF-α (median)	3.28	2.80	4.97	0.131
(P25, P75)	(1.34, 14.30)	(1.13, 12.22)	(1.39, 15.81)	
Geometric mean	4.02	3.34	4.73	
IL-6 (median)	15.74	16.37	15.59	0.242
(P25, P75)	(4.71, 51.39)	(4.15, 41.15)	(5.54, 76.24)	
Geometric mean	16.29	14.08	18.56	
IFN-α (median)	19.42	16.62	22.44	0.105
(P25, P75)	(6.86, 54.94)	(5.89, 45.91)	(7.00, 70.70)	
Geometric mean	19.46	16.17	22.93	
IL-2R (median)	141.6	127.3	156.8	0.185
(P25, P75)	(55.03, 427.8)	(47.53, 420.6)	(57.75, 436.7)	
Geometric mean	169.4	149.9	188.8	
IL-17 (median)	<LOQ	<LOQ	<LOQ	–
(P25, P75)	(<LOQ, 1.56)	(<LOQ, 1.58)	(<LOQ, 1.54)	
Geometric mean	1.19	1.35	1.06	
Regulatory				
IL-7 (median)	14.01	14.01	13.89	0.631
(P25, P75)	(2.84, 31.06)	(2.84, 32.51)	(2.84, 30.34)	
Geometric mean	12.33	12.82	11.90	

[continued]

Supplemental Table 4. Continued.

Cytokine (pg/mL)	**Total**	**Men**	**Women**	**P**[a]
Anti-inflammatory				
IL-10 (median)	3.34	3.02	4.18	0.179
(P25, P75)	(<LOQ, 30.62)	(<LOQ, 23.89)	(<LOQ, 36.85)	
Geometric mean	4.46	3.77	5.18	
IL-15 (median)	<LOQ	<LOQ	<LOQ	–
(P25, P75)	(<LOQ, 42.13)	(<LOQ, 9.90)	(<LOQ, 63.66)	
Geometric mean	9.28	7.90	10.70	
IL-1RA (median)	262.5	228.4	288.4	0.012
(P25, P75)	(166.7, 504.7)	(143.5, 390.6)	(182.4, 576.8)	
Geometric mean	295.7	257.8	334.2	

P25: percentile 25th. P75: percentile 75th.
<LOQ: value lower than the minimum concentration of the corresponding range of limits of quantification. See Supplemental Table 1.

[a] Mann-Whitney's *U* test (two-tail).

Supplemental Table 5a. Concentrations of 30 cytokines in 2016-17 by age group (N=240).

Cytokine (pg/mL)	**<45 years** (N = 88)	**45 – 64 years** (N = 105)	**≥65 years** (N = 47)	**P**[a]
Growth factors				
G-CSF (median)	70.91	22.84	22.94	0.012
(P25, P75)	(14.06, 158.9)	(<LOQ, 93.45)	(8.09, 92.29)	
Geometric mean	52.69	27.83	30.14	
EGF (median)	92.48	76.33	75.12	0.400
(P25, P75)	(52.16, 161.4)	(39.38, 195.9)	(38.41, 110.3)	
Geometric mean	81.04	78.98	80.48	
FGF (median)	14.10	12.34	10.49	0.426
(P25, P75)	(5.29, 36.18)	(4.35, 38.90)	(3.86, 22.02)	
Geometric mean	15.99	14.70	12.16	
GM-CSF (median)	11.84	9.26	4.70	0.293
(P25, P75)	(1.15, 51.59)	(0.74, 57.23)	(0.74, 32.54)	
Geometric mean	9.59	8.46	5.15	
HGF (median)	344.9	389.7	375.9	0.060
(P25, P75)	(222.2, 469.8)	(262.6, 654.2)	(310.1, 489.7)	
Geometric mean	333.0	401.6	437.9	
VEGF (median)	4.49	5.46	5.78	0.541
(P25, P75)	(2.08, 10.21)	(2.28, 9.06)	(3.06, 10.20)	
Geometric mean	4.43	4.16	5.71	
Chemokines				
IL-8 (median)	14.40	15.14	22.10	<0.001
(P25, P75)	(10.63, 21.64)	(11.93, 22.38)	(16.32, 30.70)	
Geometric mean	14.95	15.28	21.02	
IP-10 (median)	6.29	5.92	8.50	0.006
(P25, P75)	(4.23, 9.52)	(3.69, 9.64)	(6.26, 15.27)	
Geometric mean	6.21	6.26	9.17	
RANTES (median)	3331	3369	3529	0.382
(P25, P75)	(2821, 4095)	(2742, 4322)	(2992, 4760)	
Geometric mean	3497	3574	3877	
EOTAXIN (median)	62.49	81.72	95.42	<0.001
(P25, P75)	(50.28, 86.62)	(62.12, 109.0)	(66.58, 134.7)	
Geometric mean	64.33	81.03	95.68	
MIP-1α (median)	37.16	36.29	31.81	0.968
(P25, P75)	(<LOQ, 108.6)	(<LOQ, 131.2)	(<LOQ, 95.02)	
Geometric mean	34.65	39.74	36.12	
MIP-1β (median)	219.2	135.9	140.4	0.138
(P25, P75)	(124.4, 458.0)	(82.22, 513.9)	(91.02, 268.9)	
Geometric mean	240.7	213.3	182.3	
MCP-1 (median)	583.0	677.1	750.8	0.023
(P25, P75)	(471.2, 741.7)	(509.2, 930.9)	(541.1, 976.3)	
Geometric mean	628.5	743.4	792.3	
MIG (median)	<LOQ	<LOQ	6.27	–
(P25, P75)	(<LOQ, 20.63)	(<LOQ, 26.85)	(<LOQ, 44.62)	
Geometric mean	9.15	10.75	13.01	

[continued]

Supplemental Table 5a. Continued.

Cytokine (pg/mL)	**<45 years** (N = 88)	**45 – 64 years** (N = 105)	**≥65 years** (N = 47)	**P**[a]
TH1				
IL-2 (median)	18.55	11.05	6.07	0.131
(P25, P75)	(5.22, 77.37)	(1.90, 106.1)	(2.20, 32.26)	
Geometric mean	17.60	14.52	8.83	
IL-12 (median)	58.60	51.98	63.59	0.369
(P25, P75)	(42.95, 81.19)	(33.82, 87.07)	(43.60, 108.7)	
Geometric mean	58.86	57.03	71.07	
IFN-γ (median)	<LOQ	<LOQ	<LOQ	–
(P25, P75)	(<LOQ, 0.22)	(<LOQ, 0.22)	(<LOQ, 0.22)	
Geometric mean	<LOQ	<LOQ	<LOQ	
TH2				
IL-4 (median)	1.54	1.59	1.54	0.817
(P25, P75)	(1.42, 4.41)	(1.42, 7.25)	(1.42, 3.94)	
Geometric mean	3.34	3.73	3.12	
IL-5 (median)	<LOQ	0.66	0.66	–
(P25, P75)	(<LOQ, 1.11)	(<LOQ, 1.35)	(<LOQ, 0.91)	
Geometric mean	0.82	0.94	0.67	
IL-13 (median)	8.48	5.48	5.09	0.353
(P25, P75)	(1.77, 20.22)	(<LOQ, 20.12)	(1.77, 11.66)	
Geometric mean	8.41	7.04	5.33	
Pro-inflammatory				
IL-1β (median)	0.88	0.85	0.99	0.667
(P25, P75)	(<LOQ, 1.60)	(<LOQ, 2.29)	(0.52, 2.26)	
Geometric mean	0.99	1.07	1.23	
TNF-α (median)	8.84	2.43	1.74	0.001
(P25, P75)	(1.53, 25.83)	(0.44, 11.34)	(1.13, 6.84)	
Geometric mean	6.86	3.05	2.74	
IL-6 (median)	22.91	15.22	11.04	0.780
(P25, P75)	(4.36, 70.10)	(4.44, 48.57)	(5.46, 41.55)	
Geometric mean	17.18	15.63	16.20	
IFN-α (median)	23.30	17.65	15.11	0.379
(P25, P75)	(8.01, 64.43)	(4.93, 64.82)	(5.78, 35.91)	
Geometric mean	21.29	19.58	16.19	
IL-2R (median)	195.2	127.3	134.4	0.280
(P25, P75)	(63.68, 574.6)	(47.41, 382.7)	(54.48, 397.1)	
Geometric mean	194.3	152.6	165.3	
IL-17 (median)	<LOQ	<LOQ	<LOQ	–
(P25, P75)	(<LOQ, 1.54)	(<LOQ, 1.81)	(<LOQ, 1.59)	
Geometric mean	1.06	1.30	1.20	
Regulatory				
IL-7 (median)	14.01	17.36	10.51	0.616
(P25, P75)	(3.72, 32.51)	(<LOQ, 31.06)	(2.84, 28.63)	
Geometric mean	13.60	12.21	10.47	

[continued]

Supplemental Table 5a. Continued.

Cytokine (pg/mL)	**<45 years** (N = 88)	**45 – 64 years** (N = 105)	**≥65 years** (N = 47)	**P**[a]
Anti-inflammatory				
IL-10 (median)	4.14	3.54	1.24	0.508
(P25, P75)	(<LOQ, 36.49)	(<LOQ, 38.87)	(<LOQ, 14.33)	
Geometric mean	4.66	4.98	3.21	
IL-15 (median)	<LOQ	<LOQ	<LOQ	–
(P25, P75)	(<LOQ, 29.08)	(<LOQ, 108.0)	(<LOQ, <LOQ)	
Geometric mean	8.46	10.97	7.57	
IL-1RA (median)	344.2	255.0	206.5	0.105
(P25, P75)	(177.0, 514.7)	(173.0, 519.7)	(150.2, 326.5)	
Geometric mean	295.3	311.6	263.9	

P25: percentile 25th. P75: percentile 75th.
<LOQ: value lower than the minimum concentration of the corresponding range of limits of quantification. See Supplemental Table 1.

[a] Kruskal-Wallis test (two-tailed).

Supplemental Table 5b. Relative intraindividual change (%) from 2016-17 to 2020-21 of concentrations of cytokines, by age group (N=154).

Cytokine[a]	<46 years (N = 51)	46 – 62 years (N = 49)	≥63 years (N = 54)	P[b]
Growth factors				
G-CSF (median)	6.77	0.00	0.00	0.175
(P25, P75)	(-7.42, 25.67)	(0.00, 36.81)	(-5.62, 13.98)	
∆ ≥15%	39.2	36.7	24.1	
∇ ≥15%	11.8	16.3	20.4	
EGF (median)	9.29	5.47	3.85	0.038
(P25, P75)	(1.84, 27.08)	(-5.13, 30.29)	(-3.83, 12.20)	
∆ ≥15%	41.2	34.7	22.2	
∇ ≥15%	2.0	16.3	9.3	
FGF (median)	0.00	0.00	0.00	0.176
(P25, P75)	(-15.65, 30.47)	(-26.45, 12.87)	(-19.60, 23.04)	
∆ ≥15%	41.2	22.4	25.9	
∇ ≥15%	25.5	36.7	29.6	
GM-CSF (median)	0.00	0.00	0.00	0.792
(P25, P75)	(-14.42, 20.50)	(-12.63, 61.56)	(-17.94, 34.45)	
∆ ≥15%	29.4	34.7	29.6	
∇ ≥15%	23.5	22.4	25.9	
HGF (median)	7.81	4.87	4.00	0.011
(P25, P75)	(3.50, 11.94)	(0.60, 9.52)	(-1.16, 7.43)	
∆ ≥15%	13.7	10.2	3.7	
∇ ≥15%	0.0	8.2	1.9	
VEGF (median)	17.03	2.62	5.45	0.299
(P25, P75)	(-1.41, 71.24)	(-7.89, 37.69)	(-9.07, 26.70)	
∆ ≥15%	51.0	34.7	42.6	
∇ ≥15%	17.6	18.4	16.7	
Chemokines				
IL-8 (median)	8.67	6.34	9.30	0.963
(P25, P75)	(0.70, 16.87)	(-1.09, 19.17)	(0.23, 15.33)	
∆ ≥15%	29.4	30.6	25.9	
∇ ≥15%	2.0	2.0	0.0	
IP-10 (median)	-94.40	-99.23	-96.41	0.791
(P25, P75)	(-134.5, -68.95)	(-167.1, -66.62)	(-149.3, -58.51)	
∆ ≥15%	5.9	4.1	3.7	
∇ ≥15%	94.1	87.8	90.7	
RANTES (median)	0.00	-0.62	0.00	0.346
(P25, P75)	(-0.96, 0.63)	(-2.73, 0.58)	(-1.72, 0.82)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
EOTAXIN (median)	0.04	-1.14	-0.51	0.640
(P25, P75)	(-4.55, 4.70)	(-5.18, 3.62)	(-4.89, 6.88)	
∆ ≥15%	3.9	2.0	7.4	
∇ ≥15%	7.8	0.0	3.7	
MIP-1α (median)	9.03	0.00	0.00	0.026
(P25, P75)	(-1.09, 71.25)	(-17.58, 13.17)	(-7.29, 10.07)	
∆ ≥15%	41.2	24.5	18.5	
∇ ≥15%	15.7	24.5	18.5	
MIP-1β (median)	4.06	1.29	1.87	0.182
(P25, P75)	(-2.50, 16.79)	(-4.97, 10.88)	(-3.38, 6.53)	
∆ ≥15%	29.4	12.2	5.6	
∇ ≥15%	2.0	12.2	5.6	

[continued]

Supplemental Table 5b. Continued

Cytokine[a]	<46 years (N = 51)	46 – 62 years (N = 49)	≥63 years (N = 54)	P[b]
MCP-1 (median)	2.20	0.46	1.40	0.207
(P25, P75)	(-0.82, 4.27)	(-2.66, 3.13)	(-3.64, 4.42)	
Δ ≥15%	0.0	2.0	0.0	
∇ ≥15%	0.0	4.1	0.0	
MIG (median)	0.00	0.00	0.00	0.886
(P25, P75)	(0.00, 0.00)	(-24.64, 0.00)	(0.00, 1.24)	
Δ ≥15%	11.8	18.4	16.7	
∇ ≥15%	11.8	26.5	14.8	
TH1				
IL-2 (median)	25.19	-3.49	6.52	0.029
(P25, P75)	(-11.46, 71.93)	(-40.32, 38.51)	(-10.83, 58.64)	
Δ ≥15%	56.9	36.7	44.4	
∇ ≥15%	21.6	38.8	20.4	
IL-12 (median)	1.78	1.23	0.17	0.369
(P25, P75)	(-3.99, 7.13)	(-5.79, 5.37)	(-7.38, 5.17)	
Δ ≥15%	5.9	8.2	5.6	
∇ ≥15%	3.9	12.2	7.4	
IFN-γ (median)	0.00	0.00	0.00	0.631
(P25, P75)	(0.00, 0.00)	(0.00, 0.00)	(0.00, 0.00)	
Δ ≥15%	9.8	6.1	13.0	
∇ ≥15%	9.8	8.2	7.4	
TH2				
IL-4 (median)	0.00	0.00	0.00	0.315
(P25, P75)	(0.00, 33.60)	(0.00, 0.00)	(0.00, 0.00)	
Δ ≥15%	25.5	12.2	14.8	
∇ ≥15%	11.8	18.4	16.7	
IL-5 (median)	0.00	0.00	0.00	0.347
(P25, P75)	(0.00, 70.06)	(-81.09, 25.40)	(-1.52, 0.00)	
Δ ≥15%	31.4	28.6	20.4	
∇ ≥15%	17.6	26.5	20.4	
IL-13 (median)	0.00	0.00	0.00	0.617
(P25, P75)	(-11.23, 77.18)	(-1.34, 7.25)	(-23.32, 27.07)	
Δ ≥15%	37.3	20.4	27.8	
∇ ≥15%	21.6	20.4	29.6	
Pro-inflammatory				
IL-1β (median)	0.00	0.00	0.00	0.217
(P25, P75)	(-8.14, 61.01)	(-8.71, 29.21)	(-32.29, 27.73)	
Δ ≥15%	47.1	28.6	27.8	
∇ ≥15%	23.5	24.5	31.5	
TNF-α (median)	25.69	6.95	0.00	0.420
(P25, P75)	(-3.94, 89.82)	(-28.41, 86.99)	(0.00, 56.95)	
Δ ≥15%	58.8	42.9	44.4	
∇ ≥15%	17.6	26.5	16.7	
IL-6 (median)	14.37	0.64	5.15	0.520
(P25, P75)	(-7.81, 59.89)	(-6.80, 25.82)	(-7.48, 32.87)	
Δ ≥15%	49.0	28.6	38.9	
∇ ≥15%	11.8	16.3	22.2	
IFN-α (median)	14.14	0.00	0.00	0.206
(P25, P75)	(-5.32, 36.80)	(-13.51, 20.79)	(-8.66, 15.53)	
Δ ≥15%	47.1	30.6	24.1	
∇ ≥15%	13.7	18.4	14.8	

[continued]

Supplemental Table 5b. Continued

Cytokine[a]	<46 years (N = 51)	46 – 62 years (N = 49)	≥63 years (N = 54)	P[b]
IL-2R (median)	7.92	0.00	-0.55	0.081
(P25, P75)	(-7.37, 18.47)	(-12.61, 9.94)	(-10.18, 10.29)	
Δ ≥15%	29.4	16.3	22.2	
∇ ≥15%	9.8	22.4	16.7	
IL-17 (median)	0.00	0.00	0.00	0.382
(P25, P75)	(0.00, 42.26)	(0.00, 2.83)	(-6.94, 30.14)	
Δ ≥15%	29.4	20.4	29.6	
∇ ≥15%	13.7	16.3	24.1	
Regulatory				
IL-7 (median)	0.00	0.00	0.00	0.151
(P25, P75)	(-2.57, 30.93)	(-13.12, 13.82)	(-9.27, 9.64)	
Δ ≥15%	31.4	22.4	20.4	
∇ ≥15%	9.8	24.5	20.4	
Anti-inflammatory				
IL-10 (median)	0.00	0.00	0.00	0.242
(P25, P75)	(-11.37, 43.89)	(-24.55, 50.80)	(-25.23, 2.77)	
Δ ≥15%	37.3	32.7	20.4	
∇ ≥15%	19.6	28.6	27.8	
IL-15 (median)	0.00	0.00	0.00	0.035
(P25, P75)	(0.00, 190.4)	(0.00, 0.00)	(0.00, 0.00)	
Δ ≥15%	35.3	10.2	13.0	
∇ ≥15%	11.8	18.4	9.3	
IL-1RA (median)	1.06	-4.39	-2.92	0.046
(P25, P75)	(-8.24, 12.29)	(-12.23, 3.29)	(-9.62, 3.18)	
Δ ≥15%	15.7	8.2	0.0	
∇ ≥15%	11.8	12.2	11.1	

P25: percentile 25th. P75: percentile 75th.
Age groups are based on each participant's age in 2020-21.

[a] Units used for computing the relative intraindividual change for cytokine levels were pg/mL, base 10 logtransformed.
[b] Kruskal-Wallis test (two-tailed).

Supplemental Table 6a. Concentrations of 30 cytokines in 2016-17 by body mass index (N=240).

Cytokine (pg/mL)	**Underweight & Normal** (N = 101)	**Overweight** (N = 91)	**Obese** (N = 48)	**P**[a]
Growth factors				
G-CSF (median)	34.95	32.39	38.74	0.625
(P25, P75)	(12.70, 112.5)	(8.09, 125.5)	(5.68, 94.35)	
Geometric mean	39.78	32.16	34.75	
EGF (median)	86.53	77.88	89.97	0.869
(P25, P75)	(41.99, 147.4)	(42.47, 200.8)	(39.94, 105.0)	
Geometric mean	74.16	91.30	73.15	
FGF (median)	13.36	12.34	14.10	0.858
(P25, P75)	(3.70, 31.49)	(6.26, 37.98)	(5.76, 33.42)	
Geometric mean	14.05	15.06	14.98	
GM-CSF (median)	11.57	8.79	6.74	0.373
(P25, P75)	(1.09, 63.06)	(0.74, 38.19)	(0.76, 40.79)	
Geometric mean	10.32	6.78	6.56	
HGF (median)	363.6	375.9	377.1	0.935
(P25, P75)	(273.9, 556.9)	(262.6, 497.2)	(233.4, 547.8)	
Geometric mean	406.1	369.4	355.0	
VEGF (median)	5.33	5.46	5.35	0.627
(P25, P75)	(2.74, 9.32)	(1.87, 9.63)	(2.61, 9.95)	
Geometric mean	4.66	4.06	5.25	
Chemokines				
IL-8 (median)	14.40	16.42	17.68	0.057
(P25, P75)	(10.52, 22.94)	(12.31, 23.20)	(14.73, 24.59)	
Geometric mean	14.78	16.37	18.86	
IP-10 (median)	6.71	6.68	6.50	0.873
(P25, P75)	(4.10, 10.68)	(4.17, 10.18)	(3.83, 9.30)	
Geometric mean	7.12	6.62	6.15	
RANTES (median)	3288	3481	3513	0.441
(P25, P75)	(2842, 4211)	(2697, 4097)	(2995, 5061)	
Geometric mean	3483	3598	3878	
EOTAXIN (median)	73.44	78.90	82.78	0.653
(P25, P75)	(58.50, 100.3)	(60.57, 100.8)	(54.08, 111.8)	
Geometric mean	74.01	78.85	79.59	
MIP-1α (median)	28.91	38.78	37.16	0.842
(P25, P75)	(6.65, 115.8)	(5.90, 109.8)	(6.90, 132.6)	
Geometric mean	39.75	34.29	37.22	
MIP-1β (median)	151.8	186.2	202.3	0.771
(P25, P75)	(85.82, 510.3)	(104.2, 496.9)	(94.44, 338.0)	
Geometric mean	215.7	235.4	184.9	
MCP-1 (median)	645.0	647.1	695.6	0.971
(P25, P75)	(488.1, 902.0)	(495.7, 843.5)	(497.9, 943.3)	
Geometric mean	736.5	697.1	670.1	
MIG (median)	4.85	4.85	6.27	0.733
(P25, P75)	(3.67, 20.63)	(3.67, 30.51)	(3.67, 30.51)	
Geometric mean	9.78	10.99	11.32	

[continued]

Supplemental Table 6a. Continued.

Cytokine (pg/mL)	**Underweight & Normal** (N = 101)	**Overweight** (N = 91)	**Obese** (N = 48)	**P**[a]
TH1				
IL-2 (median)	11.25	13.77	13.33	0.548
(P25, P75)	(2.57, 70.52)	(3.63, 92.22)	(1.97, 47.75)	
Geometric mean	13.61	17.46	10.25	
IL-12 (median)	56.96	58.25	61.20	0.982
(P25, P75)	(41.88, 88.80)	(39.28, 89.00)	(40.14, 95.77)	
Geometric mean	64.19	56.40	59.69	
IFN-γ (median)	0.14	0.14	0.14	0.116
(P25, P75)	(0.14, 0.21)	(0.14, 0.22)	(0.14, 0.22)	
Geometric mean	0.18	0.23	0.23	
TH2				
IL-4 (median)	1.54	1.65	1.50	0.568
(P25, P75)	(1.42, 4.40)	(1.42, 4.57)	(1.42, 6.84)	
Geometric mean	3.49	3.61	3.13	
IL-5 (median)	0.66	0.70	0.56	0.626
(P25, P75)	(0.36, 1.11)	(0.37, 1.15)	(0.35, 0.95)	
Geometric mean	0.83	0.89	0.81	
IL-13 (median)	8.35	5.09	8.69	0.389
(P25, P75)	(1.77, 15.97)	(1.21, 19.18)	(1.87, 15.00)	
Geometric mean	8.03	6.03	7.56	
Pro-inflammatory				
IL-1β (median)	0.79	0.91	1.20	0.133
(P25, P75)	(0.27, 1.80)	(0.27, 1.80)	(0.57, 3.21)	
Geometric mean	1.00	0.98	1.44	
TNF-α (median)	3.28	3.10	4.20	0.925
(P25, P75)	(0.79, 13.65)	(1.34, 14.20)	(1.39, 17.89)	
Geometric mean	3.77	4.08	4.45	
IL-6 (median)	23.86	12.09	14.10	0.272
(P25, P75)	(5.42, 62.13)	(4.36, 45.05)	(4.30, 59.62)	
Geometric mean	19.56	13.78	15.26	
IFN-α (median)	15.11	22.46	21.44	0.771
(P25, P75)	(6.35, 49.84)	(3.78, 68.36)	(8.30, 44.87)	
Geometric mean	18.71	20.50	19.11	
IL-2R (median)	134.5	141.6	166.6	0.892
(P25, P75)	(58.47, 424.3)	(50.92, 397.1)	(43.04, 444.5)	
Geometric mean	178.2	157.7	174.4	
IL-17 (median)	0.58	0.61	0.58	0.512
(P25, P75)	(0.46, 1.59)	(0.55, 1.59)	(0.46, 1.51)	
Geometric mean	1.24	1.17	1.13	
Regulatory				
IL-7 (median)	15.90	12.35	16.35	0.362
(P25, P75)	(3.72, 31.06)	(2.49, 28.55)	(2.84, 40.01)	
Geometric mean	13.27	10.49	14.31	

[continued]

Supplemental Table 6a. Continued.

Cytokine (pg/mL)	**Underweight & Normal** (N = 101)	**Overweight** (N = 91)	**Obese** (N = 48)	**P**[a]
Anti-inflammatory				
IL-10 (median)	4.10	3.14	1.12	0.701
(P25, P75)	(0.46, 44.49)	(0.50, 18.10)	(0.46, 34.69)	
Geometric mean	5.53	4.15	3.25	
IL-15 (median)	2.89	2.73	2.89	0.722
(P25, P75)	(1.74, 36.91)	(1.74, 28.02)	(1.74, 79.21)	
Geometric mean	10.23	8.30	9.33	
IL-1RA (median)	264.9	262.9	231.4	0.952
(P25, P75)	(172.8, 506.6)	(163.4, 498.0)	(161.6, 500.7)	
Geometric mean	307.7	295.3	272.7	

P25: percentile 25th. P75: percentile 75th.
<LOQ: value lower than the minimum concentration of the corresponding range of limits of quantification. See Supplemental Table 1.

[a] Kruskal-Wallis test (two-tailed).

Supplemental Table 6b. Relative intraindividual change (%) from 2016-17 to 2020-21 of concentrations of 30 cytokines, by body mass index (N=153).

Cytokine[a]	Underweight & Normal (N = 68)	Overweight (N = 49)	Obese (N = 36)	P[b]
Growth factors				
G-CSF (median)	6.63	0.00	0.00	0.312
(P25, P75)	(0.00, 32.79)	(-12.54, 18.37)	(-0.64, 16.38)	
Δ ≥15%	38.2	26.5	33.3	
∇ ≥15%	13.2	22.4	13.9	
EGF (median)	8.00	5.47	6.97	0.791
(P25, P75)	(-3.54, 23.26)	(-3.77, 20.70)	(-1.65, 17.64)	
Δ ≥15%	33.8	30.6	30.6	
∇ ≥15%	5.9	10.2	5.6	
FGF (median)	0.00	0.00	0.00	0.095
(P25, P75)	(-14.89, 21.51)	(-25.87, 24.05)	(-18.05, 24.74)	
Δ ≥15%	30.9	28.6	30.6	
∇ ≥15%	25.0	38.8	27.8	
GM-CSF (median)	0.00	0.00	0.00	0.169
(P25, P75)	(-13.51, 30.87)	(-27.23, 13.33)	(0.00, 47.77)	
Δ ≥15%	33.8	24.5	36.1	
∇ ≥15%	23.5	30.6	16.7	
HGF (median)	5.42	5.38	6.50	0.197
(P25, P75)	(0.79, 10.32)	(-0.41, 9.68)	(1.92, 10.43)	
Δ ≥15%	7.4	12.2	8.3	
∇ ≥15%	5.9	2.0	0.0	
VEGF (median)	8.96	6.94	2.29	0.948
(P25, P75)	(-7.10, 39.93)	(-11.35, 32.65)	(-8.41, 27.83)	
Δ ≥15%	45.6	42.9	38.9	
∇ ≥15%	16.2	22.4	13.9	
Chemokines				
IL-8 (median)	8.44	10.54	5.78	0.203
(P25, P75)	(0.48, 13.86)	(0.00, 17.68)	(-4.79, 14.61)	
Δ ≥15%	23.5	38.8	22.2	
∇ ≥15%	1.5	0.0	2.8	
IP-10 (median)	-96.08	-86.50	-110.6	0.582
(P25, P75)	(-133.0, -64.69)	(-149.40, -56.45)	(-181.5, -67.82)	
Δ ≥15%	2.9	6.1	5.6	
∇ ≥15%	91.2	91.8	88.9	
RANTES (median)	-0.11	0.00	-0.51	0.534
(P25, P75)	(-2.06, 0.60)	(-1.94, 1.11)	(-1.68, 0.43)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
EOTAXIN (median)	-0.09	-0.53	-1.90	0.628
(P25, P75)	(-4.47, 4.50)	(-5.14, 5.96)	(-5.36, 3.89)	
Δ ≥15%	5.9	2.0	5.6	
∇ ≥15%	2.9	6.1	2.8	
MIP-1α (median)	2.02	0.00	0.00	0.120
(P25, P75)	(-11.52, 27.11)	(-8.87, 20.02)	(-2.86, 10.87)	
Δ ≥15%	30.9	28.6	22.2	
∇ ≥15%	20.6	20.4	16.7	
MIP-1β (median)	3.74	2.19	1.63	0.466
(P25, P75)	(-3.06, 13.50)	(-6.70, 9.19)	(-2.47, 6.98)	
Δ ≥15%	22.1	12.2	8.3	
∇ ≥15%	7.4	6.1	5.6	

[continued]

Supplemental Table 6b. Continued

Cytokine[a]	Underweight & Normal (N = 68)	Overweight (N = 49)	Obese (N = 36)	P[b]
MCP-1 (median)	0.11	0.87	2.19	0.749
(P25, P75)	(-2.85, 3.28)	(-2.87, 3.74)	(-1.18, 4.43)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	1.5	2.0	0.0	
MIG (median)	0.00	0.00	0.00	0.695
(P25, P75)	(-4.01, 0.00)	(0.00, 0.00)	(-0.00, 0.00)	
Δ ≥15%	16.2	18.4	11.1	
∇ ≥15%	17.6	16.3	19.4	
TH1				
IL-2 (median)	19.60	2.58	1.80	0.183
(P25, P75)	(-18.66, 55.26)	(-19.20, 63.14)	(-10.75, 42.79)	
Δ ≥15%	51.5	44.9	36.1	
∇ ≥15%	26.5	30.6	22.2	
IL-12 (median)	1.71	-1.55	1.20	0.395
(P25, P75)	(-4.63, 6.61)	(-8.64, 5.73)	(-4.88, 5.10)	
Δ ≥15%	5.9	6.1	8.3	
∇ ≥15%	8.8	10.2	2.8	
IFN-γ (median)	0.00	0.00	0.00	0.822
(P25, P75)	(0.00, 0.00)	(0.00, 0.00)	(0.00, 0.00)	
Δ ≥15%	13.2	6.1	8.3	
∇ ≥15%	10.3	8.2	5.6	
TH2				
IL-4 (median)	0.00	0.00	0.00	0.976
(P25, P75)	(0.00, 7.00)	(0.00, 0.00)	(-13.45, 0.00)	
Δ ≥15%	20.6	16.3	13.9	
∇ ≥15%	16.2	8.2	25.0	
IL-5 (median)	0.00	0.00	0.00	0.394
(P25, P75)	(-4.56, 13.80)	(-14.62, 0.00)	(0.00, 80.95)	
Δ ≥15%	23.5	22.4	38.9	
∇ ≥15%	23.5	24.5	13.9	
IL-13 (median)	0.00	0.00	0.00	0.322
(P25, P75)	(-3.85, 39.85)	(-20.97, 29.59)	(-31.71, 13.69)	
Δ ≥15%	33.8	26.5	22.2	
∇ ≥15%	17.6	26.5	33.3	
Pro-inflammatory				
IL-1β (median)	0.00	0.00	0.00	0.576
(P25, P75)	(-15.09, 48.43)	(-23.35, 4.39)	(-43.16, 61.01)	
Δ ≥15%	39.7	20.4	41.7	
∇ ≥15%	25.0	26.5	30.6	
TNF-α (median)	25.48	0.00	0.00	0.176
(P25, P75)	(0.00, 91.95)	(-1.97, 49.16)	(-10.19, 86.99)	
Δ ≥15%	55.9	44.9	38.9	
∇ ≥15%	19.1	20.4	22.2	
IL-6 (median)	2.79	3.76	5.40	0.968
(P25, P75)	(-6.69, 20.89)	(-9.10, 37.04)	(-6.64, 58.05)	
Δ ≥15%	35.3	38.8	44.4	
∇ ≥15%	10.3	22.4	22.2	
IFN-α (median)	5.22	0.00	2.24	0.300
(P25, P75)	(-8.32, 26.69)	(-9.21, 20.21)	(-9.69, 19.09)	
Δ ≥15%	36.8	28.6	33.3	
∇ ≥15%	14.7	16.3	16.7	

[continued]

Supplemental Table 6b. Continued

Cytokine[a]	Underweight & Normal (N = 68)	Overweight (N = 49)	Obese (N = 36)	P[b]
IL-2R (median)	1.51	-2.33	2.27	0.338
(P25, P75)	(-8.92, 14.73)	(-11.73, 12.89)	(-7.63, 11.88)	
∆ ≥15%	23.5	20.4	22.2	
∇ ≥15%	16.2	18.4	13.9	
IL-17 (median)	0.00	0.00	0.00	0.954
(P25, P75)	(0.00, 42.26)	(0.00, 0.00)	(-0.29, 0.00)	
∆ ≥15%	32.4	22.4	22.2	
∇ ≥15%	16.2	18.4	22.2	
Regulatory				
IL-7 (median)	0.00	0.00	0.00	0.853
(P25, P75)	(-2.20, 17.88)	(-9.06, 13.06)	(-17.34, 16.60)	
∆ ≥15%	26.5	20.4	25.0	
∇ ≥15%	13.2	18.4	27.8	
Anti-inflammatory				
IL-10 (median)	0.00	0.00	0.00	0.188
(P25, P75)	(-21.37, 55.37)	(-20.71, 2.69)	(-2.72, 34.34)	
∆ ≥15%	33.8	20.4	36.1	
∇ ≥15%	26.5	26.5	22.2	
IL-15 (median)	0.00	0.00	0.00	0.202
(P25, P75)	(-6.73, 27.57)	(0.00, 0.00)	(0.00, 2.78)	
∆ ≥15%	26.5	14.3	13.9	
∇ ≥15%	23.5	8.2	0.0	
IL-1RA (median)	-0.16	-3.44	-2.99	0.845
(P25, P75)	(-8.36, 7.83)	(-12.99, 2.47)	(-9.00, 5.42)	
∆ ≥15%	13.2	4.1	2.8	
∇ ≥15%	8.8	20.4	5.6	

P25: percentile 25th. P75: percentile 75th.
Body mass index (BMI) is based on each participant's BMI in 2020-21.

[a] Units used for computing the relative intraindividual change for cytokine levels were pg/mL, base 10 logtransformed.
[b] Kruskal-Wallis test (two-tailed).

Supplemental Table 7a. Concentrations of 30 cytokines in 2016-17 by tobacco smoking (N=240).

Cytokine (pg/mL)	**Non-smoker** (N = 93)	**Former smoker** (N = 99)	**Current smoker** (N = 48)	**P**[a]
Growth factors				
G-CSF (median)	51.85	31.84	33.67	0.625
(P25, P75)	(7.19, 125.5)	(8.09, 125.5)	(12.91, 90.89)	
Geometric mean	40.88	32.50	33.42	
EGF (median)	91.10	85.12	77.47	0.410
(P25, P75)	(44.40, 206.1)	(39.62, 120.1)	(55.86, 149.1)	
Geometric mean	93.10	71.48	75.33	
FGF (median)	13.36	12.34	12.97	0.984
(P25, P75)	(5.06, 31.33)	(4.19, 41.38)	(4.47, 25.18)	
Geometric mean	15.13	14.42	14.02	
GM-CSF (median)	7.55	6.40	15.69	0.056
(P25, P75)	(0.74, 45.18)	(1.09, 32.54)	(2.34, 172.6)	
Geometric mean	7.59	6.03	16.29	
HGF (median)	363.6	371.3	401.9	0.180
(P25, P75)	(241.1, 491.9)	(262.6, 548.1)	(313.5, 612.7)	
Geometric mean	345.4	390.1	441.0	
VEGF (median)	5.46	5.28	4.87	0.842
(P25, P75)	(2.10, 8.60)	(2.31, 10.91)	(2.60, 8.83)	
Geometric mean	4.37	4.71	4.49	
Chemokines				
IL-8 (median)	15.94	17.93	14.08	0.052
(P25, P75)	(10.90, 22.40)	(13.14, 28.20)	(11.12, 21.64)	
Geometric mean	15.42	17.87	14.28	
IP-10 (median)	6.91	7.64	4.72	0.004
(P25, P75)	(4.71, 10.08)	(4.43, 10.98)	(3.08, 8.16)	
Geometric mean	7.06	7.44	4.98	
RANTES (median)	3361	3415	3489	0.642
(P25, P75)	(2727, 4061)	(2829, 4101)	(2829, 5970)	
Geometric mean	3507	3622	3754	
EOTAXIN (median)	71.20	78.37	86.56	0.076
(P25, P75)	(53.62, 96.97)	(62.49, 101.7)	(60.18, 111.4)	
Geometric mean	72.03	78.92	82.85	
MIP-1α (median)	38.78	28.91	25.35	0.406
(P25, P75)	(7.64, 122.9)	(6.65, 113.6)	(5.90, 110.5)	
Geometric mean	44.53	33.29	32.54	
MIP-1β (median)	190.1	151.8	177.2	0.542
(P25, P75)	(103.8, 577.3)	(90.45, 332.5)	(87.12, 472.9)	
Geometric mean	247.0	200.1	196.0	
MCP-1 (median)	604.3	674.3	689.6	0.312
(P25, P75)	(494.8, 832.3)	(471.3, 903.2)	(525.2, 1010)	
Geometric mean	681.5	717.0	741.5	
MIG (median)	4.85	4.85	4.85	0.849
(P25, P75)	(3.67, 23.74)	(3.67, 30.51)	(3.84, 18.74)	
Geometric mean	10.71	11.47	8.51	

[continued]

Supplemental Table 7a. Continued.

Cytokine (pg/mL)	**Non-smoker** (N = 93)	**Former smoker** (N = 99)	**Current smoker** (N = 48)	**P**[a]
TH1				
IL-2 (median)	12.34	11.25	14.02	0.866
(P25, P75)	(3.61, 78.34)	(2.20, 51.16)	(2.20, 86.42)	
Geometric mean	15.60	12.90	14.09	
IL-12 (median)	59.04	55.76	49.35	0.256
(P25, P75)	(43.47, 92.83)	(42.88, 91.06)	(27.97, 75.77)	
Geometric mean	65.71	58.88	53.34	
IFN-γ (median)	0.14	0.14	0.14	0.571
(P25, P75)	(0.14, 0.22)	(0.14, 0.22)	(0.14, 0.22)	
Geometric mean	0.22	0.22	0.18	
TH2				
IL-4 (median)	1.54	1.65	1.54	0.590
(P25, P75)	(1.42, 3.94)	(1.42, 8.00)	(1.42, 3.88)	
Geometric mean	3.16	4.11	2.89	
IL-5 (median)	0.56	0.77	0.56	0.278
(P25, P75)	(0.35, 1.11)	(0.37, 1.19)	(0.35, 1.11)	
Geometric mean	0.77	0.92	0.85	
IL-13 (median)	8.35	5.48	8.41	0.287
(P25, P75)	(1.77, 15.34)	(1.21, 19.18)	(2.14, 19.18)	
Geometric mean	7.52	5.94	9.29	
Pro-inflammatory				
IL-1β (median)	0.91	0.91	0.82	0.904
(P25, P75)	(0.33, 2.22)	(0.27, 1.94)	(0.27, 1.91)	
Geometric mean	1.14	1.08	0.92	
TNF-α (median)	3.28	3.05	3.86	0.880
(P25, P75)	(1.36, 15.56)	(1.13, 15.51)	(1.39, 12.01)	
Geometric mean	4.32	3.70	4.15	
IL-6 (median)	14.90	15.22	21.78	0.621
(P25, P75)	(4.07, 46.71)	(4.63, 54.73)	(7.91, 66.50)	
Geometric mean	14.92	16.10	19.81	
IFN-α (median)	20.76	18.22	19.73	0.681
(P25, P75)	(5.89, 70.24)	(5.78, 47.74)	(9.02, 54.58)	
Geometric mean	21.07	17.27	21.32	
IL-2R (median)	141.6	134.5	163.1	0.983
(P25, P75)	(49.10, 502.2)	(49.91, 421.4)	(64.82, 388.8)	
Geometric mean	178.8	163.1	164.9	
IL-17 (median)	0.58	0.58	0.58	0.437
(P25, P75)	(0.46, 1.99)	(0.55, 1.56)	(0.46, 1.45)	
Geometric mean	1.22	1.34	0.87	
Regulatory				
IL-7 (median)	13.89	12.35	23.66	0.184
(P25, P75)	(2.84, 27.70)	(2.49, 31.74)	(4.23, 32.51)	
Geometric mean	12.65	10.43	16.54	

[continued]

Supplemental Table 7a. Continued.

Cytokine (pg/mL)	**Non-smoker** (N = 93)	**Former smoker** (N = 99)	**Current smoker** (N = 48)	**P**[a]
Anti-inflammatory				
IL-10 (median)	5.32	1.24	5.48	0.025
(P25, P75)	(0.50, 38.87)	(0.46, 11.71)	(0.51, 90.25)	
Geometric mean	5.66	2.70	7.95	
IL-15 (median)	2.89	2.73	2.89	0.433
(P25, P75)	(2.40, 56.60)	(1.74, 21.67)	(1.90, 48.21)	
Geometric mean	11.01	7.92	9.25	
IL-1RA (median)	255.0	250.0	312.6	0.763
(P25, P75)	(153.8, 500.7)	(166.0, 514.9)	(178.0, 474.7)	
Geometric mean	289.4	295.5	308.8	

P25: percentile 25th. P75: percentile 75th.
<LOQ: value lower than the minimum concentration of the corresponding range of limits of quantification. See Supplemental Table 1.

[a] Kruskal-Wallis test (two-tailed).

Supplemental Table 7b. Relative intraindividual change (%) from 2016-17 to 2020-21 of concentrations of cytokines, by tobacco smoking (N=154).

Cytokine[a]	Non-smoker (N = 59)	Former smoker (N = 56)	Current smoker (N = 39)	P[b]
Growth factors				
G-CSF (median)	0.00	3.68	2.68	0.046
(P25, P75)	(-15.54, 18.29)	(0.00, 36.81)	(0.00, 16.96)	
Δ ≥15%	28.8	41.1	28.2	
∇ ≥15%	25.4	10.7	10.3	
EGF (median)	6.95	8.29	7.02	0.833
(P25, P75)	(-3.40, 18.87)	(-3.00, 20.12)	(-3.59, 23.82)	
Δ ≥15%	30.5	30.4	38.5	
∇ ≥15%	10.2	5.4	5.1	
FGF (median)	0.00	0.00	0.00	0.310
(P25, P75)	(-21.74, 22.08)	(-14.61, 22.68)	(-17.74, 25.42)	
Δ ≥15%	28.8	32.1	28.2	
∇ ≥15%	39.0	25.0	25.6	
GM-CSF (median)	0.00	0.00	0.00	0.081
(P25, P75)	(-20.40, 10.39)	(0.00, 75.43)	(-18.67, 29.30)	
Δ ≥15%	22.0	41.1	30.8	
∇ ≥15%	27.1	19.6	25.6	
HGF (median)	4.87	5.38	6.00	0.575
(P25, P75)	(0.00, 10.20)	(0.60, 10.20)	(2.01, 10.28)	
Δ ≥15%	8.5	7.1	12.8	
∇ ≥15%	5.1	1.8	2.6	
VEGF (median)	5.28	8.30	5.47	0.754
(P25, P75)	(-7.48, 27.44)	(-8.41, 47.59)	(-7.91, 39.66)	
Δ ≥15%	44.1	46.4	35.9	
∇ ≥15%	18.6	16.1	17.9	
Chemokines				
IL-8 (median)	6.92	11.98	4.89	0.239
(P25, P75)	(0.00, 14.41)	(1.74, 18.57)	(0.00, 17.65)	
Δ ≥15%	23.7	32.1	30.8	
∇ ≥15%	0.0	0.0	5.1	
IP-10 (median)	-97.17	-98.79	-81.10	0.901
(P25, P75)	(-160.8, -61.36)	(-135.3, -68.52)	(-206.2, -48.71)	
Δ ≥15%	5.1	3.6	5.1	
∇ ≥15%	94.9	89.3	87.2	
RANTES (median)	0.00	-0.24	0.00	0.320
(P25, P75)	(-1.73, 1.12)	(-2.73, 0.43)	(-1.23, 0.24)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
EOTAXIN (median)	-0.74	-0.43	-0.11	0.617
(P25, P75)	(-3.95, 5.45)	(-5.06, 4.66)	(-5.12, 2.36)	
Δ ≥15%	6.8	5.4	0.0	
∇ ≥15%	5.1	1.8	5.1	
MIP-1α (median)	0.00	0.00	4.76	0.047
(P25, P75)	(-20.67, 14.62)	(-0.58, 25.14)	(0.00, 29.50)	
Δ ≥15%	23.7	28.6	33.3	
∇ ≥15%	32.2	12.5	10.3	
MIP-1β (median)	1.12	4.01	0.47	0.452
(P25, P75)	(-4.96, 7.99)	(-1.93, 10.22)	(-5.19, 14.05)	
Δ ≥15%	16.9	10.7	20.5	
∇ ≥15%	10.2	1.8	7.7	

[continued]

Supplemental Table 7b. Continued

Cytokine[a]	Non-smoker (N = 59)	Former smoker (N = 56)	Current smoker (N = 39)	P[b]
MCP-1 (median)	1.45	0.76	0.87	0.940
(P25, P75)	(-3.54, 4.35)	(-1.74, 3.58)	(-2.96, 3.82)	
Δ ≥15%	1.7	0.0	0.0	
∇ ≥15%	1.7	1.8	0.0	
MIG (median)	0.00	0.00	0.00	0.112
(P25, P75)	(-12.36, 0.00)	(0.00, 3.82)	(0.00, 0.00)	
Δ ≥15%	15.3	17.0	12.8	
∇ ≥15%	23.7	14.3	12.8	
TH1				
IL-2 (median)	5.81	17.02	0.00	0.716
(P25, P75)	(-19.69, 38.68)	(-10.58, 55.26)	(-24.46, 77.32)	
Δ ≥15%	44.1	51.8	41.0	
∇ ≥15%	28.8	19.6	33.3	
IL-12 (median)	-1.28	1.15	1.78	0.577
(P25, P75)	(-7.71, 5.45)	(-4.49, 6.14)	(-5.46, 7.13)	
Δ ≥15%	6.8	5.4	7.7	
∇ ≥15%	8.5	7.1	7.7	
IFN-γ (median)	0.00	0.00	0.00	0.814
(P25, P75)	(0.00, 0.00)	(0.00, 0.00)	(0.00, 0.00)	
Δ ≥15%	10.2	7.1	12.8	
∇ ≥15%	11.9	3.6	10.3	
TH2				
IL-4 (median)	0.00	0.00	0.00	0.359
(P25, P75)	(0.00, 0.00)	(0.00, 0.00)	(0.00, 7.93)	
Δ ≥15%	16.9	14.3	23.1	
∇ ≥15%	18.6	16.1	10.3	
IL-5 (median)	0.00	0.00	0.00	0.334
(P25, P75)	(0.00, 60.54)	(-4.56, 0.00)	(0.00, 60.21)	
Δ ≥15%	28.8	17.9	35.9	
∇ ≥15%	20.3	21.4	23.1	
IL-13 (median)	0.00	0.00	0.00	0.679
(P25, P75)	(-26.06, 34.41)	(0.00, 20.92)	(-12.03, 10.47)	
Δ ≥15%	32.2	28.6	23.1	
∇ ≥15%	32.2	16.1	23.1	
Pro-inflammatory				
IL-1β (median)	0.00	0.00	0.00	0.738
(P25, P75)	(-32.05, 56.60)	(-4.41, 27.90)	(-19.07, 47.18)	
Δ ≥15%	33.9	28.6	43.6	
∇ ≥15%	30.5	21.4	28.2	
TNF-α (median)	0.00	27.56	12.93	0.189
(P25, P75)	(-16.79, 69.46)	(0.00, 65.16)	(0.00, 109.11)	
Δ ≥15%	44.1	53.6	48.7	
∇ ≥15%	30.5	12.5	15.4	
IL-6 (median)	5.10	6.76	3.06	0.846
(P25, P75)	(-6.72, 32.02)	(-5.92, 49.58)	(-8.06, 35.44)	
Δ ≥15%	37.3	41.1	38.5	
∇ ≥15%	18.6	17.9	12.8	
IFN-α (median)	1.57	3.57	0.00	0.909
(P25, P75)	(-13.88, 36.03)	(-2.90, 20.28)	(-11.54, 62.96)	
Δ ≥15%	35.6	32.1	33.3	
∇ ≥15%	20.3	7.1	20.5	

[continued]

Supplemental Table 7b. Continued

Cytokine[a]	Non-smoker (N = 59)	Former smoker (N = 56)	Current smoker (N = 39)	P[b]
IL-2R (median)	-2.33	2.21	1.46	0.280
(P25, P75)	(-13.14, 13.33)	(-7.13, 10.49)	(-7.78, 20.57)	
Δ ≥15%	20.3	17.9	33.3	
∇ ≥15%	20.3	12.5	15.4	
IL-17 (median)	0.00	0.00	0.00	0.353
(P25, P75)	(-28.56, 27.60)	(0.00, 22.77)	(0.00, 41.87)	
Δ ≥15%	25.4	26.8	28.2	
∇ ≥15%	25.4	14.3	12.8	
Regulatory				
IL-7 (median)	0.00	0.00	0.56	0.674
(P25, P75)	(-6.19, 17.67)	(-6.89, 12.98)	(-6.78, 14.09)	
Δ ≥15%	27.1	23.2	23.1	
∇ ≥15%	18.6	21.4	12.8	
Anti-inflammatory				
IL-10 (median)	0.00	0.00	0.00	0.820
(P25, P75)	(-25.14, 32.41)	(-22.20, 29.35)	(-6.34, 43.89)	
Δ ≥15%	28.8	30.4	30.8	
∇ ≥15%	27.1	28.6	17.9	
IL-15 (median)	0.00	0.00	0.00	0.699
(P25, P75)	(0.00, 0.58)	(0.00, 0.00)	(0.00, 33.33)	
Δ ≥15%	18.6	14.3	28.2	
∇ ≥15%	13.6	10.7	15.4	
IL-1RA (median)	-1.41	-2.98	-2.32	0.758
(P25, P75)	(-12.10, 5.90)	(-9.00, 5.44)	(-7.17, 3.65)	
Δ ≥15%	8.5	7.1	7.7	
∇ ≥15%	16.9	7.1	10.3	

P25: percentile 25th. P75: percentile 75th.
Tobacco smoking groups are based on participant's data in 2020-21.

[a] Units used for computing the relative intraindividual change for cytokine levels were pg/mL, base 10 logtransformed.
[b] Kruskal-Wallis test (two-tailed).

Supplemental Table 8a. Concentrations of 30 cytokines in 2016-17 by educational level (N=240).

Cytokine (pg/mL)	**Primary or less** (N = 56)	**Secondary** (N = 65)	**University** (N = 119)	**P**[a]
Growth factors				
G-CSF (median)	33.35	27.24	34.95	0.725
(P25, P75)	(8.89, 121.1)	(5.68, 100.2)	(11.30, 122.9)	
Geometric mean	36.39	30.23	38.79	
EGF (median)	73.13	72.05	91.55	0.167
(P25, P75)	(39.06, 114.5)	(33.73, 119.7)	(48.29, 191.0)	
Geometric mean	80.21	66.53	88.42	
FGF (median)	12.09	13.36	13.85	0.525
(P25, P75)	(3.70, 30.74)	(3.86, 28.81)	(6.66, 44.71)	
Geometric mean	13.33	12.82	16.38	
GM-CSF (median)	11.68	4.70	10.25	0.225
(P25, P75)	(1.09, 57.03)	(0.53, 34.72)	(1.09, 51.60)	
Geometric mean	10.01	5.41	9.00	
HGF (median)	369.7	371.7	363.6	0.368
(P25, P75)	(310.5, 553.6)	(221.5, 584.0)	(246.0, 501.7)	
Geometric mean	450.4	375.2	355.8	
VEGF (median)	5.24	4.48	5.69	0.425
(P25, P75)	(2.55, 10.11)	(2.01, 7.95)	(2.59, 10.32)	
Geometric mean	4.95	4.07	4.60	
Chemokines				
IL-8 (median)	18.75	15.39	16.42	0.629
(P25, P75)	(12.39, 27.31)	(12.00, 22.78)	(11.17, 22.83)	
Geometric mean	15.68	15.72	16.58	
IP-10 (median)	5.95	7.64	6.68	0.470
(P25, P75)	(4.03, 9.57)	(4.30, 10.33)	(3.98, 10.18)	
Geometric mean	6.06	7.17	6.82	
RANTES (median)	3444	3361	3407	0.986
(P25, P75)	(2972, 4127)	(2694, 4706)	(2804, 4101)	
Geometric mean	3525	3686	3595	
EOTAXIN (median)	83.55	78.90	72.07	0.134
(P25, P75)	(62.94, 111.8)	(56.21, 99.45)	(57.21, 104.4)	
Geometric mean	86.90	72.08	75.26	
MIP-1α (median)	25.35	28.91	38.78	0.805
(P25, P75)	(6.65, 95.02)	(6.65, 122.9)	(6.65, 127.7)	
Geometric mean	35.54	35.73	38.62	
MIP-1β (median)	136.0	179.4	190.2	0.279
(P25, P75)	(84.12, 337.1)	(111.2, 439.4)	(91.02, 496.9)	
Geometric mean	185.6	225.1	227.3	
MCP-1 (median)	629.2	671.4	654.1	0.951
(P25, P75)	(487.2, 919.0)	(506.6, 896.4)	(492.8, 863.8)	
Geometric mean	733.7	696.7	702.0	
MIG (median)	5.56	4.85	4.85	0.425
(P25, P75)	(3.67, 20.63)	(3.67, 20.63)	(3.67, 30.51)	
Geometric mean	10.14	8.84	11.77	

[continued]

Supplemental Table 8a. Continued.

Cytokine (pg/mL)	**Primary or less** (N = 56)	**Secondary** (N = 65)	**University** (N = 119)	**P**[a]
TH1				
IL-2 (median)	7.51	13.77	14.27	0.271
(P25, P75)	(1.13, 42.65)	(2.20, 67.45)	(3.63, 79.15)	
Geometric mean	10.48	13.90	16.42	
IL-12 (median)	58.82	56.14	58.25	0.672
(P25, P75)	(44.04, 97.55)	(33.42, 87.45)	(41.86, 86.71)	
Geometric mean	67.85	56.38	59.06	
IFN-γ (median)	0.14	0.14	0.14	0.218
(P25, P75)	(0.14, 0.20)	(0.14, 0.22)	(0.14, 0.22)	
Geometric mean	0.19	0.19	0.23	
TH2				
IL-4 (median)	1.50	1.54	1.65	0.224
(P25, P75)	(1.42, 3.94)	(1.42, 4.70)	(1.42, 4.81)	
Geometric mean	2.73	3.53	3.82	
IL-5 (median)	0.56	0.56	0.77	0.307
(P25, P75)	(0.35, 1.11)	(0.37, 0.96)	(0.37, 1.15)	
Geometric mean	0.76	0.81	0.91	
IL-13 (median)	5.09	6.81	8.48	0.649
(P25, P75)	(1.77, 15.84)	(1.21, 17.45)	(1.21, 19.18)	
Geometric mean	6.23	6.45	7.99	
Pro-inflammatory				
IL-1β (median)	1.09	0.69	0.91	0.581
(P25, P75)	(0.43, 2.26)	(0.27, 1.78)	(0.27, 1.94)	
Geometric mean	1.15	0.90	1.13	
TNF-α (median)	2.92	2.91	4.37	0.556
(P25, P75)	(1.18, 10.58)	(1.34, 15.56)	(1.34, 19.53)	
Geometric mean	3.36	3.74	4.54	
IL-6 (median)	21.44	13.87	16.37	0.345
(P25, P75)	(6.23, 51.99)	(3.34, 45.39)	(4.63, 63.58)	
Geometric mean	20.73	12.41	16.88	
IFN-α (median)	15.53	18.22	22.44	0.646
(P25, P75)	(6.88, 47.25)	(7.00, 50.41)	(5.89, 66.64)	
Geometric mean	18.43	17.91	20.88	
IL-2R (median)	101.7	196.2	134.5	0.624
(P25, P75)	(49.91, 405.1)	(59.98, 416.4)	(56.65, 567.7)	
Geometric mean	148.2	172.2	178.8	
IL-17 (median)	0.58	0.61	0.58	0.419
(P25, P75)	(0.46, 1.51)	(0.46, 1.58)	(0.46, 1.83)	
Geometric mean	1.00	1.14	1.32	
Regulatory				
IL-7 (median)	20.55	12.35	14.01	0.421
(P25, P75)	(3.06, 32.51)	(2.84, 29.85)	(2.84, 30.37)	
Geometric mean	14.46	10.89	12.24	

[continued]

Supplemental Table 8a. Continued.

Cytokine (pg/mL)	**Primary or less** (N = 56)	**Secondary** (N = 65)	**University** (N = 119)	**P**[a]
Anti-inflammatory				
IL-10 (median)	4.03	0.55	4.18	0.281
(P25, P75)	(0.50, 39.83)	(0.41, 22.76)	(0.46, 36.85)	
Geometric mean	5.07	3.08	5.15	
IL-15 (median)	2.89	2.73	2.89	0.491
(P25, P75)	(1.90, 6.28)	(1.74, 21.32)	(1.74, 63.66)	
Geometric mean	7.34	8.30	11.01	
IL-1RA (median)	211.2	255.0	291.7	0.325
(P25, P75)	(155.1, 388.9)	(157.2, 511.7)	(182.4, 516.7)	
Geometric mean	271.2	292.3	310.0	

P25: percentile 25th. P75: percentile 75th.
<LOQ: value lower than the minimum concentration of the corresponding range of limits of quantification. See Supplemental Table 1.

[a] Kruskal-Wallis test (two-tailed).

Supplemental Table 8b. Relative intraindividual change (%) from 2016-17 to 2020-21 of concentrations of cytokines, by educational level (N=154).

Cytokine[a]	Primary or less (N = 35)	Secondary (N = 42)	University (N = 77)	P[b]
Growth factors				
G-CSF (median)	0.00	6.69	0.00	0.160
(P25, P75)	(-7.42, 18.29)	(0.00, 21.51)	(-10.73, 27.27)	
∆ ≥15%	28.6	40.5	31.2	
∇ ≥15%	22.9	4.8	19.5	
EGF (median)	6.95	8.54	7.88	0.597
(P25, P75)	(-3.01, 21.89)	(-0.80, 22.28)	(-4.63, 19.88)	
∆ ≥15%	31.4	35.7	31.2	
∇ ≥15%	11.4	4.8	6.5	
FGF (median)	0.00	0.00	0.00	0.733
(P25, P75)	(-21.74, 32.23)	(-16.96, 30.97)	(-20.09, 19.33)	
∆ ≥15%	34.3	31.0	27.3	
∇ ≥15%	31.4	28.6	31.2	
GM-CSF (median)	0.00	5.51	0.00	0.125
(P25, P75)	(-26.04, 30.06)	(-1.74, 85.06)	(-16.79, 17.31)	
∆ ≥15%	28.6	42.9	26.0	
∇ ≥15%	28.6	16.7	26.0	
HGF (median)	5.26	4.13	5.81	0.967
(P25, P75)	(1.88, 9.96)	(0.88, 10.23)	(0.54, 10.10)	
∆ ≥15%	5.7	7.1	11.7	
∇ ≥15%	2.9	2.4	3.9	
VEGF (median)	11.45	14.85	4.84	0.392
(P25, P75)	(-1.41, 50.11)	(-9.99, 28.53)	(-10.04, 34.84)	
∆ ≥15%	48.6	50.0	36.4	
∇ ≥15%	8.6	16.7	22.1	
Chemokines				
IL-8 (median)	6.92	5.20	9.56	0.494
(P25, P75)	(0.00, 15.21)	(-2.70, 16.03)	(1.79, 17.62)	
∆ ≥15%	25.7	28.6	29.9	
∇ ≥15%	0.0	2.4	1.3	
IP-10 (median)	-108.8	-80.59	-96.06	0.426
(P25, P75)	(-189.5, -80.11)	(-147.8, -61.16)	(-151.8, -54.12)	
∆ ≥15%	5.7	0.0	6.5	
∇ ≥15%	91.4	92.9	89.6	
RANTES (median)	0.00	0.00	-0.17	0.847
(P25, P75)	(-1.84, 1.12)	(-2.60, 0.88)	(-1.74, 0.45)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
EOTAXIN (median)	-3.52	0.29	-0.18	0.062
(P25, P75)	(-5.53, 1.75)	(-4.47, 8.07)	(-4.71, 4.63)	
∆ ≥15%	0.0	9.5	3.9	
∇ ≥15%	11.4	2.4	1.3	
MIP-1α (median)	0.00	2.58	0.00	0.275
(P25, P75)	(-17.06, 9.93)	(-5.33, 26.39)	(-7.59, 16.93)	
∆ ≥15%	22.9	33.3	27.3	
∇ ≥15%	28.6	14.3	18.2	
MIP-1β (median)	3.49	2.23	1.94	0.947
(P25, P75)	(-4.75, 12.40)	(-2.44, 6.96)	(-4.38, 11.22)	
∆ ≥15%	11.4	14.3	18.2	
∇ ≥15%	8.6	2.4	7.8	

[continued]

Supplemental Table 8b. Continued

Cytokine[a]	Primary or less (N = 35)	Secondary (N = 42)	University (N = 77)	P[b]
MCP-1 (median)	0.31	1.06	1.03	0.890
(P25, P75)	(-3.50, 4.88)	(-2.24, 4.46)	(-1.83, 3.33)	
Δ ≥15%	2.9	0.0	0.0	
∇ ≥15%	2.9	0.0	1.3	
MIG (median)	0.00	0.00	0.00	0.301
(P25, P75)	(0.00, 0.00)	(0.00, 0.50)	(-11.45, 0.00)	
Δ ≥15%	11.4	14.3	18.2	
∇ ≥15%	17.1	11.9	20.8	
TH1				
IL-2 (median)	9.53	4.71	6.66	0.956
(P25, P75)	(-19.05, 72.20)	(-13.36, 60.21)	(-16.86, 47.24)	
Δ ≥15%	48.6	45.2	45.5	
∇ ≥15%	28.6	23.8	27.3	
IL-12 (median)	2.17	0.95	0.00	0.701
(P25, P75)	(-6.25, 10.56)	(-5.14, 4.90)	(-6.22, 5.52)	
Δ ≥15%	11.4	4.8	5.2	
∇ ≥15%	11.4	7.1	6.5	
IFN-γ (median)	0.00	0.00	0.00	0.139
(P25, P75)	(0.00, 0.00)	(0.00, 0.00)	(0.00, 0.00)	
Δ ≥15%	14.3	7.1	9.1	
∇ ≥15%	2.9	4.8	13.0	
TH2				
IL-4 (median)	0.00	0.00	0.00	0.434
(P25, P75)	(0.00, 0.00)	(0.00, 1.98)	(0.00, 0.00)	
Δ ≥15%	22.9	21.4	13.0	
∇ ≥15%	5.7	16.7	19.5	
IL-5 (median)	0.00	0.00	0.00	0.746
(P25, P75)	(-84.04, 36.21)	(0.00, 12.22)	(-26.84, 63.19)	
Δ ≥15%	25.7	21.4	29.9	
∇ ≥15%	25.7	9.5	26.0	
IL-13 (median)	0.00	0.00	0.00	0.500
(P25, P75)	(-13.24, 43.30)	(-8.67, 35.02)	(-19.99, 20.51)	
Δ ≥15%	28.6	33.3	26.0	
∇ ≥15%	22.9	21.4	26.0	
Pro-inflammatory				
IL-1β (median)	12.85	0.00	0.00	0.055
(P25, P75)	(0.00, 51.78)	(-3.87, 40.73)	(-46.31, 27.43)	
Δ ≥15%	45.7	35.7	28.6	
∇ ≥15%	11.4	23.8	35.1	
TNF-α (median)	14.61	22.21	5.04	0.626
(P25, P75)	(0.00, 71.29)	(0.00, 98.15)	(-12.14, 58.65)	
Δ ≥15%	48.6	52.4	46.8	
∇ ≥15%	20.0	14.3	23.4	
IL-6 (median)	14.37	3.83	0.00	0.293
(P25, P75)	(0.00, 46.86)	(-6.73, 39.18)	(-8.44, 30.92)	
Δ ≥15%	45.7	42.9	33.8	
∇ ≥15%	11.4	19.0	18.2	
IFN-α (median)	4.22	0.84	0.00	0.982
(P25, P75)	(-11.80, 20.34)	(-8.35, 22.49)	(-9.02, 24.91)	
Δ ≥15%	34.3	35.7	32.5	
∇ ≥15%	14.3	16.7	15.6	

[continued]

Supplemental Table 8b. Continued

Cytokine[a]	Primary or less (N = 35)	Secondary (N = 42)	University (N = 77)	P[b]
IL-2R (median)	7.23	2.43	-0.71	0.186
(P25, P75)	(-10.40, 19.53)	(-5.47, 14.04)	(-11.68, 11.06)	
∆ ≥15%	34.2	23.8	16.9	
∇ ≥15%	17.1	9.5	19.5	
IL-17 (median)	0.00	0.00	0.00	0.533
(P25, P75)	(0.00, 0.00)	(0.00, 41.97)	(-0.00, 20.38)	
∆ ≥15%	22.9	31.0	26.0	
∇ ≥15%	14.3	14.3	22.1	
Regulatory				
IL-7 (median)	0.00	0.00	0.00	0.881
(P25, P75)	(-4.58, 16.01)	(-9.34, 18.55)	(-6.55, 13.69)	
∆ ≥15%	25.7	26.2	23.4	
∇ ≥15%	17.1	21.4	16.9	
Anti-inflammatory				
IL-10 (median)	0.00	0.00	0.00	0.738
(P25, P75)	(-20.85, 67.45)	(-10.75, 2.70)	(-17.49, 34.28)	
∆ ≥15%	34.3	21.4	32.5	
∇ ≥15%	28.6	23.8	24.7	
IL-15 (median)	0.00	0.00	0.00	0.861
(P25, P75)	(0.00, 3.84)	(0.00, 0.14)	(0.00, 2.50)	
∆ ≥15%	20.0	16.7	20.8	
∇ ≥15%	8.6	9.5	16.9	
IL-1RA (median)	-1.76	-1.12	-4.13	0.779
(P25, P75)	(-5.85, 5.73)	(-11.90, 7.34)	(-9.60, 3.99)	
∆ ≥15%	2.9	9.5	9.1	
∇ ≥15%	14.3	9.5	11.7	

P25: percentile 25th. P75: percentile 75th.
Educational level groups are based on participant's data in 2020-21.

[a] Units used for computing the relative intraindividual change for cytokine levels were pg/mL, base 10 logtransformed.
[b] Kruskal-Wallis test (two-tailed).

Supplemental Table 9a. Concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs in 2016-17 by sex (N=240).

Immunoglobulin (MFI)	**Total** (N = 240)	**Men** (N = 113)	**Women** (N = 127)	**P**[a]
IgA				
IgA CMV pp150 (median)	495.8	541.0	464.0	0.474
(P25, P75)	(338.2, 830.4)	(339.5, 878.8)	(332.0, 758.5)	
Geometric mean	599.6	600.3	599.0	
IgA CMV pp65 (median)	579.5	589.0	570.5	0.990
(P25, P75)	(411.4, 930.2)	(425.0, 929.5)	(404.0, 965.0)	
Geometric mean	690.2	682.7	697.0	
IgA EBV EAD (median)	424.0	426.0	422.0	0.362
(P25, P75)	(313.0, 640.2)	(320.0, 753.5)	(311.0, 611.0)	
Geometric mean	501.2	536.5	471.8	
IgA VCAp18 (median)	488.0	533.0	450.0	0.276
(P25, P75)	(337.0, 753.8)	(340.5, 769.2)	(334.0, 696.5)	
Geometric mean	566.2	568.6	564.2	
IgA N 229E (median)	1366	1330	1386	0.539
(P25, P75)	(718.9, 3547)	(725.0, 4670)	(716.0, 3495)	
Geometric mean	1788	1961	1648	
IgA N HKU1 (median)	690.5	693.0	684.0	0.738
(P25, P75)	(470.8, 1160)	(453.0, 1046)	(476.0, 1184)	
Geometric mean	816.7	799.6	832.2	
IgA NL 63 (median)	1532	2078	1409	0.115
(P25, P75)	(788.6, 4593)	(806.0, 6944)	(756.0, 3280)	
Geometric mean	2064	2394	1809	
IgA N OC43 (median)	1420	1528	1372	0.423
(P25, P75)	(887.8, 2806)	(880.5, 3120)	(896.0, 2623)	
Geometric mean	1639	1746	1548	

[continued]

Supplemental Table 9a. Continued.

Immunoglobulin (MFI)	**Total** (N = 240)	**Men** (N = 113)	**Women** (N = 127)	**P**[a]
IgG				
IgG CMV pp150 (median)	15201	12441	18278	0.111
(P25, P75)	(5334, 30751)	(4934, 26052)	(5496, 39185)	
Geometric mean	13591	12106	15065	
IgG CMV pp65 (median)	6819	6954	6658	0.918
(P25, P75)	(4441, 10864)	(4783, 10402)	(4120, 11066)	
Geometric mean	6774	6766	6782	
IgG EBV EAD (median)	6926	6858	6962	0.910
(P25, P75)	(4760, 9991)	(4918, 9561)	(4748, 10196)	
Geometric mean	6846	6806	6881	
IgG VCAp18 (median)	7810	7400	8092	0.799
(P25, P75)	(4280, 14308)	(4281, 14284)	(4274, 14419)	
Geometric mean	8528	8869	8236	
IgG N 229E (median)	44429	52588	36615	0.009
(P25, P75)	(25747, 73639)	(28715, 77569)	(22440, 68493)	
Geometric mean	41828	47429	37403	
IgG N HKU1 (median)	14094	13620	14351	0.681
(P25, P75)	(10031, 18800)	(9967, 18550)	(10087, 19685)	
Geometric mean	13710	13749	13675	
IgG NL 63 (median)	36644	39669	32016	0.166
(P25, P75)	(20923, 58452)	(22704, 58558)	(19408, 58443)	
Geometric mean	34243	36698	32197	
IgG N OC43 (median)	21454	21540	21367	0.314
(P25, P75)	(11238, 37532)	(11454, 42493)	(10888, 34667)	
Geometric mean	21072	22278	20054	

[continued]

Supplemental Table 9a. Continued.

Immunoglobulin (MFI)	**Total** (N = 240)	**Men** (N = 113)	**Women** (N = 127)	**P**[a]
IgM				
IgM CMV pp150 (median)	1277	1020	1596	<0.001
(P25, P75)	(724.4, 2420)	(564.8, 1604)	(930.5, 3382)	
Geometric mean	1378	1060	1742	
IgM CMV pp65 (median)	655.0	517.5	804.0	<0.001
(P25, P75)	(427.6, 1036)	(398.2, 809.5)	(525.5, 1155)	
Geometric mean	698.2	577.3	826.8	
IgM EBV EAD (median)	423.8	363.5	488.0	<0.001
(P25, P75)	(304.2, 590.5)	(268.5, 506.0)	(338.0, 680.0)	
Geometric mean	435.8	377.8	494.7	
IgM VCAp18 (median)	861.2	677.0	1020	<0.001
(P25, P75)	(498.8, 1599)	(442.2, 1118)	(592.0, 1872)	
Geometric mean	911.1	749.3	1084	
IgM N 229E (median)	1431	1162	1680	<0.001
(P25, P75)	(863.6, 2378)	(707.0, 1708)	(1189, 3009)	
Geometric mean	1444	1140	1783	
IgM N HKU1 (median)	1022	850.5	1147	<0.001
(P25, P75)	(705.6, 1652)	(567.5, 1360)	(866.5, 1757)	
Geometric mean	1079	922.6	1239	
IgM NL 63 (median)	948.2	697.5	1249	<0.001
(P25, P75)	(591.5, 1568)	(448.2, 1104)	(783.0, 1918)	
Geometric mean	1012	764.0	1298	
IgM N OC43 (median)	2060	1510	2664	<0.001
(P25, P75)	(1121, 3496)	(991.2, 2569)	(1288, 3948)	
Geometric mean	2065	1630	2547	

[continued]

Supplemental Table 9a. Continued.

Immunoglobulin (µg/mL)	**Total** (N = 240)	**Men** (N = 113)	**Women** (N = 127)	**P**[a]
Total immunoglobulin				
IgG1 (median)	6111	5777	6648	0.029
(P25, P75)	(4744, 8220)	(4410, 7388)	(5108, 8403)	
Geometric mean	6250	5917	6561	
IgG2 (median)	3119	2857	3206	0.005
(P25, P75)	(2486, 3690)	(2380, 3544)	(2595, 3783)	
Geometric mean	3040	2867	3202	
IgG3 (median)	1237	1158	1294	0.015
(P25, P75)	(1004, 1472)	(960.4, 1413)	(1073, 1531)	
Geometric mean	1198	1134	1258	
IgG4 (median)	228.7	225.1	231.6	0.628
(P25, P75)	(122.7, 400.8)	(129.1, 407.6)	(107.6, 396.8)	
Geometric mean	226.7	234.7	219.9	
Sum of IgGs (median)	10900	10447	11426	0.010
(P25, P75)	(8865, 13719)	(8474, 12684)	(9425, 14123)	
Geometric mean	11035	10479	11555	
IgE (median)	0.23	0.24	0.21	0.242
(P25, P75)	(0.15, 0.33)	(0.15, 0.35)	(0.14, 0.32)	
Geometric mean	0.23	0.24	0.22	
IgA (median)	246.1	244.9	247.1	0.418
(P25, P75)	(193.7, 301.7)	(185.2, 293.5)	(196.2, 313.2)	
Geometric mean	239.9	233.9	245.5	
IgM (median)	918.7	809.7	1158	<0.001
(P25, P75)	(690.0, 1325)	(654.1, 986.9)	(773.3, 1480)	
Geometric mean	946.7	806.7	1091	

P25: percentile 25th. P75: percentile 75th.
<LOQ: value lower than the minimum concentration of the corresponding range of limits of quantification. See Supplemental Table 1.

[a] Mann-Whitney's *U* test (two-tail).

Supplemental Table 9b. Relative intraindividual change (%) from 2016-17 to 2020-21 of concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs, by sex (N=154).

Immunoglobulin[a]	Men (N = 72)	Women (N = 82)	P[b]
IgA			
IgA CMV pp150 (median)	0.36	0.48	0.735
(P25, P75)	(-2.17, 4.47)	(-2.81, 3.15)	
∆ ≥15%	4.2	4.9	
∇ ≥15%	0.0	1.2	
IgA CMV pp65 (median)	0.66	1.06	0.648
(P25, P75)	(-2.29, 4.61)	(-2.33, 3.27)	
∆ ≥15%	2.8	1.2	
∇ ≥15%	0.0	1.2	
IgA EBV EAD (median)	-0.16	0.74	0.957
(P25, P75)	(-2.64, 4.10)	(-1.99, 2.82)	
∆ ≥15%	2.8	0.0	
∇ ≥15%	1.4	1.2	
IgA VCAp18 (median)	0.45	1.05	0.991
(P25, P75)	(-2.08, 3.62)	(-2.16, 3.48)	
∆ ≥15%	4.2	0.0	
∇ ≥15%	0.0	1.2	
IgA N 229E (median)	0.28	-0.93	0.250
(P25, P75)	(-4.05, 5.01)	(-3.74, 2.84)	
∆ ≥15%	5.6	0.0	
∇ ≥15%	5.6	4.9	
IgA N HKU1 (median)	1.51	0.70	0.393
(P25, P75)	(-2.11, 3.41)	(-2.68, 2.72)	
∆ ≥15%	4.2	1.2	
∇ ≥15%	0.0	0.0	
IgA NL 63 (median)	-0.55	-0.19	0.761
(P25, P75)	(-3.79, 6.79)	(-3.55, 3.65)	
∆ ≥15%	9.7	3.7	
∇ ≥15%	2.8	6.1	
IgA N OC43 (median)	0.03	0.39	0.797
(P25, P75)	(-2.84, 3.37)	(-2.60, 2.91)	
∆ ≥15%	1.4	3.7	
∇ ≥15%	0.0	1.2	

[continued]

Supplemental Table 9b. Continued

Immunoglobulin[a]	Men (N = 72)	Women (N = 82)	P[b]
IgG			
IgG CMV pp150 (median)	1.57	1.31	0.744
(P25, P75)	(-1.69, 3.99)	(-1.04, 4.04)	
Δ ≥15%	2.8	1.2	
∇ ≥15%	0.0	0.0	
IgG CMV pp65 (median)	1.51	0.31	0.222
(P25, P75)	(-1.33, 3.91)	(-2.88, 2.79)	
Δ ≥15%	0.0	1.2	
∇ ≥15%	0.0	0.0	
IgG EBV EAD (median)	0.85	-0.22	0.659
(P25, P75)	(-2.27, 2.56)	(-2.20, 3.24)	
Δ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG VCAp18 (median)	0.87	0.33	0.232
(P25, P75)	(-1.79, 4.17)	(-2.17, 3.13)	
Δ ≥15%	2.8	1.2	
∇ ≥15%	0.0	1.2	
IgG N 229E (median)	0.15	0.15	0.888
(P25, P75)	(-2.56, 2.77)	(-2.56, 2.70)	
Δ ≥15%	1.4	0.0	
∇ ≥15%	0.0	0.0	
IgG N HKU1 (median)	1.15	0.23	0.244
(P25, P75)	(-1.37, 2.48)	(-1.63, 2.39)	
Δ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG NL 63 (median)	0.36	-0.35	0.199
(P25, P75)	(-2.57, 3.58)	(-2.48, 2.26)	
Δ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG N OC43 (median)	0.36	-0.24	0.187
(P25, P75)	(-2.21, 3.97)	(-4.18, 2.65)	
Δ ≥15%	0.0	1.2	
∇ ≥15%	1.4	3.7	

[continued]

Supplemental Table 9b. Continued

Immunoglobulin[a]	Men (N = 72)	Women (N = 82)	P[b]
IgM			
IgM CMV pp150 (median)	1.08	1.01	0.415
(P25, P75)	(-1.58, 5.13)	(-2.32, 3.36)	
∆ ≥15%	2.8	0.0	
∇ ≥15%	1.4	0.0	
IgM CMV pp65 (median)	0.78	0.44	0.853
(P25, P75)	(-2.02, 2.82)	(-1.59, 4.04)	
∆ ≥15%	1.4	0.0	
∇ ≥15%	0.0	0.0	
IgM EBV EAD (median)	0.73	0.14	0.245
(P25, P75)	(-1.68, 3.76)	(-2.99, 3.31)	
∆ ≥15%	2.8	0.0	
∇ ≥15%	0.0	0.0	
IgM VCAp18 (median)	1.72	0.43	0.107
(P25, P75)	(-1.72, 5.21)	(-2.57, 3.79)	
∆ ≥15%	1.4	2.4	
∇ ≥15%	0.0	0.0	
IgM N 229E (median)	1.85	-0.16	0.046
(P25, P75)	(-1.24, 5.42)	(-3.28, 3.97)	
∆ ≥15%	4.2	1.2	
∇ ≥15%	0.0	1.2	
IgM N HKU1 (median)	1.34	0.71	0.407
(P25, P75)	(-1.59, 4.38)	(-1.42, 2.82)	
∆ ≥15%	2.8	1.2	
∇ ≥15%	0.0	0.0	
IgM NL 63 (median)	1.56	0.63	0.221
(P25, P75)	(-2.20, 4.23)	(-3.92, 5.56)	
∆ ≥15%	2.8	0.0	
∇ ≥15%	1.4	0.0	
IgM N OC43 (median)	0.27	1.26	0.567
(P25, P75)	(-2.68, 4.23)	(-2.53, 4.35)	
∆ ≥15%	1.4	2.4	
∇ ≥15%	0.0	0.0	

[continued]

Supplemental Table 9b. Continued

Immunoglobulin[a]	Men (N = 72)	Women (N = 82)	P[b]
Total immunoglobulin			
IgG1 (median)	1.91	0.62	0.025
(P25, P75)	(-0.83, 3.58)	(-2.22, 2.32)	
∆ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG2 (median)	0.88	0.46	0.087
(P25, P75)	(-0.26, 2.38)	(-0.86, 1.88)	
∆ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG3 (median)	-0.01	-0.80	0.297
(P25, P75)	(-2.65, 1.77)	(-2.65, 1.09)	
∆ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG4 (median)	0.96	1.17	0.640
(P25, P75)	(-2.27, 4.59)	(-1.42, 4.27)	
∆ ≥15%	2.8	0.0	
∇ ≥15%	1.4	0.0	
Sum of IgGs (median)	1.20	0.26	0.014
(P25, P75)	(-0.46, 2.79)	(-1.63, 1.98)	
∆ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgE (median)	4.11	1.39	0.262
(P25, P75)	(-4.47, 13.02)	(-3.65, 9.36)	
∆ ≥15%	20.8	13.4	
∇ ≥15%	8.3	9.8	
IgA (median)	1.17	0.29	0.074
(P25, P75)	(-1.32, 4.60)	(-2.81, 3.61)	
∆ ≥15%	1.4	0.0	
∇ ≥15%	0.0	0.0	
IgM (median)	1.05	-0.25	0.069
(P25, P75)	(-1.05, 3.53)	(-1.89, 2.82)	
∆ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	

P25: percentile 25th. P75: percentile 75th.

[a] Units used for computing the relative intraindividual change were base 10 logtransformed MFI for levels of isotype-antigen combinations, and base 10 logtransformed µg/mL for levels of total Igs.
[b] Mann-Whitney's *U* test (two-tail).

Supplemental Table 10a. Concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs in 2016-17 by age group (N=240).

Immunoglobulin (MFI)	**<45 years** (N = 88)	**45 – 64 years** (N = 105)	**≥65 years** (N = 47)	**P**[a]
IgA				
IgA CMV pp150 (median)	488.5	439.0	677.5	0.029
(P25, P75)	(330.1, 826.8)	(329.8, 696.2)	(427.0, 1063)	
Geometric mean	586.5	558.4	732.5	
IgA CMV pp65 (median)	523.0	569.0	802.5	0.005
(P25, P75)	(397.2, 851.0)	(397.2, 792.0)	(466.0, 1702)	
Geometric mean	592.9	664.4	999.0	
IgA EBV EAD (median)	372.8	407.0	580.0	0.029
(P25, P75)	(305.4, 579.8)	(308.5, 631.8)	(330.0, 1244)	
Geometric mean	439.2	499.9	645.8	
IgA VCAp18 (median)	447.0	488.5	590.0	0.096
(P25, P75)	(328.1, 680.5)	(351.0, 734.2)	(332.0, 973.0)	
Geometric mean	490.4	591.5	672.3	
IgA N 229E (median)	1290	1558	1546	0.651
(P25, P75)	(727.8, 3471)	(716.5, 3986)	(717.5, 3504)	
Geometric mean	1607	1819	2105	
IgA N HKU1 (median)	629.0	684.0	932.0	0.031
(P25, P75)	(441.0, 1031)	(478.2, 1094)	(476.0, 1706)	
Geometric mean	707.2	824.9	1046	
IgA NL 63 (median)	1635	1345	2285	0.329
(P25, P75)	(785.4, 4157)	(772.2, 4336)	(793.0, 8840)	
Geometric mean	1949	1905	2750	
IgA N OC43 (median)	1382	1377	1544	0.830
(P25, P75)	(899.2, 2679)	(835.2, 2725)	(913.0, 3345)	
Geometric mean	1562	1600	1891	

[continued]

Supplemental Table 10a. Continued.

Immunoglobulin (MFI)	**<45 years** (N = 88)	**45 – 64 years** (N = 105)	**≥65 years** (N = 47)	**P**[a]
IgG				
IgG CMV pp150 (median)	13886	14982	19973	0.226
(P25, P75)	(5481, 27945)	(5107, 29360)	(5937, 46896)	
Geometric mean	12606	12813	17854	
IgG CMV pp65 (median)	7754	6572	6756	0.099
(P25, P75)	(5060, 12612)	(4068, 9177)	(3615, 11066)	
Geometric mean	7559	6321	6440	
IgG EBV EAD (median)	7287	7007	5914	0.388
(P25, P75)	(4976, 10318)	(4714, 9310)	(4630, 9082)	
Geometric mean	7145	6825	6363	
IgG VCAp18 (median)	8100	7815	7066	0.620
(P25, P75)	(4338, 13503)	(4300, 14532)	(3334, 14772)	
Geometric mean	8139	9105	8041	
IgG N 229E (median)	46607	43472	42804	0.717
(P25, P75)	(24041, 76984)	(28790, 70706)	(23096, 72216)	
Geometric mean	41710	43992	37568	
IgG N HKU1 (median)	13666	14345	12973	0.671
(P25, P75)	(9492, 19883)	(10446, 19091)	(10408, 16628)	
Geometric mean	13290	14347	13129	
IgG NL 63 (median)	41605	31820	33123	0.124
(P25, P75)	(24773, 63705)	(22243, 57049)	(17438, 50410)	
Geometric mean	38088	33284	29893	
IgG N OC43 (median)	24018	19178	21886	0.451
(P25, P75)	(11440, 38243)	(10664, 36361)	(13010, 40084)	
Geometric mean	21870	19811	22560	

[continued]

Supplemental Table 10a. Continued.

Immunoglobulin (MFI)	**<45 years** (N = 88)	**45 – 64 years** (N = 105)	**≥65 years** (N = 47)	**P**[a]
IgM				
IgM CMV pp150 (median)	1512	1130	997.0	0.005
(P25, P75)	(923.8, 3278)	(702.2, 2064)	(527.0, 1754)	
Geometric mean	1692	1275	1117	
IgM CMV pp65 (median)	818.8	605.0	446.5	<0.001
(P25, P75)	(574.5, 1356)	(404.2, 919.2)	(351.0, 718.0)	
Geometric mean	905.4	628.6	542.7	
IgM EBV EAD (median)	475.8	424.0	338.0	0.011
(P25, P75)	(328.0, 657.8)	(300.5, 546.0)	(250.0, 496.0)	
Geometric mean	482.6	431.5	367.8	
IgM VCAp18 (median)	1176	805.0	510.0	<0.001
(P25, P75)	(747.6, 1934)	(495.0, 1450)	(359.5, 870.5)	
Geometric mean	1226	868.4	582.1	
IgM N 229E (median)	1608	1402	1094	0.002
(P25, P75)	(1234, 2688)	(730.0, 2185)	(682.0, 2311)	
Geometric mean	1762	1336	1185	
IgM N HKU1 (median)	1242	932.0	919.0	0.002
(P25, P75)	(846.6, 1831)	(638.5, 1474)	(601.0, 1522)	
Geometric mean	1261	1021	910.8	
IgM NL 63 (median)	1157	783.0	778.5	<0.001
(P25, P75)	(785.0, 1980)	(502.0, 1381)	(509.0, 1203)	
Geometric mean	1292	895.2	841.0	
IgM N OC43 (median)	2313	1980	1796	0.062
(P25, P75)	(1439, 3708)	(988.8, 3545)	(992.0, 3074)	
Geometric mean	2363	1986	1748	

[continued]

Supplemental Table 10a. Continued.

Immunoglobulin (µg/mL)	**<45 years** (N = 88)	**45 – 64 years** (N = 105)	**≥65 years** (N = 47)	**P**[a]
Total immunoglobulin				
IgG1 (median)	6671	5777	5993	0.113
(P25, P75)	(5063, 8957)	(4536, 7943)	(4624, 8412)	
Geometric mean	6699	5902	6236	
IgG2 (median)	3200	3055	3098	0.168
(P25, P75)	(2659, 3741)	(2413, 3579)	(2376, 3732)	
Geometric mean	3201	2905	3053	
IgG3 (median)	1236	1251	1198	0.767
(P25, P75)	(1012, 1519)	(989.8, 1488)	(1055, 1406)	
Geometric mean	1232	1186	1164	
IgG4 (median)	270.3	199.7	253.2	0.047
(P25, P75)	(138.8, 479.4)	(103.4, 351.8)	(134.6, 398.6)	
Geometric mean	264.0	196.8	234.1	
Sum of IgGs (median)	11466	10344	10891	0.033
(P25, P75)	(9775, 14612)	(8433, 12781)	(9094, 13705)	
Geometric mean	11730	10482	11041	
IgE (median)	0.26	0.20	0.24	0.041
(P25, P75)	(0.18, 0.35)	(0.14, 0.31)	(0.17, 0.35)	
Geometric mean	0.25	0.21	0.24	
IgA (median)	238.2	249.6	246.8	0.736
(P25, P75)	(194.2, 300.2)	(192.8, 304.1)	(188.5, 307.2)	
Geometric mean	234.2	240.5	249.7	
IgM (median)	1056	885.1	816.5	0.005
(P25, P75)	(777.9, 1347)	(635.1, 1407)	(612.5, 1151)	
Geometric mean	1054	926.2	812.9	

P25: percentile 25th. P75: percentile 75th.
<LOQ: value lower than the minimum concentration of the corresponding range of limits of quantification. See Supplemental Table 1.

[a] Kruskal-Wallis test (two-tailed).

Supplemental Table 10b. Relative intraindividual change (%) from 2016-17 to 2020-21 of concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs, by age group (N=154).

Immunoglobulin[a]	<46 years (N = 51)	46 – 62 years (N = 49)	≥63 years (N = 54)	P[b]
IgA				
IgA CMV pp150 (median)	0.62	0.55	0.25	0.326
(P25, P75)	(-1.99, 5.97)	(-2.77, 3.16)	(-2.85, 2.87)	
∆ ≥15%	11.8	0.0	1.9	
∇ ≥15%	0.0	0.0	1.9	
IgA CMV pp65 (median)	0.47	1.10	0.88	0.793
(P25, P75)	(-2.86, 3.39)	(-2.06, 2.57)	(-2.18, 5.33)	
∆ ≥15%	3.9	0.0	1.9	
∇ ≥15%	0.0	0.0	1.9	
IgA EBV EAD (median)	-0.09	0.63	0.74	0.998
(P25, P75)	(-2.37, 3.84)	(-1.96, 3.44)	(-2.84, 3.98)	
∆ ≥15%	3.9	0.0	0.0	
∇ ≥15%	0.0	2.0	1.9	
IgA VCAp18 (median)	0.54	1.12	0.45	0.808
(P25, P75)	(-2.07, 2.96)	(-2.02, 4.62)	(-2.55, 3.46)	
∆ ≥15%	3.9	2.0	0.0	
∇ ≥15%	0.0	0.0	1.9	
IgA N 229E (median)	-0.02	-0.23	-0.76	0.929
(P25, P75)	(-3.32, 3.50)	(-4.98, 3.14)	(-3.90, 5.12)	
∆ ≥15%	2.0	6.1	0.0	
∇ ≥15%	7.8	2.0	5.6	
IgA N HKU1 (median)	0.80	0.94	1.02	0.550
(P25, P75)	(-1.69, 2.31)	(-2.67, 2.76)	(-1.86, 3.73)	
∆ ≥15%	5.9	0.0	1.9	
∇ ≥15%	0.0	0.0	0.0	
IgA NL 63 (median)	-0.32	-0.41	-0.53	0.780
(P25, P75)	(-3.98, 6.09)	(-2.18, 3.65)	(-5.44, 4.88)	
∆ ≥15%	7.8	8.2	3.7	
∇ ≥15%	3.9	4.1	5.6	
IgA N OC43 (median)	-0.36	0.37	0.92	0.958
(P25, P75)	(-2.86, 3.62)	(-2.46, 3.69)	(-2.73, 2.58)	
∆ ≥15%	5.9	0.0	1.9	
∇ ≥15%	0.0	0.0	1.9	

[continued]

Supplemental Table 10b. Continued

Immunoglobulin[a]	<46 years (N = 51)	46 – 62 years (N = 49)	≥63 years (N = 54)	P[b]
IgG				
IgG CMV pp150 (median)	2.25	0.83	1.12	0.717
(P25, P75)	(-1.50, 3.99)	(-2.06, 3.98)	(-0.72, 4.13)	
Δ ≥15%	5.9	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG CMV pp65 (median)	0.46	0.82	0.95	0.565
(P25, P75)	(-3.18, 2.51)	(-1.82, 2.92)	(-1.92, 3.95)	
Δ ≥15%	0.0	0.0	1.9	
∇ ≥15%	0.0	0.0	0.0	
IgG EBV EAD (median)	0.97	0.80	-0.21	0.278
(P25, P75)	(-2.97, 3.17)	(-1.12, 3.37)	(-2.89, 2.17)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG VCAp18 (median)	0.62	0.67	0.73	0.836
(P25, P75)	(-3.19, 3.18)	(-1.25, 2.88)	(-2.35, 3.47)	
Δ ≥15%	2.0	2.0	1.9	
∇ ≥15%	2.0	0.0	0.0	
IgG N 229E (median)	0.15	-0.18	0.35	0.886
(P25, P75)	(-2.91, 2.71)	(-2.35, 2.35)	(-2.53, 3.03)	
Δ ≥15%	2.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG N HKU1 (median)	0.69	0.43	0.75	0.979
(P25, P75)	(-1.60, 2.40)	(-1.55, 2.59)	(-1.49, 2.15)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG NL 63 (median)	-0.35	0.10	-0.33	0.923
(P25, P75)	(-2.89, 3.44)	(-1.41, 2.35)	(-2.41, 3.06)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG N OC43 (median)	-0.12	0.09	0.42	0.992
(P25, P75)	(-3.75, 3.25)	(-2.03, 2.39)	(-4.40, 3.62)	
Δ ≥15%	2.0	0.0	0.0	
∇ ≥15%	2.0	0.0	5.6	

[continued]

Supplemental Table 10b. Continued

Immunoglobulin[a]	< 46 years (N = 51)	46 – 62 years (N = 49)	≥ 63 years (N = 54)	P[b]
IgM				
IgM CMV pp150 (median)	0.80	1.29	0.96	0.850
(P25, P75)	(-2.61, 3.99)	(-2.96, 3.74)	(-0.89, 3.43)	
Δ ≥15%	2.0	0.0	1.9	
∇ ≥15%	0.0	0.0	1.9	
IgM CMV pp65 (median)	0.19	0.95	0.59	0.620
(P25, P75)	(-2.39, 2.94)	(-1.46, 3.48)	(-1.62, 4.39)	
Δ ≥15%	0.0	0.0	1.9	
∇ ≥15%	0.0	0.0	0.0	
IgM EBV EAD (median)	0.57	0.38	0.51	0.796
(P25, P75)	(-2.45, 3.07)	(-2.16, 3.81)	(-2.47, 4.10)	
Δ ≥15%	2.0	0.0	1.9	
∇ ≥15%	0.0	0.0	0.0	
IgM VCAp18 (median)	1.34	0.89	1.30	0.695
(P25, P75)	(-3.38, 4.11)	(-2.32, 3.89)	(-1.70, 5.04)	
Δ ≥15%	2.0	2.0	1.9	
∇ ≥15%	0.0	0.0	0.0	
IgM N 229E (median)	-0.14	0.14	1.81	0.105
(P25, P75)	(-3.47, 4.20)	(-3.39, 3.29)	(-1.26, 6.55)	
Δ ≥15%	3.9	0.0	3.7	
∇ ≥15%	0.0	0.0	1.9	
IgM N HKU1 (median)	-0.11	0.61	1.78	0.047
(P25, P75)	(-2.28, 3.65)	(-1.66, 3.30)	(-0.23, 4.41)	
Δ ≥15%	2.0	2.0	1.9	
∇ ≥15%	0.0	0.0	0.0	
IgM NL 63 (median)	0.86	0.79	2.17	0.237
(P25, P75)	(-2.61, 4.92)	(-3.81, 3.63)	(-2.20, 4.93)	
Δ ≥15%	2.0	0.0	1.9	
∇ ≥15%	0.0	2.0	0.0	
IgM N OC43 (median)	-0.99	0.26	1.52	0.013
(P25, P75)	(-4.03, 2.30)	(-2.35, 6.55)	(-0.93, 4.59)	
Δ ≥15%	0.0	4.1	1.9	
∇ ≥15%	0.0	0.0	0.0	

[continued]

Supplemental Table 10b. Continued

Immunoglobulin[a]	< 46 years (N = 51)	46 – 62 years (N = 49)	≥ 63 years (N = 54)	P[b]
Total immunoglobulin				
IgG1 (median)	0.46	1.06	1.48	0.072
(P25, P75)	(-3.66, 2.30)	(-0.91, 3.45)	(-1.03, 4.36)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG2 (median)	0.51	0.80	0.73	0.496
(P25, P75)	(-0.82, 1.79)	(-1.31, 2.50)	(-0.09, 2.52)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG3 (median)	-0.73	-1.18	0.54	0.004
(P25, P75)	(-3.05, 0.80)	(-3.26, 0.38)	(-0.94, 2.39)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG4 (median)	-0.02	2.22	2.38	0.052
(P25, P75)	(-3.14, 3.26)	(-1.71, 5.47)	(-0.39, 4.48)	
∆ ≥15%	2.0	2.0	0.0	
∇ ≥15%	0.0	2.0	0.0	
Sum of IgGs (median)	0.31	0.59	1.24	0.034
(P25, P75)	(-2.34, 1.98)	(-0.90, 2.81)	(0.05, 2.51)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgE (median)	1.90	0.57	4.36	0.198
(P25, P75)	(-6.25, 8.94)	(-4.87, 11.12)	(-0.34, 13.39)	
∆ ≥15%	15.7	14.3	20.4	
∇ ≥15%	13.7	10.2	3.7	
IgA (median)	0.08	0.74	1.60	0.197
(P25, P75)	(-2.58, 2.83)	(-2.52, 4.35)	(-0.25, 3.95)	
∆ ≥15%	0.0	0.0	1.9	
∇ ≥15%	0.0	0.0	0.0	
IgM (median)	-0.43	-0.15	1.15	0.100
(P25, P75)	(-2.03, 1.92)	(-1.49, 3.01)	(-0.69, 4.03)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	

P25: percentile 25th. P75: percentile 75th.
Age groups are based on each participants' age in 2020-21.

[a] Units used for computing the relative intraindividual change were base 10 logtransformed MFI for levels of isotype-antigen combinations, and base 10 logtransformed µg/mL for levels of total Igs.
[b] Kruskal-Wallis test (two-tailed).

Supplemental Table 11a. Concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs in 2016-17 by body mass index (N=240).

Immunoglobulin (MFI)	**Underweight or normal weight** (N = 101)	**Overweight** (N = 91)	**Obese** (N = 48)	**P**[a]
IgA				
IgA CMV pp150 (median)	490.0	521.0	486.5	0.780
(P25, P75)	(325.0, 790.0)	(357.5, 874.5)	(334.5, 985.2)	
Geometric mean	593.0	611.2	591.7	
IgA CMV pp65 (median)	501.0	611.0	704.2	0.007
(P25, P75)	(370.5, 814.8)	(430.5, 878.0)	(510.6, 1267)	
Geometric mean	613.1	726.2	804.4	
IgA EBV EAD (median)	372.0	443.5	544.5	0.013
(P25, P75)	(286.5, 567.8)	(328.0, 666.5)	(362.4, 877.6)	
Geometric mean	441.8	530.0	587.8	
IgA VCAp18 (median)	448.0	509.0	526.2	0.464
(P25, P75)	(322.0, 716.0)	(337.5, 758.0)	(355.5, 806.1)	
Geometric mean	554.7	567.8	588.3	
IgA N 229E (median)	1420	1224	1855	0.384
(P25, P75)	(737.5, 3728)	(701.5, 3495)	(790.9, 4700)	
Geometric mean	1816	1618	2093	
IgA N HKU1 (median)	611.0	693.0	907.0	0.034
(P25, P75)	(438.5, 1061)	(476.0, 1159)	(537.5, 1563)	
Geometric mean	727.6	843.5	979.5	
IgA NL 63 (median)	1185	1952	2206	0.125
(P25, P75)	(702.0, 3939)	(793.0, 5048)	(954.6, 7442)	
Geometric mean	1757	2125	2743	
IgA N OC43 (median)	1342	1732	1384	0.143
(P25, P75)	(826.2, 2394)	(914.0, 3784)	(946.1, 2927)	
Geometric mean	1407	1843	1805	

[continued]

Supplemental Table 11a. Continued.

Immunoglobulin (MFI)	**Underweight or normal weight** (N = 101)	**Overweight** (N = 91)	**Obese** (N = 48)	**P**[a]
IgG				
IgG CMV pp150 (median)	13592	18463	16981	0.406
(P25, P75)	(4770, 30230)	(5960, 31786)	(8276, 38419)	
Geometric mean	11939	14745	15298	
IgG CMV pp65 (median)	6850	6756	7013	0.936
(P25, P75)	(4046, 11684)	(4492, 10900)	(4569, 10398)	
Geometric mean	6873	6871	6397	
IgG EBV EAD (median)	7146	6602	7211	0.915
(P25, P75)	(4450, 9854)	(4937, 9529)	(4749, 10385)	
Geometric mean	6785	6853	6962	
IgG VCAp18 (median)	8092	7400	7313	0.828
(P25, P75)	(4150, 13639)	(4274, 15657)	(4612, 14398)	
Geometric mean	8145	8864	8733	
IgG N 229E (median)	44878	45478	43887	0.986
(P25, P75)	(24226, 81574)	(27383, 68320)	(25828, 77814)	
Geometric mean	40858	42476	42682	
IgG N HKU1 (median)	13882	14011	15078	0.910
(P25, P75)	(9585, 19788)	(10910, 18268)	(9378, 19036)	
Geometric mean	13539	13876	13757	
IgG NL 63 (median)	33353	39508	34875	0.810
(P25, P75)	(20757, 58449)	(22601, 57348)	(20336, 60506)	
Geometric mean	33179	35673	33864	
IgG N OC43 (median)	19362	23511	21454	0.333
(P25, P75)	(9866, 34673)	(11831, 40084)	(10896, 43680)	
Geometric mean	19254	22709	22108	

[continued]

Supplemental Table 11a. Continued.

Immunoglobulin (MFI)	**Underweight or normal weight** (N = 101)	**Overweight** (N = 91)	**Obese** (N = 48)	**P**[a]
IgM				
IgM CMV pp150 (median)	1476	1113	1277	0.003
(P25, P75)	(962.2, 2991)	(676.0, 2192)	(724.4, 2420)	
Geometric mean	1666	1280	1378	
IgM CMV pp65 (median)	825.0	604.5	655.0	<0.001
(P25, P75)	(545.5, 1186)	(422.0, 920.0)	(427.6, 1036)	
Geometric mean	858.0	655.5	698.2	
IgM EBV EAD (median)	456.0	427.0	423.8	0.013
(P25, P75)	(352.5, 607.0)	(278.0, 623.0)	(304.2, 590.5)	
Geometric mean	482.8	426.9	435.8	
IgM VCAp18 (median)	1089	831.0	861.2	<0.001
(P25, P75)	(648.8, 1902)	(471.5, 1365)	(498.8, 1599)	
Geometric mean	1147	849.3	911.1	
IgM N 229E (median)	1680	1305	1431	<0.001
(P25, P75)	(1176, 3103)	(800.0, 2040)	(863.6, 2378)	
Geometric mean	1769	1307	1444	
IgM N HKU1 (median)	1147	953.5	1022	0.003
(P25, P75)	(827.5, 1805)	(627.0, 1524)	(705.6, 1652)	
Geometric mean	1245	993.4	1079	
IgM NL 63 (median)	1132	779.0	948.2	0.001
(P25, P75)	(758.8, 1799)	(513.0, 1328)	(591.5, 1568)	
Geometric mean	1234	891.7	1012	
IgM N OC43 (median)	2550	1860	2060	0.003
(P25, P75)	(1319, 4096)	(1076, 3074)	(1121, 3496)	
Geometric mean	2526	1812	2065	

[continued]

Supplemental Table 11a. Continued.

Immunoglobulin (µg/mL)	**Underweight or normal weight** (N = 101)	**Overweight** (N = 91)	**Obese** (N = 48)	**P**[a]
Total immunoglobulin				
IgG1 (median)	6149	6080	6079	0.950
(P25, P75)	(4727, 8308)	(4624, 8189)	(4910, 8088)	
Geometric mean	6292	6184	6285	
IgG2 (median)	3077	3059	3259	0.936
(P25, P75)	(2439, 3640)	(2544, 3692)	(2509, 3731)	
Geometric mean	3037	3056	3015	
IgG3 (median)	1261	1198	1209	0.448
(P25, P75)	(1051, 1479)	(964.8, 1480)	(1004, 1460)	
Geometric mean	1248	1135	1221	
IgG4 (median)	226.9	216.9	269.8	0.318
(P25, P75)	(126.6, 336.7)	(113.0, 401.6)	(132.0, 544.8)	
Geometric mean	219.8	214.4	269.1	
Sum of IgGs (median)	10929	10834	10963	0.902
(P25, P75)	(8573, 13802)	(8851, 13721)	(9544, 13649)	
Geometric mean	11068	10933	11161	
IgE (median)	0.23	0.21	0.25	0.759
(P25, P75)	(0.15, 0.33)	(0.15, 0.35)	(0.15, 0.34)	
Geometric mean	0.23	0.23	0.24	
IgA (median)	231.2	266.3	249.7	0.039
(P25, P75)	(183.3, 282.0)	(200.5, 319.3)	(199.9, 288.7)	
Geometric mean	223.5	257.7	243.3	
IgM (median)	1139	885.8	787.0	<0.001
(P25, P75)	(737.2, 1473)	(700.7, 1194)	(578.3, 1042)	
Geometric mean	1072	909.7	786.3	

P25: percentile 25th. P75: percentile 75th.
<LOQ: value lower than the minimum concentration of the corresponding range of limits of quantification. See Supplemental Table 1.

[a] Kruskal-Wallis test (two-tailed).

Supplemental Table 11b. Relative intraindividual change (%) from 2016-17 to 2020-21 of concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs, by body mass index (N=154).

Immunoglobulin[a]	Normal weight (N = 68)	Overweight (N = 49)	Obese (N = 36)	P[b]
IgA				
IgA CMV pp150 (median)	0.69	0.37	0.25	0.793
(P25, P75)	(-2.30, 3.75)	(-3.74, 3.95)	(-2.03, 2.96)	
∆ ≥15%	5.9	4.1	2.8	
∇ ≥15%	0.0	2.0	0.0	
IgA CMV pp65 (median)	1.06	0.52	1.87	0.245
(P25, P75)	(-2.45, 2.58)	(-2.35, 4.79)	(-1.99, 6.63)	
∆ ≥15%	1.5	2.0	2.8	
∇ ≥15%	1.5	0.0	0.0	
IgA EBV EAD (median)	0.57	0.09	0.30	0.893
(P25, P75)	(-1.91, 3.46)	(-2.67, 4.60)	(-2.61, 2.89)	
∆ ≥15%	1.5	2.0	0.0	
∇ ≥15%	1.5	2.0	0.0	
IgA VCAp18 (median)	0.95	0.45	0.59	0.977
(P25, P75)	(-2.06, 3.37)	(-2.91, 5.20)	(-2.00, 2.95)	
∆ ≥15%	1.5	2.0	0.0	
∇ ≥15%	1.5	0.0	0.0	
IgA N 229E (median)	-0.71	-2.14	1.61	0.336
(P25, P75)	(-4.29, 3.21)	(-4.67, 4.57)	(-1.58, 3.23)	
∆ ≥15%	2.9	4.1	0.0	
∇ ≥15%	4.4	10.2	0.0	
IgA N HKU1 (median)	0.70	0.80	1.19	0.659
(P25, P75)	(-2.53, 2.61)	(-2.55, 3.18)	(-2.38, 3.60)	
∆ ≥15%	2.9	4.1	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgA NL 63 (median)	-0.19	-0.76	-0.47	0.932
(P25, P75)	(-3.18, 4.57)	(-6.03, 7.74)	(-3.63, 3.55)	
∆ ≥15%	4.4	10.2	5.6	
∇ ≥15%	4.4	6.1	2.8	
IgA N OC43 (median)	0.45	0.31	-0.31	0.698
(P25, P75)	(-2.19, 3.46)	(-3.67, 3.03)	(-2.51, 2.46)	
∆ ≥15%	4.4	2.0	0.0	
∇ ≥15%	0.0	0.0	2.8	

[continued]

Supplemental Table 11b. Continued

Immunoglobulin[a]	Normal weight (N = 68)	Overweight (N = 49)	Obese (N = 36)	P[b]
IgG				
IgG CMV pp150 (median)	2.30	0.92	0.79	0.047
(P25, P75)	(-0.34, 5.26)	(-2.79, 2.48)	(-1.30, 3.80)	
∆ ≥15%	2.9	2.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG CMV pp65 (median)	1.45	0.92	0.31	0.769
(P25, P75)	(-2.18, 4.12)	(-1.89, 2.50)	(-2.98, 2.76)	
∆ ≥15%	0.0	0.0	2.8	
∇ ≥15%	0.0	0.0	0.0	
IgG EBV EAD (median)	1.54	-0.51	-0.21	0.075
(P25, P75)	(-2.14, 3.94)	(-2.17, 1.91)	(-3.07, 1.29)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG VCAp18 (median)	1.09	0.23	0.13	0.363
(P25, P75)	(-1.31, 4.57)	(-1.32, 2.68)	(-2.66, 2.41)	
∆ ≥15%	2.9	0.0	2.8	
∇ ≥15%	1.5	0.0	0.0	
IgG N 229E (median)	0.90	-0.38	-0.46	0.329
(P25, P75)	(-2.30, 4.34)	(-2.52, 1.99)	(-3.00, 2.23)	
∆ ≥15%	1.5	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG N HKU1 (median)	1.10	0.74	0.15	0.294
(P25, P75)	(-1.45, 2.94)	(-1.74, 1.97)	(-2.14, 1.80)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG NL 63 (median)	0.26	-0.21	-0.50	0.740
(P25, P75)	(-2.67, 2.99)	(-2.94, 2.46)	(-2.11, 2.05)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG N OC43 (median)	0.87	0.09	-0.84	0.176
(P25, P75)	(-1.91, 3.48)	(-3.85, 2.71)	(-4.17, 2.04)	
∆ ≥15%	1.5	0.0	0.0	
∇ ≥15%	1.5	4.1	2.8	

[continued]

Supplemental Table 11b. Continued

Immunoglobulin[a]	Normal weight (N = 68)	Overweight (N = 49)	Obese (N = 36)	P[b]
IgM				
IgM CMV pp150 (median)	1.56	1.43	-0.79	0.140
(P25, P75)	(-2.05, 5.73)	(-0.78, 3.85)	(-2.86, 1.71)	
∆ ≥15%	0.0	2.0	2.8	
∇ ≥15%	0.0	2.0	0.0	
IgM CMV pp65 (median)	0.93	-0.10	0.39	0.491
(P25, P75)	(-1.37, 3.70)	(-2.77, 2.24)	(-2.20, 4.35)	
∆ ≥15%	0.0	2.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgM EBV EAD (median)	1.31	-0.22	0.05	0.539
(P25, P75)	(-1.88, 3.69)	(-2.86, 3.15)	(-2.80, 3.65)	
∆ ≥15%	0.0	4.1	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgM VCAp18 (median)	1.54	0.33	0.42	0.532
(P25, P75)	(-2.08, 4.10)	(-2.53, 4.24)	(-2.34, 3.71)	
∆ ≥15%	4.4	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgM N 229E (median)	0.55	0.81	1.60	0.628
(P25, P75)	(-3.24, 4.55)	(-3.35, 4.06)	(-2.15, 5.99)	
∆ ≥15%	1.5	4.1	2.8	
∇ ≥15%	0.0	0.0	2.8	
IgM N HKU1 (median)	0.53	1.19	1.16	0.870
(P25, P75)	(-1.55, 3.48)	(-1.46, 2.75)	(-1.56, 4.85)	
∆ ≥15%	1.5	0.0	2.8	
∇ ≥15%	0.0	0.0	0.0	
IgM NL 63 (median)	0.73	0.66	3.34	0.130
(P25, P75)	(-3.85, 3.70)	(-2.89, 4.57)	(-0.71, 5.33)	
∆ ≥15%	0.0	4.1	0.0	
∇ ≥15%	1.5	0.0	0.0	
IgM N OC43 (median)	0.12	0.70	0.92	0.765
(P25, P75)	(-3.28, 4.69)	(-2.92, 3.34)	(-1.75, 4.51)	
∆ ≥15%	2.9	2.0	0.0	
∇ ≥15%	0.0	0.0	0.0	

[continued]

Supplemental Table 11b. Continued

Immunoglobulin[a]	Normal weight (N = 68)	Overweight (N = 49)	Obese (N = 36)	P[b]
Total immunoglobulin				
IgG1 (median)	0.84	1.89	0.54	0.514
(P25, P75)	(-1.92, 2.98)	(-1.18, 4.20)	(-1.30, 2.78)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG2 (median)	0.66	0.81	0.60	0.920
(P25, P75)	(-0.61, 1.84)	(-0.69, 2.58)	(-0.91, 2.49)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG3 (median)	-0.87	-0.01	0.09	0.140
(P25, P75)	(-3.21, 0.71)	(-1.62, 2.14)	(-2.66, 1.99)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG4 (median)	0.71	1.21	1.97	0.979
(P25, P75)	(-1.82, 4.55)	(-1.55, 4.49)	(-2.14, 4.08)	
Δ ≥15%	0.0	2	0.0	
∇ ≥15%	1.5	0.0	0.0	
Sum of IgGs (median)	0.43	1.21	0.51	0.452
(P25, P75)	(-1.10, 2.02)	(-0.74, 2.77)	(-1.05, 2.31)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgE (median)	0.75	3.15	3.61	0.287
(P25, P75)	(-11.14, 10.24)	(-2.16, 10.67)	(-0.50, 13.53)	
Δ ≥15%	14.7	16.3	22.2	
∇ ≥15%	13.2	6.1	5.6	
IgA (median)	0.77	0.74	1.75	0.420
(P25, P75)	(-2.31, 3.91)	(-2.18, 2.24)	(-1.67, 4.55)	
Δ ≥15%	0.0	2	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgM (median)	-0.03	0.50	0.90	0.960
(P25, P75)	(-1.48, 3.94)	(-1.52, 2.38)	(-2.05, 3.34)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	

P25: percentile 25th. P75: percentile 75th.
Body mass index (BMI) is based on each participant's BMI in 2020-21.

[a] Units used for computing the relative intraindividual change were base 10 logtransformed MFI for levels of isotype-antigen combinations, and base 10 logtransformed µg/mL for levels of total Igs.
[b] Kruskal-Wallis test (two-tailed).

Supplemental Table 12a. Concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs in 2016-17 by tobacco smoking (N=240).

Immunoglobulin (MFI)	**Non-smoker** (N = 93)	**Former smoker** (N = 99)	**Current smoker** (N = 48)	**P**[a]
IgA				
IgA CMV pp150 (median)	507.0	498.5	463.2	0.657
(P25, P75)	(331.8, 838.5)	(358.0, 828.5)	(318.2, 822.2)	
Geometric mean	574.2	614.7	619.3	
IgA CMV pp65 (median)	563.0	685.0	497.5	0.001
(P25, P75)	(402.2, 847.5)	(438.0, 1440)	(366.2, 681.6)	
Geometric mean	624.1	865.1	526.6	
IgA EBV EAD (median)	388.0	477.5	390.0	0.082
(P25, P75)	(293.8, 585.2)	(345.0, 741.5)	(311.9, 621.0)	
Geometric mean	463.6	564.9	455.6	
IgA VCAp18 (median)	418.0	521.5	503.2	0.086
(P25, P75)	(311.0, 749.0)	(359.0, 834.5)	(340.4, 653.1)	
Geometric mean	527.9	633.1	515.3	
IgA N 229E (median)	1069	1838	1320	0.084
(P25, P75)	(645.5, 3705)	(830.5, 3753)	(739.0, 3547)	
Geometric mean	1581	2141	1567	
IgA N HKU1 (median)	597.0	767.0	630.2	0.033
(P25, P75)	(431.0, 1163)	(534.0, 1184)	(436.5, 1092)	
Geometric mean	706.6	916.3	852.7	
IgA NL 63 (median)	1538	1461	1837	0.854
(P25, P75)	(735.2, 4995)	(809.0, 4285)	(765.5, 6754)	
Geometric mean	2015	2049	2195	
IgA N OC43 (median)	1290	1647	1440	0.199
(P25, P75)	(824.8, 2558)	(944.5, 2950)	(832.5, 3304)	
Geometric mean	1410	1899	1616	

[continued]

Supplemental Table 12a. Continued.

Immunoglobulin (MFI)	**Non-smoker** (N = 93)	**Former smoker** (N = 99)	**Current smoker** (N = 48)	**P**[a]
IgG				
IgG CMV pp150 (median)	15073	14680	17841	0.731
(P25, P75)	(5167, 28634)	(5303, 36396)	(5456, 37335)	
Geometric mean	12628	14086	14558	
IgG CMV pp65 (median)	7057	6697	6767	0.334
(P25, P75)	(4968, 11166)	(3661, 11865)	(4133, 8664)	
Geometric mean	7339	6473	6372	
IgG EBV EAD (median)	7177	6720	7540	0.542
(P25, P75)	(4890, 10081)	(4748, 9164)	(4657, 11198)	
Geometric mean	7019	6524	7203	
IgG VCAp18 (median)	7815	7400	8888	0.477
(P25, P75)	(4230, 13933)	(4146, 13412)	(4856, 15721)	
Geometric mean	8175	8355	9658	
IgG N 229E (median)	42135	43472	48624	0.410
(P25, P75)	(22762, 77039)	(26623, 68493)	(29682, 80750)	
Geometric mean	40586	40555	47261	
IgG N HKU1 (median)	13536	14752	13974	0.459
(P25, P75)	(9878, 17230)	(10606, 19962)	(8573, 21359)	
Geometric mean	13313	14037	13824	
IgG NL 63 (median)	39623	33123	42683	0.111
(P25, P75)	(20941, 60736)	(19039, 49903)	(23546, 65837)	
Geometric mean	35345	30877	39864	
IgG N OC43 (median)	21022	19339	26634	0.081
(P25, P75)	(11287, 36880)	(10624, 35728)	(16411, 46987)	
Geometric mean	20059	19680	26692	

[continued]

Supplemental Table 12a. Continued.

Immunoglobulin (MFI)	**Non-smoker** (N = 93)	**Former smoker** (N = 99)	**Current smoker** (N = 48)	**P**[a]
IgM				
IgM CMV pp150 (median)	1310	1115	1328	0.218
(P25, P75)	(877.0, 2702)	(616.0, 2020)	(741.5, 2225)	
Geometric mean	1561	1235	1358	
IgM CMV pp65 (median)	726.0	584.0	654.5	0.051
(P25, P75)	(454.2, 1175)	(401.0, 852.0)	(418.4, 1076)	
Geometric mean	800.3	611.8	703.6	
IgM EBV EAD (median)	449.5	387.0	484.2	0.007
(P25, P75)	(323.8, 730.2)	(276.5, 513.0)	(328.6, 675.0)	
Geometric mean	476.2	383.4	477.9	
IgM VCAp18 (median)	899.0	685.5	1030	0.041
(P25, P75)	(525.8, 1848)	(466.0, 1348)	(547.0, 1578)	
Geometric mean	996.5	802.7	994.6	
IgM N 229E (median)	1469	1305	1681	0.107
(P25, P75)	(942.8, 2448)	(752.0, 2264)	(1129, 2688)	
Geometric mean	1548	1292	1588	
IgM N HKU1 (median)	1076	975.0	1027	0.070
(P25, P75)	(771.5, 1746)	(627.0, 1522)	(754.6, 1705)	
Geometric mean	1159	952.4	1214	
IgM NL 63 (median)	1118	769.0	1032	0.005
(P25, P75)	(696.5, 1763)	(496.0, 1321)	(675.4, 1866)	
Geometric mean	1161	842.2	1130	
IgM N OC43 (median)	2122	1606	2390	0.089
(P25, P75)	(1159, 3525)	(1013, 3370)	(1279, 4194)	
Geometric mean	2134	1848	2433	

[continued]

Supplemental Table 12a. Continued.

Immunoglobulin (µg/mL)	**Non-smoker** (N = 93)	**Former smoker** (N = 99)	**Current smoker** (N = 48)	**P**[a]
Total immunoglobulin				
IgG1 (median)	6120	6166	5870	0.724
(P25, P75)	(4649, 8451)	(4793, 8376)	(4630, 7742)	
Geometric mean	6429	6176	6063	
IgG2 (median)	3077	3212	2738	0.060
(P25, P75)	(2479, 3701)	(2485, 3800)	(2465, 3399)	
Geometric mean	3110	3092	2808	
IgG3 (median)	1203	1284	1187	0.858
(P25, P75)	(989.4, 1501)	(990.8, 1473)	(1063, 1415)	
Geometric mean	1225	1185	1176	
IgG4 (median)	238.3	221.1	224.7	0.878
(P25, P75)	(130.7, 388.1)	(111.0, 464.6)	(127.5, 346.9)	
Geometric mean	233.3	227.1	213.8	
Sum of IgGs (median)	11256	10903	10450	0.240
(P25, P75)	(8766, 13993)	(9403, 13721)	(8393, 12520)	
Geometric mean	11368	10996	10494	
IgE (median)	0.22	0.24	0.21	0.878
(P25, P75)	(0.16, 0.34)	(0.14, 0.34)	(0.14, 0.33)	
Geometric mean	0.23	0.23	0.23	
IgA (median)	243.9	250.1	239.0	0.360
(P25, P75)	(188.7, 291.3)	(200.5, 304.2)	(162.3, 320.8)	
Geometric mean	233.7	251.5	229.1	
IgM (median)	1124	846.1	1024	0.025
(P25, P75)	(747.6, 1459)	(686.0, 1052)	(582.4, 1347)	
Geometric mean	1028	880.1	937.3	

P25: percentile 25th. P75: percentile 75th.
<LOQ: value lower than the minimum concentration of the corresponding range of limits of quantification. See Supplemental Table 1.

[a] Kruskal-Wallis test (two-tailed).

Supplemental Table 12b. Relative intraindividual change (%) from 2016-17 to 2020-21 of concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs, by tobacco smoking (N=154).

Immunoglobulin[a]	Non-smoker (N = 59)	Former smoker (N = 56)	Current smoker (N = 39)	P[b]
IgA				
IgA CMV pp150 (median)	-0.02	-0.16	1.61	0.173
(P25, P75)	(-2.75, 3.12)	(-2.21, 3.24)	(-0.91, 4.61)	
∆ ≥15%	1.7	3.6	10.3	
∇ ≥15%	1.7	0.0	0.0	
IgA CMV pp65 (median)	0.74	0.32	1.74	0.386
(P25, P75)	(-2.50, 2.76)	(-2.37, 4.56)	(-1.17, 4.80)	
∆ ≥15%	0.0	1.8	5.1	
∇ ≥15%	0.0	0.0	2.6	
IgA EBV EAD (median)	0.09	-0.40	0.75	0.680
(P25, P75)	(-1.83, 3.04)	(-2.38, 3.87)	(-1.79, 4.07)	
∆ ≥15%	0.0	0.0	5.1	
∇ ≥15%	0.0	0.0	5.1	
IgA VCAp18 (median)	0.45	0.82	1.12	0.289
(P25, P75)	(-2.73, 2.25)	(-2.38, 4.20)	(-0.49, 3.85)	
∆ ≥15%	1.7	0.0	5.1	
∇ ≥15%	0.0	0.0	2.6	
IgA N 229E (median)	0.58	-1.35	1.37	0.332
(P25, P75)	(-3.22, 5.33)	(-3.87, 2.20)	(-4.15, 3.44)	
∆ ≥15%	1.7	0.0	7.7	
∇ ≥15%	6.8	3.6	5.1	
IgA N HKU1 (median)	0.90	-0.55	1.86	0.134
(P25, P75)	(-2.49, 2.86)	(-2.78, 2.91)	(-0.43, 3.59)	
∆ ≥15%	0.0	3.6	5.1	
∇ ≥15%	0.0	0.0	0.0	
IgA NL 63 (median)	-0.76	-0.65	0.27	0.454
(P25, P75)	(-5.25, 7.02)	(-3.63, 2.78)	(-1.98, 6.09)	
∆ ≥15%	6.8	5.4	7.7	
∇ ≥15%	6.8	0.0	7.7	
IgA N OC43 (median)	0.48	0.18	0.78	0.819
(P25, P75)	(-2.96, 3.83)	(-2.69, 2.48)	(-2.86, 3.62)	
∆ ≥15%	1.7	1.8	5.1	
∇ ≥15%	1.7	0.0	0.0	

[continued]

Supplemental Table 12b. Continued

Immunoglobulin[a]	Non-smoker (N = 59)	Former smoker (N = 56)	Current smoker (N = 39)	P[b]
IgG				
IgG CMV pp150 (median)	1.33	1.25	1.76	0.571
(P25, P75)	(-1.70, 3.99)	(-0.35, 3.80)	(-2.45, 4.55)	
Δ ≥15%	0.0	1.8	5.1	
∇ ≥15%	0.0	0.0	0.0	
IgG CMV pp65 (median)	0.67	1.00	1.68	0.682
(P25, P75)	(-1.88, 2.51)	(-1.74, 2.59)	(-4.12, 5.69)	
Δ ≥15%	1.7	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG EBV EAD (median)	0.37	0.71	0.51	0.840
(P25, P75)	(-2.09, 2.61)	(-2.25, 2.34)	(-2.82, 3.95)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG VCAp18 (median)	0.23	0.73	1.05	0.652
(P25, P75)	(-1.47, 3.13)	(-1.88, 2.23)	(-2.28, 4.82)	
Δ ≥15%	5.1	0.0	0.0	
∇ ≥15%	1.7	0.0	0.0	
IgG N 229E (median)	0.30	-0.50	0.48	0.702
(P25, P75)	(-2.50, 2.88)	(-2.56, 2.39)	(-2.75, 3.35)	
Δ ≥15%	0.0	0.0	2.6	
∇ ≥15%	0.0	0.0	0.0	
IgG N HKU1 (median)	0.48	0.44	1.11	0.850
(P25, P75)	(-1.60, 2.40)	(-1.48, 2.10)	(-2.34, 2.95)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG NL 63 (median)	-0.28	0.12	0.40	0.620
(P25, P75)	(-2.92, 2.48)	(-2.24, 2.32)	(-2.89, 3.64)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG N OC43 (median)	-0.23	0.46	-0.13	0.837
(P25, P75)	(-2.97, 3.38)	(-3.41, 2.73)	(-3.75, 2.68)	
Δ ≥15%	0.0	0.0	2.6	
∇ ≥15%	5.1	0.0	2.6	

[continued]

Supplemental Table 12b. Continued

Immunoglobulin[a]	Non-smoker (N = 59)	Former smoker (N = 56)	Current smoker (N = 39)	P[b]
IgM				
IgM CMV pp150 (median)	-0.01	1.08	2.32	0.039
(P25, P75)	(-2.25, 2.80)	(-2.25, 3.45)	(-1.59, 6.59)	
Δ ≥15%	0.0	1.8	2.6	
∇ ≥15%	1.7	0.0	0.0	
IgM CMV pp65 (median)	0.66	0.10	0.57	0.768
(P25, P75)	(-1.64, 4.24)	(-1.93, 2.16)	(-1.95, 4.09)	
Δ ≥15%	1.7	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgM EBV EAD (median)	1.34	0.38	0.35	0.979
(P25, P75)	(-2.92, 3.85)	(-2.18, 3.57)	(-1.76, 3.58)	
Δ ≥15%	1.7	1.8	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgM VCAp18 (median)	0.64	1.35	1.23	0.886
(P25, P75)	(-2.77, 4.96)	(-2.08, 2.55)	(-2.33, 5.42)	
Δ ≥15%	1.7	1.8	2.6	
∇ ≥15%	0.0	0.0	0.0	
IgM N 229E (median)	0.21	1.28	1.31	0.386
(P25, P75)	(-3.37, 3.37)	(-1.73, 4.44)	(-2.62, 6.58)	
Δ ≥15%	0.0	3.6	5.1	
∇ ≥15%	0.0	1.8	0.0	
IgM N HKU1 (median)	1.32	0.95	0.57	0.897
(P25, P75)	(-2.20, 3.11)	(-1.26, 4.37)	(-1.15, 3.65)	
Δ ≥15%	1.7	1.8	2.6	
∇ ≥15%	0.0	0.0	0.0	
IgM NL 63 (median)	1.28	0.93	2.42	0.240
(P25, P75)	(-2.39, 4.32)	(-3.49, 3.72)	(-0.62, 6.88)	
Δ ≥15%	0.0	3.6	0.0	
∇ ≥15%	1.7	0.0	0.0	
IgM N OC43 (median)	1.36	-0.34	1.26	0.538
(P25, P75)	(-2.70, 3.60)	(-3.01, 4.50)	(-1.70, 5.80)	
Δ ≥15%	0.0	5.4	0.0	
∇ ≥15%	0.0	0.0	0.0	

[continued]

Supplemental Table 12b. Continued

Immunoglobulin[a]	Non-smoker (N = 59)	Former smoker (N = 56)	Current smoker (N = 39)	P[b]
Total immunoglobulin				
IgG1 (median)	0.06	1.13	1.66	0.065
(P25, P75)	(-2.55, 2.36)	(-1.32, 3.57)	(0.14, 3.42)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG2 (median)	0.79	0.41	0.87	0.608
(P25, P75)	(-0.47, 2.03)	(-0.75, 2.50)	(-0.63, 2.04)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG3 (median)	-0.28	0.11	-0.91	0.569
(P25, P75)	(-2.41, 1.65)	(-3.14, 2.24)	(-3.05, 1.45)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG4 (median)	1.39	2.48	0.17	0.130
(P25, P75)	(-1.41, 3.94)	(-1.30, 5.51)	(-2.35, 2.94)	
∆ ≥15%	1.7	1.8	0.0	
∇ ≥15%	1.7	0.0	0.0	
Sum of IgGs (median)	0.34	0.75	1.19	0.085
(P25, P75)	(-1.97, 1.99)	(-0.73, 3.07)	(0.11, 2.39)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgE (median)	1.90	4.30	2.57	0.434
(P25, P75)	(-4.62, 9.61)	(-2.57, 12.90)	(-3.86, 8.94)	
∆ ≥15%	13.6	21.4	15.4	
∇ ≥15%	10.2	10.7	5.1	
IgA (median)	0.53	1.50	1.36	0.115
(P25, P75)	(-2.42, 2.18)	(-1.87, 5.26)	(-0.84, 4.94)	
∆ ≥15%	0.0	1.8	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgM (median)	-0.23	-0.23	1.20	0.329
(P25, P75)	(-2.03, 3.40)	(-1.52, 3.15)	(-0.90, 3.53)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	

P25: percentile 25th. P75: percentile 75th.
Tobacco smoking groups are based on participants' data in 2020-21.

[a] Units used for computing the relative intraindividual change were base 10 logtransformed MFI for levels of isotype-antigen combinations, and base 10 logtransformed µg/mL for levels of total Igs.
[b] Kruskal-Wallis test (two-tailed).

Supplemental Table 13. Relative intraindividual change (%) from 2016-17 to 2020-21 of concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs, by presence or absence of dyslipidemia (N=154).

	Dyslipidemia		
Immunoglobulin[a]	**No** (N = 117)	**Yes** (N = 37)	**P**[b]
IgA			
IgA CMV pp150 (median)	0.26	1.16	0.511
(P25, P75)	(-2.69, 3.38)	(-1.83, 3.86)	
Δ ≥15%	6	0.0	
∇ ≥15%	0.9	0.0	
IgA CMV pp65 (median)	1.01	0.85	0.948
(P25, P75)	(-2.30, 3.50)	(-2.16, 4.98)	
Δ ≥15%	2.6	0.0	
∇ ≥15%	0.0	2.7	
IgA EBV EAD (median)	0.23	0.63	0.743
(P25, P75)	(-2.21, 3.54)	(-2.50, 5.08)	
Δ ≥15%	1.7	0.0	
∇ ≥15%	0.9	2.7	
IgA VCAp18 (median)	0.57	1.24	0.533
(P25, P75)	(-2.06, 3.11)	(-2.64, 4.18)	
Δ ≥15%	1.7	2.7	
∇ ≥15%	0.0	2.7	
IgA N 229E (median)	0.14	-0.98	0.538
(P25, P75)	(-3.28, 3.39)	(-5.61, 5.33)	
Δ ≥15%	3.4	0.0	
∇ ≥15%	4.3	8.1	
IgA N HKU1 (median)	0.90	1.65	0.495
(P25, P75)	(-1.99, 2.68)	(-2.71, 3.61)	
Δ ≥15%	2.6	2.7	
∇ ≥15%	0.0	0.0	
IgA NL 63 (median)	-0.41	-0.53	0.864
(P25, P75)	(-3.71, 4.25)	(-3.78, 6.27)	
Δ ≥15%	7.7	2.7	
∇ ≥15%	4.3	5.4	
IgA N OC43 (median)	0.40	0.37	0.655
(P25, P75)	(-2.68, 3.35)	(-3.96, 2.53)	
Δ ≥15%	3.4	0.0	
∇ ≥15%	0.0	2.7	

[continued]

Supplemental Table 13. Continued

Immunoglobulin[a]	Dyslipidemia No (N = 117)	Dyslipidemia Yes (N = 37)	
IgG			
IgG CMV pp150 (median)	1.66	0.80	0.552
(P25, P75)	(-1.01, 3.93)	(-2.48, 4.90)	
Δ ≥15%	2.6	0.0	
∇ ≥15%	0.0	0.0	
IgG CMV pp65 (median)	0.92	0.31	0.497
(P25, P75)	(-1.65, 2.92)	(-4.08, 5.46)	
Δ ≥15%	0.9	0.0	
∇ ≥15%	0.0	0.0	
IgG EBV EAD (median)	0.75	-0.61	0.151
(P25, P75)	(-1.84, 3.13)	(-3.39, 2.40)	
Δ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG VCAp18 (median)	0.77	0.41	0.677
(P25, P75)	(-1.69, 3.40)	(-2.34, 3.12)	
Δ ≥15%	2.6	0.0	
∇ ≥15%	0.9	0.0	
IgG N 229E (median)	0.30	-0.45	0.671
(P25, P75)	(-2.42, 2.71)	(-3.17, 2.67)	
Δ ≥15%	0.9	0.0	
∇ ≥15%	0.0	0.0	
IgG N HKU1 (median)	0.69	0.18	0.155
(P25, P75)	(-1.56, 2.51)	(-2.08, 1.70)	
Δ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG NL 63 (median)	0.10	-0.39	0.740
(P25, P75)	(-2.23, 2.59)	(-2.95, 2.72)	
Δ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG N OC43 (median)	0.24	-0.63	0.455
(P25, P75)	(-2.97, 3.00)	(-3.85, 3.38)	
Δ ≥15%	0.9	0.0	
∇ ≥15%	1.7	5.4	

[continued]

Supplemental Table 13. Continued.

Immunoglobulin[a]	Dyslipidemia No (N = 117)	Dyslipidemia Yes (N = 37)	P[b]
IgM			
IgM CMV pp150 (median)	0.95	1.58	0.538
(P25, P75)	(-2.10, 3.53)	(-1.70, 5.91)	
∆ ≥15%	0.9	2.7	
∇ ≥15%	0.0	2.7	
IgM CMV pp65 (median)	0.62	0.21	0.721
(P25, P75)	(-1.66, 3.03)	(-2.33, 4.50)	
∆ ≥15%	0.0	2.7	
∇ ≥15%	0.0	0.0	
IgM EBV EAD (median)	0.45	0.55	0.340
(P25, P75)	(-2.33, 3.11)	(-2.07, 4.38)	
∆ ≥15%	0.9	2.7	
∇ ≥15%	0.0	0.0	
IgM VCAp18 (median)	0.89	1.68	0.097
(P25, P75)	(-2.53, 3.89)	(0.03, 5.94)	
∆ ≥15%	1.7	2.7	
∇ ≥15%	0.0	0.0	
IgM N 229E (median)	0.40	2.97	0.050
(P25, P75)	(-3.29, 4.08)	(-1.63, 7.92)	
∆ ≥15%	2.6	2.7	
∇ ≥15%	0.9	0.0	
IgM N HKU1 (median)	0.61	1.77	0.054
(P25, P75)	(-1.92, 3.12)	(-0.64, 6.39)	
∆ ≥15%	0.9	5.4	
∇ ≥15%	0.0	0.0	
IgM NL 63 (median)	0.90	2.24	0.204
(P25, P75)	(-2.89, 4.21)	(-2.37, 6.52)	
∆ ≥15%	0.9	2.7	
∇ ≥15%	0.9	0.0	
IgM N OC43 (median)	-0.19	3.26	0.004
(P25, P75)	(-3.27, 3.29)	(-1.06, 6.91)	
∆ ≥15%	1.7	2.7	
∇ ≥15%	0.0	0.0	

[continued]

Supplemental Table 13. Continued.

Immunoglobulin[a]	Dyslipidemia No (N = 117)	Dyslipidemia Yes (N = 37)	P[b]
Total Immunoglobulin			
IgG1 (median)	0.84	1.96	0.050
(P25, P75)	(-1.78, 2.96)	(-0.07, 4.20)	
∆ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG2 (median)	0.51	0.79	0.071
(P25, P75)	(-0.97, 1.99)	(0.29, 2.76)	
∆ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG3 (median)	-0.43	0.18	0.046
(P25, P75)	(-3.09, 1.21)	(-1.18, 2.21)	
∆ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgG4 (median)	0.36	2.92	0.016
(P25, P75)	(-2.07, 3.83)	(-0.07, 5.97)	
∆ ≥15%	0.9	2.7	
∇ ≥15%	0.9	0.0	
Sum of IgGs (median)	0.55	1.65	0.032
(P25, P75)	(-1.49, 2.23)	(0.11, 2.98)	
∆ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	
IgE (median)	2.57	3.98	0.338
(P25, P75)	(-5.08, 10.67)	(-1.34, 13.31)	
∆ ≥15%	16.2	18.9	
∇ ≥15%	11.1	2.7	
IgA (median)	0.16	3.09	0.002
(P25, P75)	(-2.61, 3.23)	(0.68, 5.20)	
∆ ≥15%	0.9	0.0	
∇ ≥15%	0.0	0.0	
IgM (median)	-0.18	1.73	0.046
(P25, P75)	(-1.67, 2.87)	(-0.85, 4.35)	
∆ ≥15%	0.0	0.0	
∇ ≥15%	0.0	0.0	

P25: percentile 25th. P75: percentile 75th.
The presence or absence of dyslipidemia was determined from data provided by the Data Analytics Program for Health Research and Innovation (PADRIS) and the Barcelona Health Survey (BHS) of 2016 (section 2.7). The following ICD-10 codes were used: E78.00 (pure hypercholesterolemia, unspecified, N=19), E78.1 (pure hyperglyceridemia, N=3), E78.2 (mixed hyperlipidemia, N=1), E78.5 (hyperlipidemia, unspecified, N=3), and E78.9 (disorder of lipoprotein metabolism, unspecified, N=11).

[a] Units used for computing the relative intraindividual change were base 10 logtransformed MFI for levels of isotype-antigen combinations, and base 10 logtransformed µg/mL for levels of total Igs.
[b] Mann-Whitney's *U* test (two-tail).

Supplemental Table 14a. Concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs in 2016-17 by educational level (N=240).

Immunoglobulin (MFI)	**Primary or less** (N = 56)	**Secondary** (N = 65)	**University** (N = 119)	**P**[a]
IgA				
IgA CMV pp150 (median)	487.5	502.5	490.0	0.966
(P25, P75)	(337.5, 872.6)	(342.5, 884.5)	(332.0, 821.5)	
Geometric mean	609.6	616.6	585.9	
IgA CMV pp65 (median)	592.0	535.5	599.0	0.994
(P25, P75)	(405.6, 1098)	(417.2, 1060)	(408.5, 836.0)	
Geometric mean	769.9	672.6	665.0	
IgA EBV EAD (median)	414.5	426.0	426.5	0.868
(P25, P75)	(288.2, 744.5)	(291.5, 622.2)	(328.0, 636.5)	
Geometric mean	516.9	485.4	502.7	
IgA VCAp18 (median)	459.5	499.0	481.5	0.866
(P25, P75)	(333.4, 746.2)	(320.5, 808.2)	(340.0, 730.0)	
Geometric mean	578.7	573.5	556.6	
IgA N 229E (median)	1476	1139	1420	0.438
(P25, P75)	(711.4, 3668)	(679.5, 3412)	(747.5, 4162)	
Geometric mean	1823	1551	1916	
IgA N HKU1 (median)	737.8	634.0	684.0	0.731
(P25, P75)	(478.0, 1429)	(436.5, 1143)	(476.0, 1094)	
Geometric mean	883.7	790.6	801.0	
IgA NL 63 (median)	1532	1523	1680	0.835
(P25, P75)	(661.5, 7030)	(790.2, 4409)	(801.0, 4550)	
Geometric mean	1991	2003	2135	
IgA N OC43 (median)	1328	1544	1377	0.610
(P25, P75)	(784.5, 2617)	(945.5, 2699)	(900.5, 3005)	
Geometric mean	1441	1684	1715	

[continued]

Supplemental Table 14a. Continued.

Immunoglobulin (MFI)	**Primary or less** (N = 56)	**Secondary** (N = 65)	**University** (N = 119)	**P**[a]
IgG				
IgG CMV pp150 (median)	17454	18278	13403	0.045
(P25, P75)	(6094, 32650)	(8736, 44660)	(4962, 24583)	
Geometric mean	15082	17448	11291	
IgG CMV pp65 (median)	6114	7390	6747	0.051
(P25, P75)	(3150, 10210)	(5167, 12529)	(4465, 10435)	
Geometric mean	5995	7964	6568	
IgG EBV EAD (median)	5878	6983	7070	0.678
(P25, P75)	(4659, 10964)	(5272, 9630)	(4712, 9901)	
Geometric mean	6740	7209	6704	
IgG VCAp18 (median)	7949	7699	7815	0.901
(P25, P75)	(3777, 14112)	(4283, 14120)	(4381, 14461)	
Geometric mean	7885	8545	8839	
IgG N 229E (median)	56887	46238	43104	0.519
(P25, P75)	(23160, 87043)	(24571, 73910)	(26070, 69012)	
Geometric mean	45839	42941	39493	
IgG N HKU1 (median)	14313	14732	13611	0.258
(P25, P75)	(9857, 19927)	(11426, 19512)	(9517, 18774)	
Geometric mean	14479	14703	12861	
IgG NL 63 (median)	31365	41283	36258	0.317
(P25, P75)	(18233, 58213)	(25159, 57890)	(20630, 58662)	
Geometric mean	31003	38633	33594	
IgG N OC43 (median)	19823	25113	21367	0.385
(P25, P75)	(11482, 40084)	(12352, 39554)	(10710, 36189)	
Geometric mean	22025	23238	19564	

[continued]

Supplemental Table 14a. Continued.

Immunoglobulin (MFI)	**Primary or less** (N = 56)	**Secondary** (N = 65)	**University** (N = 119)	**P**[a]
IgM				
IgM CMV pp150 (median)	1308	1129	1300	0.770
(P25, P75)	(578.5, 2498)	(719.5, 2058)	(798.5, 2539)	
Geometric mean	1397	1315	1405	
IgM CMV pp65 (median)	548.8	676.0	718.0	0.323
(P25, P75)	(394.6, 1097)	(514.2, 942.0)	(425.5, 1102)	
Geometric mean	649.6	704.3	718.9	
IgM EBV EAD (median)	412.2	432.0	415.0	0.529
(P25, P75)	(277.6, 583.5)	(334.5, 586.0)	(286.0, 594.0)	
Geometric mean	414.8	458.9	433.6	
IgM VCAp18 (median)	641.0	884.0	895.0	0.103
(P25, P75)	(425.5, 1284)	(592.5, 1534)	(517.0, 1780)	
Geometric mean	756.7	985.3	952.8	
IgM N 229E (median)	1380	1347	1532	0.397
(P25, P75)	(832.1, 2425)	(863.8, 1888)	(928.0, 2638)	
Geometric mean	1390	1363	1518	
IgM N HKU1 (median)	1160	1022	987.5	0.902
(P25, P75)	(682.8, 1708)	(747.8, 1482)	(713.0, 1654)	
Geometric mean	1126	1051	1072	
IgM NL 63 (median)	1036	1029	930.5	0.748
(P25, P75)	(523.2, 1509)	(579.8, 1862)	(597.5, 1541)	
Geometric mean	966.2	1094	990.3	
IgM N OC43 (median)	2110	2378	1980	0.648
(P25, P75)	(997.2, 3411)	(1132, 3600)	(1126, 3562)	
Geometric mean	1964	2220	2031	

[continued]

Supplemental Table 14a. Continued.

Immunoglobulin (µg/mL)	**Primary or less** (N = 56)	**Secondary** (N = 65)	**University** (N = 119)	**P**[a]
Total immunoglobulin				
IgG1 (median)	5970	6707	5848	0.059
(P25, P75)	(4380, 7728)	(5109, 8955)	(4592, 7940)	
Geometric mean	6110	6881	5993	
IgG2 (median)	2918	3033	3192	0.595
(P25, P75)	(2506, 3601)	(2507, 3530)	(2436, 3752)	
Geometric mean	2978	3043	3067	
IgG3 (median)	1272	1235	1236	0.783
(P25, P75)	(1022, 1557)	(990.0, 1438)	(1011, 1472)	
Geometric mean	1199	1187	1204	
IgG4 (median)	201.4	222.7	258.0	0.659
(P25, P75)	(112.9, 387.1)	(132.2, 474.1)	(118.4, 377.1)	
Geometric mean	214.5	232.0	229.8	
Sum of IgGs (median)	10888	11833	10760	0.124
(P25, P75)	(8659, 13751)	(9414, 14743)	(8545, 13370)	
Geometric mean	10840	11698	10779	
IgE (median)	0.21	0.26	0.23	0.685
(P25, P75)	(0.14, 0.36)	(0.15, 0.36)	(0.15, 0.33)	
Geometric mean	0.23	0.24	0.22	
IgA (median)	241.1	254.9	245.4	0.738
(P25, P75)	(175.8, 298.5)	(194.9, 310.1)	(194.3, 297.1)	
Geometric mean	239.2	244.9	237.6	
IgM (median)	885.4	916.8	946.5	0.340
(P25, P75)	(613.3, 1198)	(755.8, 1220)	(688.7, 1382)	
Geometric mean	875.8	972.2	967.8	

P25: percentile 25th. P75: percentile 75th.
<LOQ: value lower than the minimum concentration of the corresponding range of limits of quantification. See Supplemental Table 1.

[a] Kruskal-Wallis test (two-tailed).

Supplemental Table 14b. Relative intraindividual change (%) from 2016-17 to 2020-21 of concentrations of 24 isotype-antigen combinations for cytomegalovirus, Epstein-Barr and common cold infections, and of total Igs, by educational level (N=154).

Immunoglobulin[a]	Primary or less (N = 35)	Secondary (N = 42)	University (N = 77)	P[b]
IgA				
IgA CMV pp150 (median)	1.12	0.67	0.00	0.513
(P25, P75)	(-2.23, 4.79)	(-2.06, 3.43)	(-2.82, 2.82)	
∆ ≥15%	2.9	4.8	5.2	
∇ ≥15%	2.9	0.0	0.0	
IgA CMV pp65 (median)	1.74	0.38	0.74	0.157
(P25, P75)	(-0.83, 6.87)	(-3.25, 2.73)	(-2.29, 3.50)	
∆ ≥15%	0.0	2.4	2.6	
∇ ≥15%	0.0	2.4	0.0	
IgA EBV EAD (median)	0.75	-0.35	0.45	0.437
(P25, P75)	(-0.96, 4.45)	(-2.98, 3.79)	(-2.31, 3.19)	
∆ ≥15%	0.0	0.0	2.6	
∇ ≥15%	0.0	2.4	1.3	
IgA VCAp18 (median)	1.47	-0.61	1.04	0.143
(P25, P75)	(-1.43, 3.85)	(-3.33, 3.17)	(-2.00, 3.58)	
∆ ≥15%	2.9	0.0	2.6	
∇ ≥15%	0.0	2.4	0.0	
IgA N 229E (median)	1.36	-0.21	-0.74	0.963
(P25, P75)	(-3.77, 3.07)	(-4.92, 5.53)	(-3.53, 3.46)	
∆ ≥15%	0.0	4.8	2.6	
∇ ≥15%	8.6	4.8	3.9	
IgA N HKU1 (median)	1.97	0.29	0.76	0.385
(P25, P75)	(-1.47, 3.96)	(-2.94, 3.46)	(-2.52, 2.68)	
∆ ≥15%	2.9	2.4	2.6	
∇ ≥15%	0.0	0.0	0.0	
IgA NL 63 (median)	0.11	-1.02	-0.52	0.454
(P25, P75)	(-1.92, 6.60)	(-4.97, 6.78)	(-3.70, 3.74)	
∆ ≥15%	8.6	9.5	3.9	
∇ ≥15%	0.0	7.1	5.2	
IgA N OC43 (median)	-0.22	-0.16	0.85	0.641
(P25, P75)	(-3.29, 2.38)	(-2.71, 3.94)	(-2.58, 3.77)	
∆ ≥15%	0.0	4.8	2.6	
∇ ≥15%	2.9	0.0	0.0	

[continued]

Supplemental Table 14b. Continued

Immunoglobulin[a]	Primary or less (N = 35)	Secondary (N = 42)	University (N = 77)	P[b]
IgG				
IgG CMV pp150 (median)	1.22	0.90	1.55	0.939
(P25, P75)	(-3.19, 4.66)	(-0.52, 3.99)	(-1.60, 3.83)	
Δ ≥15%	2.9	2.4	1.3	
∇ ≥15%	0.0	0.0	0.0	
IgG CMV pp65 (median)	0.90	1.08	0.79	0.683
(P25, P75)	(-2.56, 4.31)	(-1.79, 2.55)	(-2.88, 2.47)	
Δ ≥15%	0.0	2.4	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG EBV EAD (median)	0.24	1.02	-0.18	0.591
(P25, P75)	(-2.27, 3.17)	(-1.98, 3.17)	(-2.26, 2.40)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG VCAp18 (median)	0.42	1.38	0.41	0.180
(P25, P75)	(-2.29, 4.28)	(-0.26, 4.97)	(-2.35, 2.61)	
Δ ≥15%	0.0	4.8	1.3	
∇ ≥15%	0.0	0.0	1.3	
IgG N 229E (median)	-0.78	0.54	0.15	0.565
(P25, P75)	(-3.58, 2.96)	(-1.79, 2.67)	(-2.64, 2.59)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG N HKU1 (median)	-0.05	0.94	0.60	0.624
(P25, P75)	(-1.59, 2.46)	(-1.49, 2.65)	(-1.72, 1.90)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG NL 63 (median)	-0.47	0.14	0.35	0.830
(P25, P75)	(-1.89, 2.87)	(-2.25, 3.11)	(-2.90, 2.32)	
Δ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG N OC43 (median)	0.52	0.15	-0.13	0.669
(P25, P75)	(-4.17, 3.16)	(-2.18, 3.45)	(-3.89, 2.96)	
Δ ≥15%	0.0	0.0	1.3	
∇ ≥15%	5.7	2.4	1.3	

[continued]

Supplemental Table 14b. Continued

Immunoglobulin[a]	Primary or less (N = 35)	Secondary (N = 42)	University (N = 77)	P[b]
IgM				
IgM CMV pp150 (median)	-0.18	1.99	1.22	0.444
(P25, P75)	(-1.48, 3.71)	(-1.69, 4.53)	(-2.50, 3.45)	
Δ ≥15%	2.9	2.4	0.0	
∇ ≥15%	0.0	2.4	0.0	
IgM CMV pp65 (median)	0.82	-0.03	0.66	0.674
(P25, P75)	(-2.63, 4.22)	(-2.68, 3.62)	(-1.39, 3.66)	
Δ ≥15%	0.0	0.0	1.3	
∇ ≥15%	0.0	0.0	0.0	
IgM EBV EAD (median)	1.23	0.52	0.38	0.776
(P25, P75)	(-1.68, 3.58)	(-1.96, 4.12)	(-2.92, 3.67)	
Δ ≥15%	0.0	2.4	1.3	
∇ ≥15%	0.0	0.0	0.0	
IgM VCAp18 (median)	0.89	1.57	0.98	0.692
(P25, P75)	(-2.37, 6.56)	(-0.70, 3.97)	(-2.56, 3.51)	
Δ ≥15%	2.9	0.0	2.6	
∇ ≥15%	0.0	0.0	0.0	
IgM N 229E (median)	1.90	1.27	-0.02	0.540
(P25, P75)	(-2.46, 4.79)	(-1.86, 4.03)	(-3.41, 4.25)	
Δ ≥15%	2.9	2.4	2.6	
∇ ≥15%	0.0	0.0	1.3	
IgM N HKU1 (median)	1.32	0.95	0.61	0.722
(P25, P75)	(-0.76, 4.32)	(-1.01, 4.18)	(-1.89, 3.57)	
Δ ≥15%	2.9	2.4	1.3	
∇ ≥15%	0.0	0.0	0.0	
IgM NL 63 (median)	0.99	1.12	1.26	0.543
(P25, P75)	(-3.17, 3.90)	(-2.21, 6.08)	(-3.39, 4.06)	
Δ ≥15%	0.0	4.8	0.0	
∇ ≥15%	2.9	0.0	0.0	
IgM N OC43 (median)	1.34	0.67	0.43	0.830
(P25, P75)	(-3.21, 5.42)	(-2.50, 4.37)	(-2.65, 3.77)	
Δ ≥15%	2.9	2.4	1.3	
∇ ≥15%	0.0	0.0	0.0	

[continued]

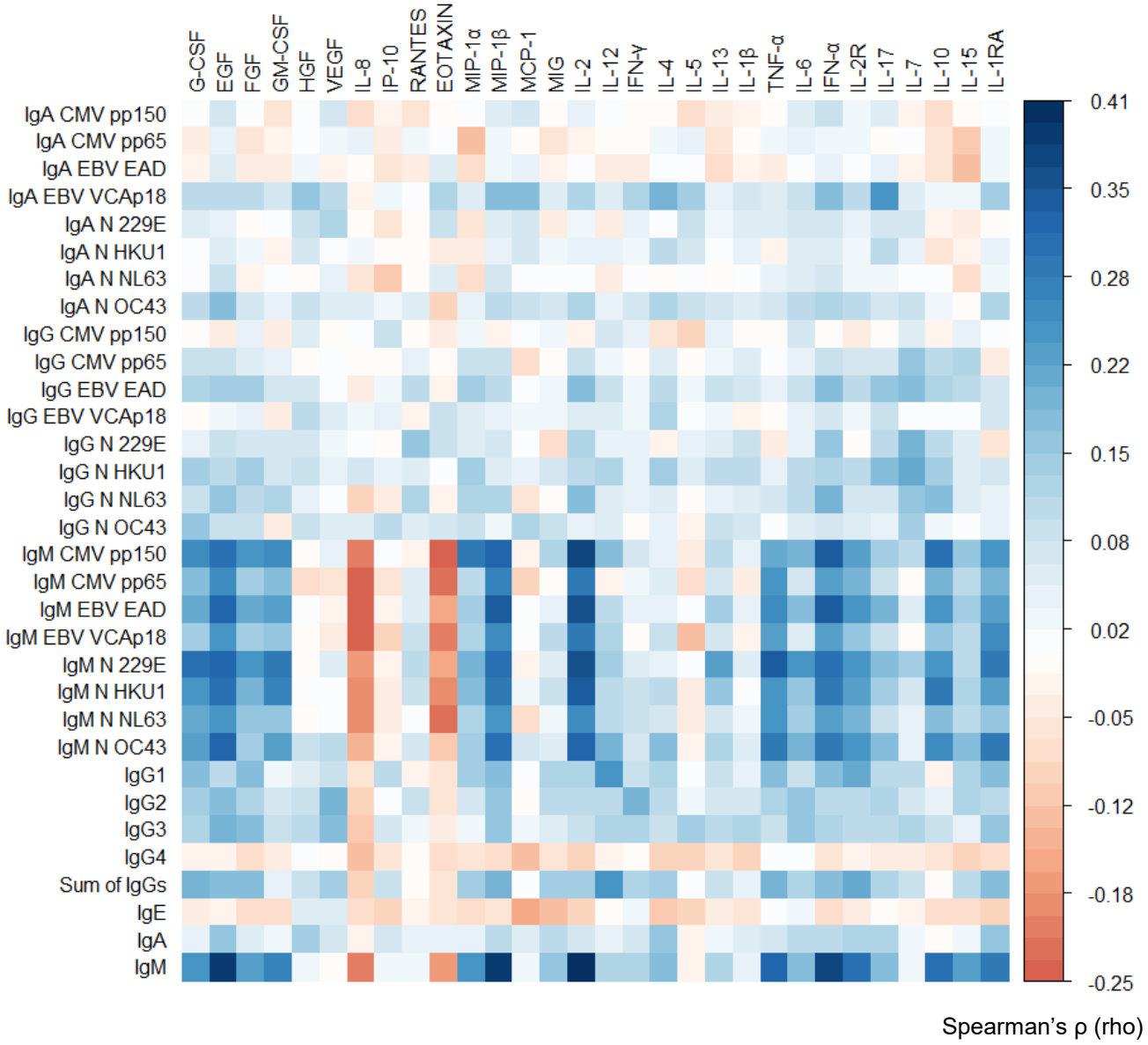
Supplemental Table 14b. Continued

Immunoglobulin[a]	Primary or less (N = 35)	Secondary (N = 42)	University (N = 77)	P[b]
Total immunoglobulin				
IgG1 (median)	1.43	0.54	1.26	0.835
(P25, P75)	(-2.40, 3.73)	(-1.58, 3.06)	(-1.47, 3.14)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG2 (median)	0.83	0.41	0.68	0.967
(P25, P75)	(-1.91, 2.04)	(-0.71, 2.79)	(-0.43, 1.98)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG3 (median)	-0.74	-0.09	-0.28	0.630
(P25, P75)	(-2.67, 1.34)	(-3.36, 2.51)	(-1.89, 0.86)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgG4 (median)	2.03	1.76	0.75	0.984
(P25, P75)	(-3.17, 4.06)	(-1.39, 4.48)	(-1.72, 4.76)	
∆ ≥15%	2.9	2.4	0.0	
∇ ≥15%	2.9	0.0	0.0	
Sum of IgGs (median)	0.73	0.58	0.85	0.975
(P25, P75)	(-1.61, 2.46)	(-0.91, 2.45)	(-1.01, 2.20)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	
IgE (median)	2.91	3.35	1.90	0.682
(P25, P75)	(-2.35, 12.49)	(-0.68, 12.02)	(-5.70, 10.56)	
∆ ≥15%	17.1	19	15.6	
∇ ≥15%	2.9	11.9	10.4	
IgA (median)	2.02	0.80	0.74	0.486
(P25, P75)	(-1.43, 4.94)	(-3.12, 4.05)	(-1.68, 3.61)	
∆ ≥15%	0.0	0.0	1.3	
∇ ≥15%	0.0	0.0	0.0	
IgM (median)	-0.15	0.18	0.35	0.819
(P25, P75)	(-1.56, 3.04)	(-1.63, 4.10)	(-1.33, 3.00)	
∆ ≥15%	0.0	0.0	0.0	
∇ ≥15%	0.0	0.0	0.0	

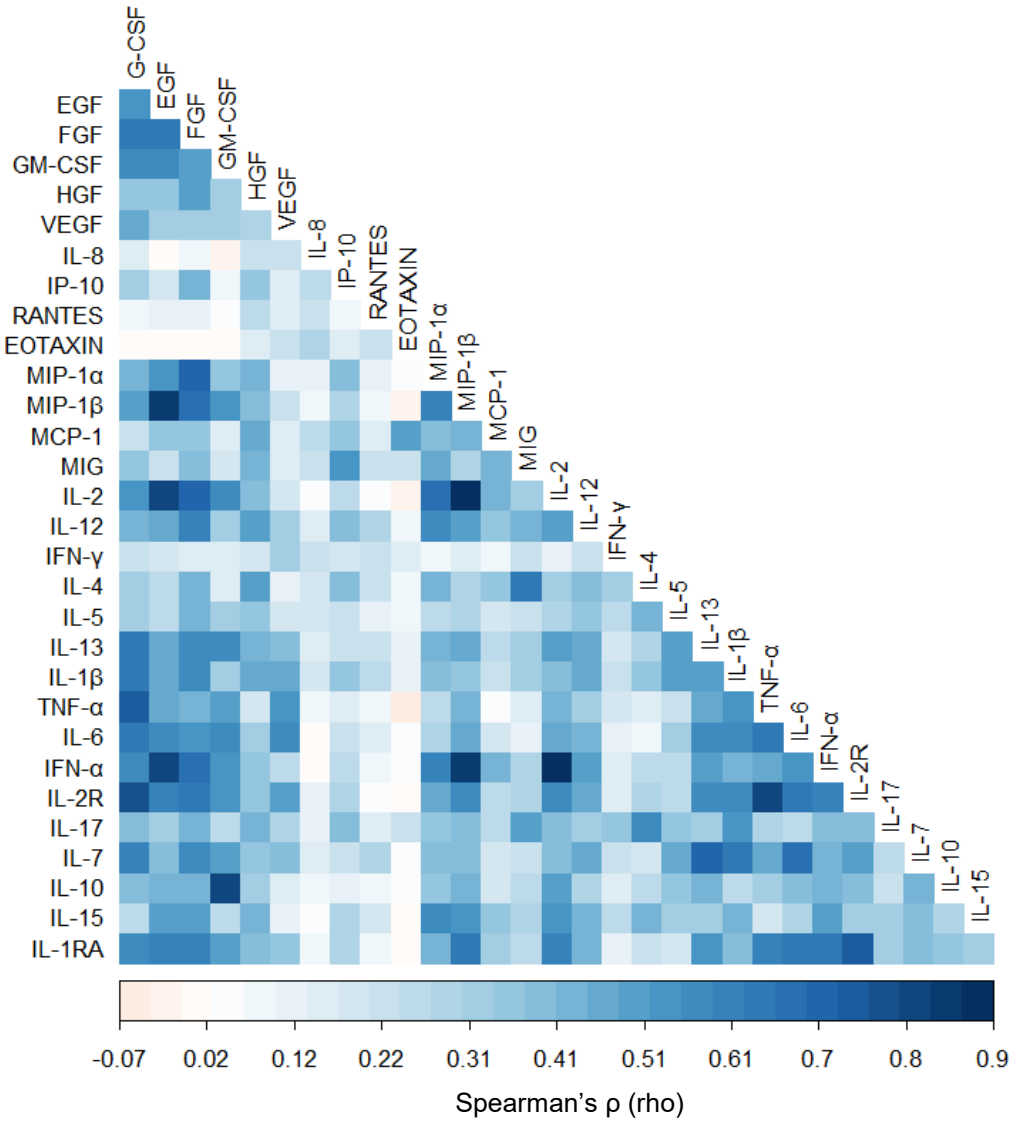
P25: percentile 25th. P75: percentile 75th.
Educational levels groups are based on participants' data in 2020-21.

[a] Units used for computing the relative intraindividual change were base 10 logtransformed MFI for levels of isotype-antigen combinations, and base 10 logtransformed µg/mL for levels of total Igs.
[b] Kruskal-Wallis test (two-tailed).

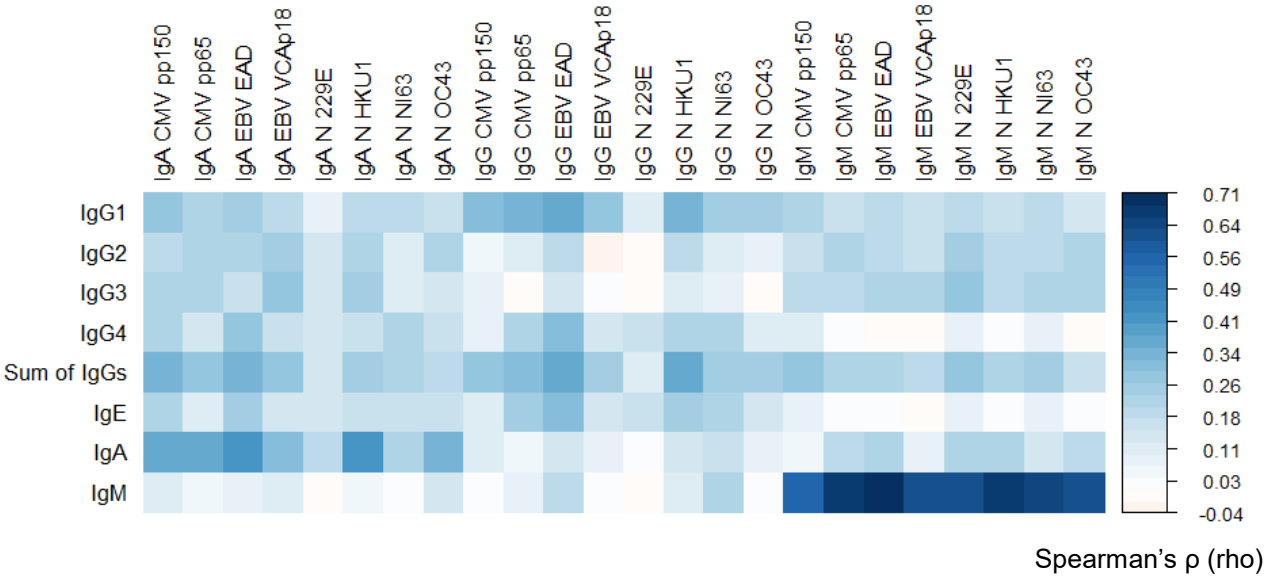
Supplemental Figure 1. Correlations between concentrations of cytokines and immunoglobulins in 2016-2017.



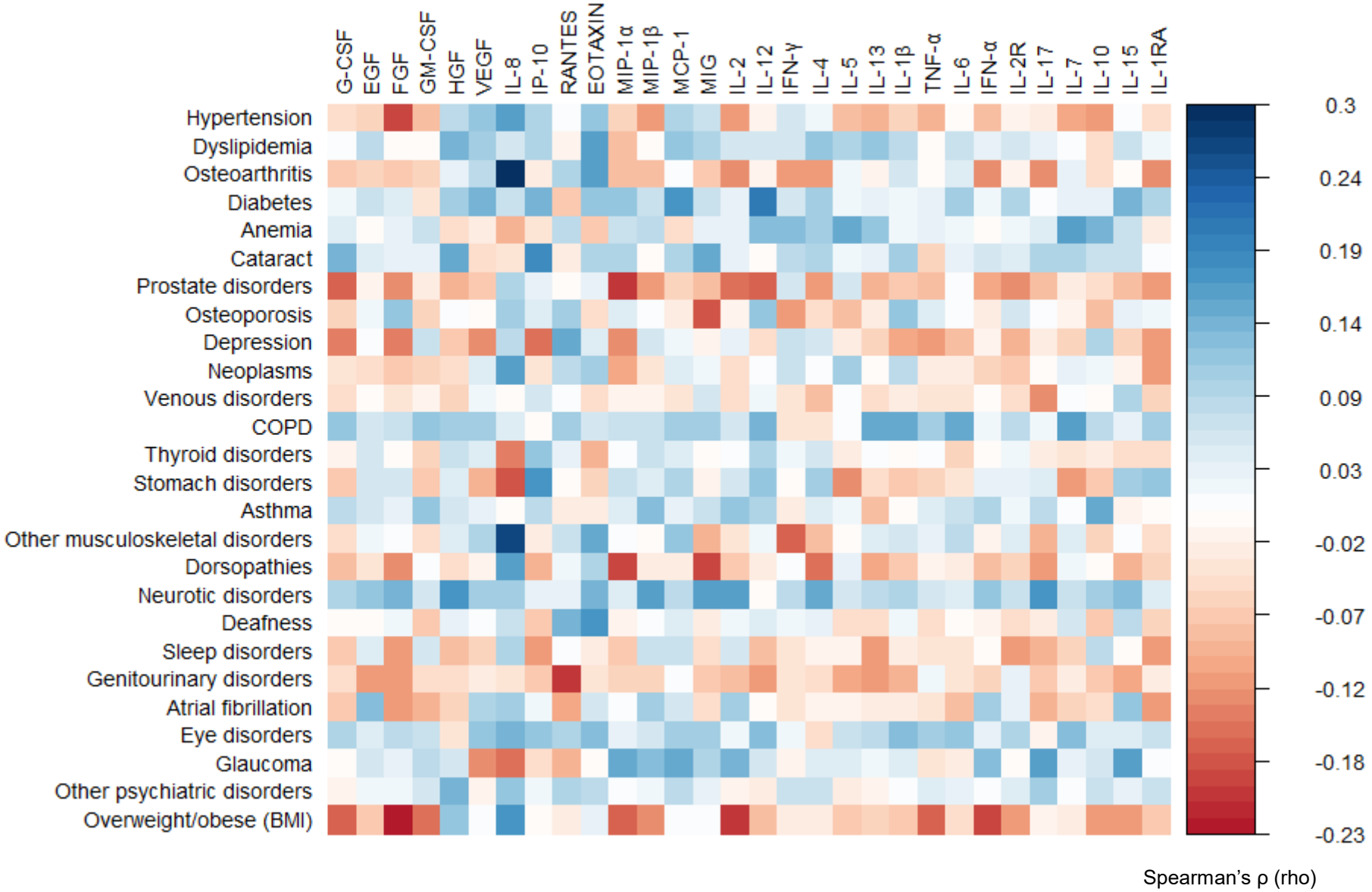
Supplemental Figure 2. Correlations between concentrations of cytokines in 2016-2017.



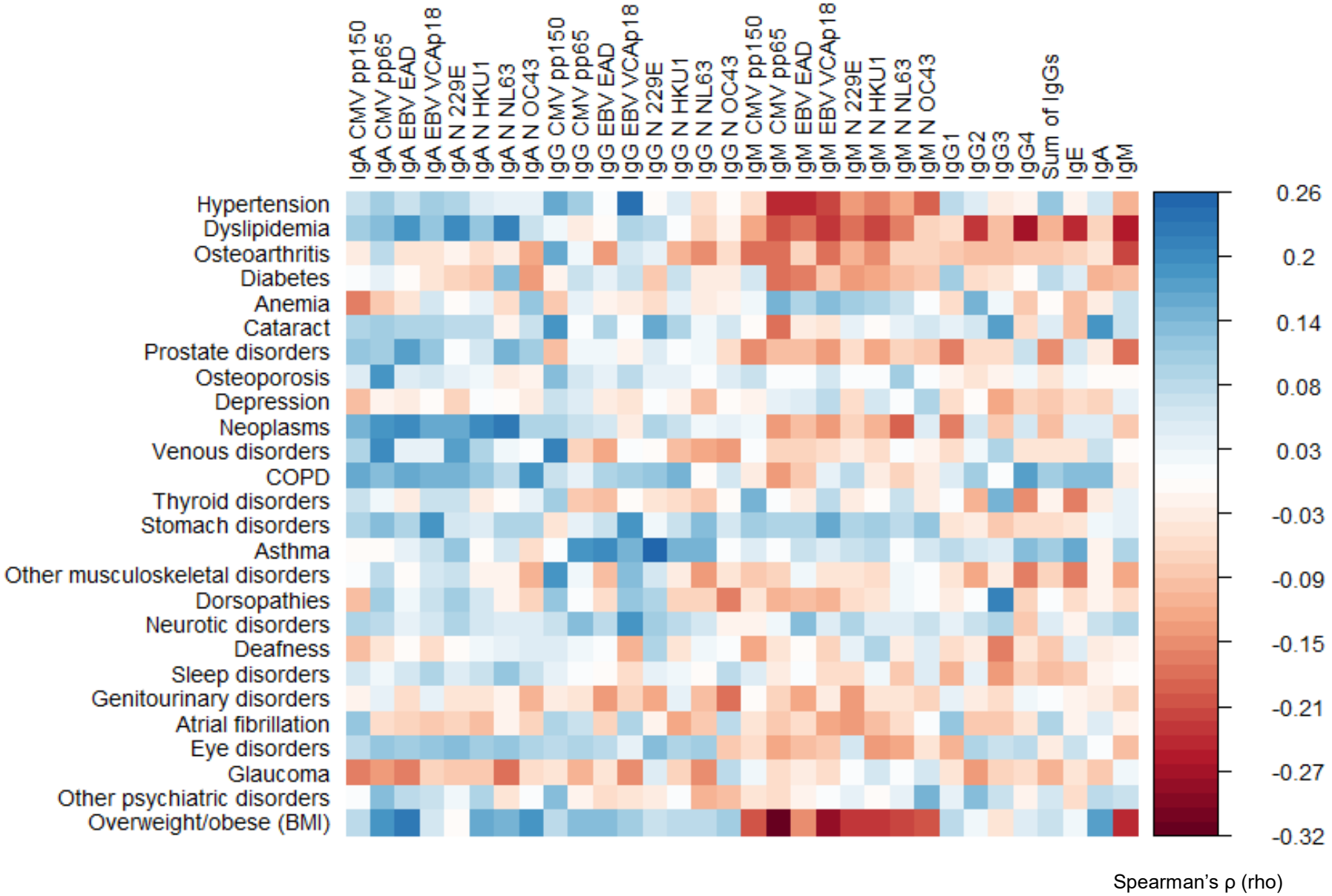
Supplemental Figure 3. Correlations between concentrations of immunoglobulins against CMV, EBV and HCoV, and total immunoglobulins in 2016-2017.



Supplemental Figure 4a. Correlations between concentrations of cytokines and comorbidities in 2016-2017.



Supplemental Figure 4b. Correlations between concentrations of immunoglobulins against CMV, EBV and HCoV, total immunoglobulins and comorbidities in 2016-2017.



Supplemental Figure 5. Concentrations of IL-8, IL-2R, IL-6 and TNF-α in 2020-21 in the 20 participants who developed COVID-19 (panel a); and relative intraindividual change of IL-8, IL-2R, IL-6 and TNF-α concentrations in 2020-21 with respect to concentrations in 2016-17 in the 20 participants who developed COVID-19 (panel b). Vertical axis (panel a): Concentrations in 2020-21 (pg/mL). Vertical axis (panel b): Relative intraindividual change (%). Horizontal axis: Time interval from COVID-19 symptom onset to blood extraction (months).