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High seroprevalence but short-lived immune response to SARS-CoV-2 infection in Paris

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Anna et al,

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ABSTRACT

Although the COVID-19 pandemic peaked in March/April 2020 in France, the prevalence of infection is barely known. Using high-throughput methods, we assessed herein the serological response against the SARS-CoV-2 virus of 1847 participants working in three sites of an institution in Paris conurbation.

In May-July 2020, 11% (95% CI: 9.7-12.6) of serums were positive for IgG against the SARS-CoV-2 N and S proteins, and 9.5% (CI:8.2-11.0) were neutralizer in pseudo-typed virus assays. The prevalence of seroconversion was 11.6% (CI:10.2-13.2) when considering positivity in at least one assays. In 5% of RT-qPCR positive individuals, no systemic IgGs were detected. Among immune individuals, 21% had been asymptomatic. Anosmia (loss of smell) and ageusia (loss of taste) occurred in 52% of the IgG-positive individuals and in 3% of the negative ones. In contrast, 30% of the anosmia-ageusia cases were seronegative suggesting that the true prevalence of infection may have reached 16.6%.

In sera obtained 4-8 weeks after the first sampling anti-N and anti-S IgG titers and neutralization activity in pseudo-virus assay declined by 31%, 17% and 53%, resulting thus in half-life of respectively 35, 87 and 28 days.

The population studied is representative of active workers in Paris. The short lifespan of the serological systemic responses suggests an underestimation of the true prevalence of infection.
INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the coronavirus disease 2019 (COVID-19) emerged in 2019 in China [1-3] before being detected in a patient living in the Paris conurbation in December 2019 [4]. From January 2020, the virus spread exponentially leading to a risk of Paris conurbation intensive care units saturation. Accordingly, on March 17th, a lockdown was imposed by the French authorities to slow down virus progression. To date, the exposure of the French population during that period remains poorly documented. In contrast with RT-qPCR assays which are positive for only 2-3 weeks after infection [5], a more efficient way to monitor virus propagation is a serological study of representative populations since specific and lasting antibodies are generated in the great majority of infected subjects [6, 7]. However, studying the anti-SARS-CoV-2 serological response of large cohorts is challenging and require robustness, specificities, sensitivities and high-throughput capabilities of the measurement methods which are exceeding the performance of currently marketed serological assays.

Here, we developed original bioluminescence-based serological assays allowing a high throughput assessment of the specific antibody responses to the Spike (S) and Nucleoprotein (N) proteins of SARS-CoV-2 and their ability to neutralize the virus fusion with a permissive human cell line.

We monitored individual serology against SARS-CoV-2 in a large cohort of workers in three institution sites following the March-April 2020 peak of the COVID-19 pandemic in Paris (France) and over the next six months. More than half of Institut Curie workers (n=1847), a hospital and research
RESULTS

COHORT DESCRIPTION, ASSAY DEVELOPMENT AND VALIDATION

Blood samples were collected from 1847 volunteers at the three sites of the Institut Curie located in three cities of Paris conurbation (Ile-de-France): Paris, Saint Cloud and Orsay from April 28th until July 31st for the initial time-point. None of the individuals showed clinical signs of COVID-19 or had been subjected to a standard RNA detection of SARS-CoV-2, using RT-qPCR, within 14 days prior to blood sampling. All participants were invited to complete a web-based questionnaire which included demographic variables, symptom occurrences and whether these had led to a sick leave, treatment and/or hospitalization. The participant cohort had a strong (77.4%) female bias (Table 1); the mean age was 38 and ranged between 19 and 75 years old. The hospital-working staff represented 72.7% of the volunteers, the rest being researchers and administrative staff.

Three serological assays were carried out on these 1847 sera samples in multi-well plates at the Institut Pasteur. Luciferase-linked immuno-sorbent assays (LuLISA) were used to assess specific IgG for SARS-CoV-2 Nucleoprotein (N) and Spike (S) proteins in these serum samples. LuLISA [10] is expanding the sensitivity, the dynamic range and the scalability in comparison with the gold standard ELISA [8] as detailed in Supporting Information (Figures S1 and S2). A neutralization activity assay using pseudo-typed virus, also named pseudo-neutralization test (PNT), was undertaken [9] to assess in parallel of LuLISA, the ability of serum components to neutralize the fusion of a SARS-CoV-2 Spike pseudo-typed lentiviral vector encoding a luciferase gene using a permissive human cell line...
RLU/s) and to a confidence level of 99% in the case of PNT assay (28,783 RLU/s) established on prepandemic negative sera.

The robustness of the specificity thresholds and dynamic ranges were assessed using dilution series of COVID-19 positive sera (Supporting Information Figures S2 and S3). The specificity for SARS-CoV-2 anti-N IgG was assessed against purified Nucleoproteins of SARS-CoV-1 as well as seasonal coronaviruses (HCoV) HKU, OC43, NL63, 229E (Supporting Information Figures S4 and S5).

HIGH PREVALENCE OF ANTI-SARS-CoV-2 IgG RESPONSE IN THE STUDY COHORT

For the Institut Curie workers, using a 98% specificity threshold, the seroprevalence of IgG directed against N and S proteins was 9.9% (183/1847, 95% CI: 8.6-11.4) and 9.8% (181/1847, 95% CI: 8.5-11.3), respectively (Figure 1A-B and Table 1). Amongst all the serums tested, 9.5% (176/1847, 95% CI: 8.2-11.0) displayed a pseudo-neutralization activity against the pseudo-virus (Figure 1C).

Considering each of these assays independently as a marker of specific immune response leads to a 11.6% (215/1847, 95% CI: 10.2-13.2) positivity of immunization.

The correlative plots (Figure 1D) indicates that the responses against the N and S are linked when both are above their respective threshold ($R^2=0.57$). Correlation between PNT and LuLISA is mainly detectable when high levels of both IgG against N and S are detected (red dots in Figure 1D).

Moreover, above the 98% specificity threshold, a higher correlation is observed between PNT and LuLISA IgG/S ($R^2=0.60$) (Figure 1F) than between PNT and LuLISA IgG/N ($R^2=0.47$) (Figure 1E).
Based on the web-based survey, 54% (1007/1847) participants mentioned at least one symptom (Supporting Information Table S1). Symptomatic workers were more seropositive (16.8%, 170/1007, CI 95%: 14.6-19.3) than asymptomatic workers (5.3%, 45/840, CI 95%: 3.9-7.1) (Table 2 and Figure 2A). Hence, SARS-CoV-2 infection may have been asymptomatic in at least 20.9% (45/215, 95% CI: 16.5-28.2) of the cases (Figure 2B). The amount of anti-N IgG was higher in the symptomatic versus asymptomatic patients while the levels of anti-S or the neutralization capacity in pseudo-virus assay did not differ (Supporting Information Figure S6). This discrepancy suggests that anti-N IgG may be generated in the course of a mild infection.

A correlation between serological tests, RT-qPCR and symptoms was performed (Figure 2C). In the 171 individuals tested by RT-qPCR, 169 (99%) reported symptoms, only 76 (44.4%, CI 95%: 37.9-53.1) were positive in serological assays. Among these 171 RT-qPCR tested workers, 55% of them were RT-qPCR negative, seronegative for anti-N and anti-S IgG and PNT, but symptomatic whereas 35% were positive, seropositive and symptomatic. Moreover, no IgG antibodies were detected in 3 subjects out of 63 with a positive SARS-CoV-2 RT-qPCR indicating that a systemic anti-N or S IgG response may not always be present following a proven SARS-CoV-2 infection. However, low levels of anti-SARS-CoV-2 IgM, were detected using a commercial lateral flow assay, in one of these three subjects (data not shown). Except for one case, all anosmia/ageusia cases without detectable systemic IgG (n=48) were associated with other COVID-19 typical symptoms and occurred in late February, March or April suggesting that they represent true SARS-CoV-2 infections. Indeed, one of them was associated with a positive SARS-CoV-2 RT-qPCR test and, in 7 cases anti-SARS-CoV-2 IgM were
A date for the symptom onset was mentioned in 885 out of 1007 cases. Symptoms were mostly (61%) reported in March 2020 (Figure 2D), consistent with the reported epidemic development as well as the number of Parisian hospital admissions published daily by Santé Publique France (the French governmental public health agency) [10]. The intensity of immune responses according to the date of symptom occurrence is reported in Figure 2E-G. The decrease seen in April (14.8%) probably reflects the efficacy of the population lockdown on the disease spread. The March peak of symptom occurrence represented 82% of the seropositive individuals compared to 56% in people devoid of COVID-19 specific IgG. Although some workers displayed an immune response corresponding to symptoms dated as early as the first week of February 2020, a sharp peak of seropositive individuals corresponded to symptoms declared in March. These results indicate that the virus was circulating in early February in the Paris conurbation and achieved a high prevalence in March.

The frequency of declared symptoms was significantly much higher in seropositive workers (79%) than in those devoid of COVID-19 specific IgG (51%) (Table 2). If fever (66%, 142/215) was the most frequent symptoms in the seropositive population, it was also noted in individuals lacking antibodies (37%, 599/1632) suggesting a low correlation with a COVID-19 infection (chi-square scores 2E\(^{-16}\)) (Table 2). In contrast, anosmia/ageusia and myalgia symptoms were highly prevalent (52%, 111/215 and 48%, 103/215 respectively) in the seropositive group but were rare in the seronegative group 3% (48/1632) and 15.7% (256/1632) respectively (Figure 2H-J), resulting in a high correlation with COVID-19 (chi-square scores 5E\(^{-76}\) and 3E\(^{29}\)) (Table 2). Only anosmia/ageusia symptoms were temporally correlated with the epidemic peak in March whereas other symptoms such as myalgia, cough, and dyspnea were not.
To follow over time the antibody titers and neutralizing activity, a second blood sample \( t_2 \) was obtained 4-8 weeks after the first one \( t_0 \) from more than 1000 individuals. For the 120 samples of individuals previously found positive, the results are reported in Figure 3A, D, G according to the time interval between symptom onset and sampling. A clear decrease in the antibody titers and neutralization activity in pseudo-virus assay was observed. The half-lives of the antibody titers were 35, 87 and 28 days for anti-N, anti-S IgG and neutralization activity in pseudo-virus assay, respectively. A paired analysis showed a systematic decreased response (p<0.0005) (Figure 3B, E, H). The titers of antibodies decreased by 31% and 17% for anti-N and anti-S IgG, respectively for a majority of workers (>75%) and this correlated with a major decrease of the neutralization activity in pseudo-virus assay (53%) (Figure 3C, F, I). Interestingly, some workers sera became negative in our assays: 15% (16/107) for LuLiSA IgG/N (Figure 3C), 14% (10/71) for PNT (Figure 3I) and 5% (4/84) for LuLiSA IgG/S (Figure 3F). Thus, past a few months, a serological-based survey of SARS-CoV-2 may run a risk of underestimating the number of formerly infected individuals.

**DISCUSSION**

We report here the longitudinal study Curie-O-SA describing the natural immune response against the SARS-CoV-2 in a large population of healthy subjects working in the Paris conurbation following the March 2020 peak. Three bioluminescence-based and sensitive high-throughput assays including a pseudo-typed virus neutralization activity assay allowed repeated measurements on a large
correlated beyond twice their positive thresholds as well as with the viral pseudo-neutralization
capacity beyond three times their positive thresholds. 2) 21% of infections had been asymptomatic.
3) At least 5% of the RT-qPCR confirmed infections and 30% (92 of 307) of the very probable
infection cases according to symptoms did not develop any detectable anti-N or anti-S IgG
antibodies nor a serum neutralization capacity. 4) The systemic IgG/N and IgG/S immunity associated
with neutralization activity on pseudo-typed virus decreased rapidly with a half-life between 4 to 12
weeks following infection.

Still, there are some limitations in our study. 1) High throughput methods for assessing IgM or IgA
responses were not ready at the time of the study. Assessing these isotypes may indeed be relevant
since a commercial lateral flow assay detected anti-SARS-CoV-2 IgM in 7 out of 48 individuals
featuring anosmia/ageusia but devoid of IgG response. 2) For regulatory and logistic reasons, the
blood sampling started 4-6 weeks after the epidemic peak and were performed over a 2.5-month
period. The interval between the infection and the blood draw varied between 2 to 18 weeks. We
may thus have missed the antibody response peak in some cases and, again, underestimated the
true prevalence of the infection. 3) For logistic reasons, the blood samplings from research center
staff were delayed by an average 2 weeks, possibly leading to a slight underestimation of the true
prevalence of SARS-CoV-2 infection in the research center. 4) The use of a web-based auto-
questionnaire leaves some space for inaccurate or selective memories as well as input errors and
missing values. Indeed, despite the high response rate, the symptom onset date was missing in 18%
of the cases (187/1007). Symptoms were declared by 79% of the immunized individuals but also by
could not be done using SARS-CoV-2 active viruses on such a large number of samples within a biosafety level (BCL3) laboratory. In accordance with recent studies 38/1847 individuals were RT-qPCR positive but negative for serological tests [17] and all subject among the 215/1847 did not display a common scheme of coordinated immune response [18] which included all the parameters studied. Indeed, the largest response was the sequential occurrence of anti-N IgG, followed by the anti-S IgG and then the pseudo-typed virus neutralizing activity. A few individuals were endowed with neutralizing sera without any detectable IgG against S, either because their S-specific IgG had high affinity and a very low concentration or because other Ig than IgG were responsible for the pseudo-typed virus neutralization activity as recently suggested for S-specific IgA or IgM [19]. The lower prevalence of the PNT activity could be either related to a lower sensitivity of the assay or to an immune response decrease of the as evidenced in Figure 3. Indeed, although most of the symptoms occurred from March to early April, the blood samplings were performed between May and July, leading to a variable interval between an eventual infection and the antibody response study.

Our experiments pointed out a clear cross-recognition of IgG for SARS-CoV-1 and -2 Nucleoproteins but none with any of the seasonal HCoV, aside from samples displaying a very high IgG/N content (Supporting Information Figure S4). This may be expected in view of the relatively few short peptide patterns common to all the aligned antigens sequences (Supporting Information Figure S5). Altogether, since there are no documented cases of SARS-CoV-1 observed in France during the 2003
residences (n=127, 88.7%) in Paris conurbation performed with the same LuLISA and PNT methods in July [21]. Our results are consistent with other large-scale serological studies (mostly with single time-point and no symptom reports) in conurbations that have been subjected to the SARS-CoV-2 epidemic. Higher seroprevalence was found in New-York City (USA, n=5129, 22.7%, March [22]; n=72 401, 41.5%, March [23]), Madrid (Spain, n=2590, 31.6%, April) [24], similar in Saint Petersburg (Russia, n=1038, 10.8%, May) [25] and lower in Geneva (Swiss, n= 2766, 8.5%, April) [26], Wuhan (China, n=17368, 3.8%, March-April) [27] or nine cities of Rio Grande do Sul (Brazil, n=4500, 0.22%, May) [28]. Seroprevalence in healthcare workers (HCW) highly exposed to COVID is also contrasted. It was high in London (n=2167, 31.6%, May-June 2020) [12] and Paris (n=154, 21.1%, March) [29] but lower in Essen (Germany, n=316, 1.6%, March) [13]; Madrid (Spain, n=578, 9.3%, April) [11]; Milano (Italia, n=789, 10.8%, March) [15].

Among seropositive individuals, 20% had been asymptomatic in this study which is less than what has been mentioned in other studies reports although this is highly dependent on the recording and reporting methods: 40% in Madrid area [11], 50% in Boston area [30] and up to 80% locally in France on September 2020. This last result where it is a likely consequence of reinforced mask wearing policies [31] since such efficacy was also observed in Wuhan with 86% of asymptomatic in January 2020 [32], plausibly due to mask-attenuated infectious load [31].

The pattern of symptoms displayed by the immune subjects are consistent with those reported elsewhere [33, 34]. Our results further emphasize the predictive value and specificity of the
Anna et al,

the hospital and research center is mainly due to the health staff priority access to SARS-CoV-2 RT-qPCR assays in March and April 2020.

In this first report, we studied the immune response of 120 positive individuals 6-12 weeks after the first blood sampling. A majority of these individuals (>75%) displayed a diminishing anti-SARS Cov2 response. Notably, the anti-N IgG decreased faster (31%) than anti-S IgG (17%) suggesting that if anti-

N IgG titration is a reliable marker for prevalence follow-up during the early stage of the COVID-19 pandemic, this assay may be less relevant for longer studies and should be seconded with an anti-S IgG assay. This observation emphasizes the difficulty to estimate the real seroprevalence in a large population. Interestingly, the very slow drop of anti-S IgG titer also reported in other studies [23, 35] did not correlate with the major decrease of neutralization activity on pseudo-typed viruses observed herein (53%). Since our pseudo-neutralization assay is exclusively associated with anti-S response, the neutralization activity we observed might be explained by the occurrence of other Ig isotypes, such as IgM or IgA, which would disappear much faster than the IgG from subsequent blood samples [19]. This illustrates again the serological complexity of any long-lasting immunity.

From an epidemiological perspective, the 11.6-16.6% seroprevalence results may still underestimate the number of individuals who have been infected by the SARS-CoV-2 because, as discussed earlier, we also observed a lack of systemic IgG response among the RT-qPCR positive individuals along with a gradual loss of the virus-specific IgG titer. In the present epidemic, the rather fast decrease in
MATERIALS AND METHODS

PARTICIPANTS AND WEB-BASED QUESTIONNAIRE

This study was registered and received ethical approval by the Comité de Protection des Personnes Méditerranée III (2020.04.18 bis 20.04.16.49458, 27/4/2020) registered in the clinical trial database (NCT04369066). Following informed consent, 18 years of age or older volunteer participant outside of any SARS-CoV-2 acute infectious episode in the last 7 days, working at one of the three Institut Curie locations (Paris, Orsay or Saint Cloud) completed a web-based questionnaire (Ennov Clinical) detailed in Supporting Information. A 5 mL blood sample was taken from all participants in dry tubes. After clotting, blood was centrifuged 10 min at 2000 g. Supernatant serum was separated and frozen. Sera to be tested were thawed and distributed into 96 well plates at the Institut Curie. An aliquot of a pool of positive (or negative) sera was distributed into 6 wells of each plate in a unique dispatching pattern allowing an unambiguous identification of plates.

These positive and negative wells were used to control for any drift of the measurements. The plates were then assessed at the Institut Pasteur. For the Figure 3 longitudinal analysis, the $t_1$ and $t_2$ samples were analyzed in the same experiment from frozen serum samples.

SARS-CoV-2 SPECIFIC IgG ASSAYS

Development and validation of the LuLISA method are described in the methods section of the Supporting Information. Briefly, N- and S- specific IgG were assessed using an ELISA-based assays on sera incubated in antigen-coated wells (Supporting Information Figure S1-2) [36-38]. Antigens have
a size-exclusion chromatography. White 384-well plates with flat bottoms (Fluoronunc C384 Maxisorp, Nunc) were coated with either 1 μg/mL of Nucleoprotein or Spike protein in PBS buffer, 50 μL/well for 3 hours at room temperature or overnight at 4°C. Wells were washed using a plate washer (Zoom, Berthold Technologies, Germany) two cycles of three times with 100 μL of PBS/Tween 20 0.1%. Sera were diluted 200 times in PBS, non-fat milk 3% and Tween 20 0.1%. 50 μL of serum dilutions were incubated 1 hour at room temperature in their respective wells. Wells were washed two cycles of three times with 100 μL of PBS/Tween 20 0.1%. The Anti-Fc IgG VHH (Fc1) was derived from an antibody from immunized alpaca (Vicugna pacos) [39] and expressed as a tandem with an optimized catalytic domain nanoKAZ from Ophlophorus gracilirostris luciferase [40]. Purified Fc1-nanoKAZ 1 ng/mL (400·10^6 RLU·s⁻¹·mL⁻¹) in PBS, non-fat milk 3% and Tween 20 0.1% was loaded (50 μL/well) and incubated 30 min at room temperature. Wells were washed two cycles of three times with 100 μL of PBS/Tween 20 0.1%. Emptied wells were loaded with 50 μL of the luciferin solution, resulting from hikarazine-108 hydrolysis (Q-108) at 13 μM. The plate was orbitaly shaken for 3 seconds and the collected photons were counted during 0.5 sec per well and measured 2 times in a plate luminometer (Mithras2, Berthold, Wildbad, Germany).

PSEUDO-NEUTRALIZATION ASSAYS

Pseudo-typed vectors were produced and titrated as previously described [41]. Inhibition assay of neutralization of pseudo-typed virus--cell fusion by serum contents [9] are detailed in the methods section of the Supporting Information and Figure S3. Briefly, sera were decomplemented at 56°C
removed by aspiration and bioluminescence is measured using a Luciferase Assay System (Promega) on an EnSpire plate reader (PerkinElmer).

STATISTICAL ANALYSIS

Seropositivity was defined as the presence of detectable anti-SARS-CoV-2 antibodies against N or S. The proportion of seropositive samples was compared by time between onset of symptoms and collection of blood sample using chi-square test.

LuLISA, and pseudo-neutralization of sera were compared by delay since onset of symptoms using the Kruskall-Wallis non-parametric test. The chi-square test was used to evaluate the association between investigated factors and neutralization levels.

All analyses were performed using GraphPad Prism 8 (GraphPad Software, LLC). These results and the raw data of the LuLISA IgG/N and IgG/S and PNT are provided in the Supporting Information Table S1.

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AUTHORS

PNT design: FA, PC; PNT processing: FA, PS; LuLISA design: SG; LuLISA processing: SG, TR; Target
design: MG, ZC, NE; Target production: FD, ED, OR-LG, SP; Substrate synthesis: YJ; Study logistics and
sampling collection: DL, VG, FC-B, AS, MDG, OL; Automate and plate handling: OH; Data analysis: FA,
TR, AIL, OL; Contribution to text and figure editing: FA, SG, AIL, YJ, TR; writing manuscript: FA, TR, OL.

Data availability statement:

All raw data are available in the article Supporting Information.

CONFLICT OF INTEREST

YJ, SG and TR have patented the pro-luciferins (hikarazines) synthesis and uses (EP 3395803 / WO
2018197727, 2018) and applied for a patent which includes claims describing the LuLISA. FA and PC
have applied for a patent claiming the PNT. The rest of the authors declare no commercial or
financial conflict of interest.
Figure 1: Serological responses to SARS-CoV-2 among Institut Curie workers using LuLISA IgG/N, IgG/S and PNT assays

(A-C) Sera from prepandemic samples from healthy donors (blood bank), prepandemic patients (breast cancer), COVID-19 patients (RT-PCR positive) and Institut Curie workers were evaluated in LuLISA IgG/N (A) or IgG/S (B) and PNT (C) assays. For LuLISA, raw values are represented. Sera were considered positive for anti-N or -S IgG if the value was above the 98% threshold (See Supporting Information Figure S1 for calculation details). For PNT assay, values after ID50 calculation are represented (See Supporting Information Figure S3 for calculation details and S7 for raw values).
Anna et al,

Figure 2: Temporal distribution of symptoms appearance and serology correlates with COVID-19 outbreak in France

(A) Seroprevalence among asymptomatic and symptomatic workers. (B) Proportion of asymptomatic and symptomatic seropositive workers. (C) Correlation between symptom reporting (blue circle), RT-qPCR testing (red circle) and seroconversion. (D) Temporal variations of urine pH for symptomatic and asymptomatic workers.
date of symptom onset. (E) LuLISA IgG/N, (F) LuLISA IgG/S (G) PNT. (H-J) Prevalence of symptoms according to serology status. Seropositive workers are represented in red and seronegative in blue. Symptomatic workers are represented in orange/yellow. Number of workers for each area is indicated. Percentage represents the proportion of symptomatic in seropositive (orange area) individuals and symptomatic in seronegative ones (yellow area). (K-M) Prevalence of symptom during pandemic outbreak according to serology status. Plots represent the number of workers reporting symptoms (y-axis) per week in 2020 (x-axis). Only the 3 most representative symptoms from Table 2 are plotted: Anosmia/Ageusia as an example of temporally and clinically correlated to COVID-19 (H, K), Myalgia as clinically only correlated (I, L) and rhinitis as poorly correlated (J, M).
Figure 3: Serological profile follow-up overtime

Workers whose serum was positive for IgG anti-N (A-B-C), anti-S (D-E-F) and pseudo-neutralisation (G-H-I) activities.
### Table Section

#### Table 1: Serological assay results and working groups

|                | Institute |          | Hospital |          | Research center |          |
|----------------|-----------|----------|----------|----------|-----------------|----------|
|                | n         | %        | n        | %        | n              | %        |
| **Total**      | 1847      | 100      | 1342     | 72.7     | 505             | 27.3     |
| Female         | 1429      | 77.4     | 1074     | 80.0     | 355             | 70.3     |
| Male           | 418       | 22.6     | 268      | 20.0     | 150             | 29.7     |
| Age (mean)     | 38        |          | 40       |          | 38              |          |
| 0-38 yrs       | 943       | 51.1     | 656      | 48.9     | 287             | 56.8     |
| >38 yrs        | 904       | 48.9     | 686      | 51.1     | 218             | 43.2     |
| RT-qPCR        | 189       | 10.2     | 181      | 13.5     | 8               | 1.2      |
| Positive       | 66        | 34.9     | 63       | 34.8     | 3               | 37.5     |
| **Serological tests** |          |          |          |          |                 |          |
| 1847           | 100.0     | 1342     | 100.0    | 505      | 100.0           |          |
| IgG/N Positive | 183       | 9.9      | 151      | 11.3     | 32              | 6.3      |
| IgG/S Positive | 181       | 9.8      | 149      | 11.1     | 32              | 6.3      |
| PTN Positive   | 176       | 9.5      | 146      | 10.9     | 30              | 5.9      |
| **Sero. Positive** | 215       | 11.6     | 171      | 12.7     | 44              | 8.7      |
| Female         | 160       | 11.2     | 134      | 12.5     | 26              | 7.3      |
| Male           | 55        | 13.2     | 37       | 13.8     | 18              | 12.0     |
| 0-38 yrs       | 107       | 11.3     | 80       | 12.2     | 27              | 9.4      |
| >38 yrs        | 108       | 11.9     | 91       | 13.3     | 17              | 7.8      |
Tiredness    741    40    142    66    599    37    2E-16
Cough        239    13    61    28    178    11    7E-13
Unusual Headache  416    23    88    41    328    20    6E-12
Shortness of breath  663    36    119    55    544    33    2E-10
Rhinitis     380    21    74    34    306    19    9E-08
Intestinal symptoms  259    14    50    23    209    13    3E-05
Conjunctivitis  91     5    17     8    74     5    3E-02

ABBREVIATIONS

COVID-19, Coronavirus Disease 2019

ELISA, Enzyme-linked immunosorbent assay

Ig, Immunoglobulin

IgG/N, Anti-Nucleoprotein immunoglobulin G

IgG/S, Anti-Spike immunoglobulin G

LuLISA, Luciferase-linked immunosorbent assay

N protein, SARS-CoV-2 Nucleoprotein or Nucleocapsid protein

PNT, Pseudo-neutralization test or neutralization activity assay performed on pseudo-typed virus

RT-qPCR, Reverse transcriptase quantitative polymerase chain reaction

SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2
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