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Contribution of rapid lateral flow assays from capillary blood specimens to the diagnosis of COVID-19 in symptomatic healthcare workers: a pilot study in a university hospital, Paris, France

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ABSTRACT

Background: This study aimed to assess, by rapid tests, the immune status against COVID-19 among Healthcare Workers (HCW) with history of symptoms, and for whom SARS-CoV-2 detection was either not documented or negative.

Methods: Whole blood by finger prick and serum samples were taken from HCW for use with 2 rapid lateral flow tests and an automated immunoassay.

Results: Seventy-two HCWs were included, median duration between symptoms onset and serology sampling was 68 days. Anti-SARS-CoV-2 antibodies were detected by rapid test in 11 HCW (15.3%) and confirmed in the 10 with available serum by the automated immunoassay. The frequency of ageusia or anosmia was higher in participants with SARS-CoV-2 antibodies (P = 0.0006 and P = 0.029, respectively).

Conclusions: This study, among symptomatic HCW during the first wave in France, showed that 15% had IgG anti-SARS-CoV-2, a higher seroprevalence than in the general population. Rapid lateral flow tests were highly concordant with automated immunoassay.

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1. Introduction

Since the detection of the first cluster in Wuhan, China on December 31, 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide reaching the pandemic stage at the beginning of March (WHO, 2020). France has been heavily affected by the epidemic and went into first lockdown on March 17, 2020 (Santé publique France, 2020). Front-line healthcare workers (HCW) are particularly exposed to the risk of contracting SARS-CoV-2, and subsequently transmitting the disease to others (patients, co-workers and the community) (Ali et al., 2020; Chou et al., 2020; Nguyen et al., 2020). Risk-informed preventive measures are critical to protect HCW, to maintain a fully functional healthcare system, and to control rates of secondary transmission (Adams and Walls, 2020). Nasopharyngeal swab reverse transcriptase-polymerase chain reaction (RT-PCR) is still the reference to detect SARS-CoV-2 RNA during acute infection. SARS-CoV-2 RNA testing for prevalent infection is a key part of the preventive strategy. In the early time of pandemic stage, RT-PCR was first performed only on symptomatic individuals (patients and HCW), leading to an underestimation of the risk as many of those infected are asymptomatic (Pan et al., 2020; Wu et al., 2020; Zhao et al., 2020).

People infected with SARS-CoV-2 develop antibodies specific to proteins of the virus approximately 1 to 2 weeks after the onset of illness (Amanat et al., 2020; Xiang et al., 2020; Zhao et al., 2020). Immunoglobulin M (IgM) and IgG seem to appear in the same time, from around 10 days after the onset of illness. In the weeks that followed infection, IgG specific of SARS-CoV-2 will persist for a period as yet difficult to determine and dependent of the severity of the infection (Duysburgh et al., 2021). Currently, a large number of serological tests are available in various formats, measuring different antibody subclasses. Serological tests can help to detect cases not identified during the acute phase of infection (Abbasi, 2020; Bryan et al., 2020). Fingerstick-based rapid tests detecting IgG, IgM and total antibodies have also been developed. The presence of antibody shows evidence of...
either past infection by carrying IgG only, or early infection (IgM only), or current infection (IgG and IgM). Negativity of serological detection substantiates the absence of infection, or a very early stage of infection. Healthcare organization in a context of epidemic crisis and ability to deliver effective care to patients would be significantly improved by the knowledge of immunization status. This information is critical in implementing preventive measures around managing of Covid-19 patients in healthcare-settings, ensuring patient and staff safety.

This study aimed to assess by rapid SARS-CoV-2 serological tests, the immune status against COVID-19 in a group of HCW with past history of symptoms suggestive of the disease, and for whom SARS-CoV-2 detection was either not documented or negative. Within this framework, the study also compared the performance characteristics of an immunochromatographic rapid test with a chemiluminescent microparticle immunoassay serological test.

2. Patients and methods

2.1. Study participants and samples

An open-labeled prospective nonrandomized monocentric study was conducted in the infectious diseases ward, Bichat university hospital, Paris, France, and recruited between May 12 and June 2, 2020. Recruitment was timed to enroll 100 healthcare workers with clinical practice, of which 50 among those working in the hospital, and 50 among physicians in private practices working in Northern Paris. The inclusion criteria were as follows: HCW who experienced symptoms suggestive of COVID-19, for whom infection was: (i) either not documented (PCR test not performed) or (ii) cleared (PCR test negative), with an onset of symptoms between February 1 and March 30 and the end date older than 15 days: dry cough and fever with or without chest pain, anosmia, ageusia, fatigue, severe headache. Non-inclusion criteria were non-HCW, pregnant women and protected adults.

Initial medical consultation at Bichat Hospital was provided to each subject enrolled in the study from May 12 to June 2, 2020, during which a clinical examination and an interview were carried out. For each HCW, socio-demographic (age, sex, occupation, place of work), clinical (comorbidities, date of onset of the symptoms, description of signs, duration of fewer and of other symptoms, any documented COVID-19 case in family or professional environment), biological (PCR test if performed and result) data were collected by medical personnel by mean of a standardized form.

For testing the specificity of applied serological tests, pre-pandemic negative serum samples from 35 patients in the COBRA cohort (Asthma and Airway Obstruction French Multicentric Cohort Study coordinated by Inserm), collected 2 years prior to the current COVID-19 pandemic and stored at -20°C in the Centre for biological resources within the hospital, were also used and served as a control group.

2.2. Rapid lateral flow test and automated immunoassay

A sample of whole blood by finger prick (10 μL) was taken for use with the COVID-Presto® test rapid Covid-19 IgG/IgM (AAZ, Boulogne-Billancourt, France). A venous blood sampling (5 mL) was performed to test serum with two rapid lateral flow test Presto® and NG-Test® IgM-IgG COVID-19 (NG Biotech, Guipry, France) and with the automated Abbott SARS-CoV-2 IgG kit (chemiluminescent microparticle immunoassay, CMIA) (Abbott, IL, USA) according to the manufacturer’s instructions. The assay cut-off of the Abbott automated assay is an index of 1.68 and the assigned grey zone is comprised between 0.49 and 1.68.

2.3. Statistical analysis

Descriptive analysis included the calculation of medians (plus standard deviation; SD) for continuous variables and numbers (n, %) for categorical variables. Chi-squared tests or Fisher exact tests were performed on categorical data. Sensitivity and specificity were calculated to determine the diagnostic accuracy of assays for COVID-19.

2.4. Ethics

The study received ethical approval from French independent Committee for the protection of persons (CPP Sud-Est III, Identifier: 20.04.09.87252). Informed consent was obtained from all study subjects prior to study entry. Trial registration: ClinicalTrials.gov, Identifier NCT04525417.

3. Results

A total of 72 HCWs who underwent the study procedure were eligible and included. Out of them, 43 (60%) were working exclusively in the Hospital and 29 were private practice professionals. The median participant age was 43 years (interquartile range [IQR] = 32–52); 76% (n = 55) were female. One to 8 different symptoms have been recorded in patients and the median number of symptoms was 4, with fatigue (89%), headache (67%), and cough (53%) most commonly reported. The median duration between the day of onset of symptoms and the day of serology sampling was 68 days (IQR = 56–78).

Among the 72 HCWs included in the study, anti-SARS-CoV-2 antibodies were detected by finger prick in 11 with the Presto® rapid test (15.3%, CI 95% = 6.9–23.7) (Table 1), 10 were positive for IgG only and 1 was positive for IgM + IgG. The automated Abbott assay was performed for 71 of the 72 HCWs, showing positive IgG for 11 (15.3%), IgG were positive by the automated Abbott assay for all 10 of the 11 HCWs with a positive Presto® rapid test for whom serum was available, including 2 with a ratio in the grey zone. The automated Abbott assay detected IgG anti-SARS-CoV-2 in one additional sample compared to the finger prick Presto® rapid test, with a ratio in the grey zone (0.58). There was no difference depending on the serology sampling time from the symptoms onset, since 6 positive samples were collected before the median of 68 days and 5 after this median. Median days from symptoms onset was 64 days (IQR = 58–74) and 68 days (IQR = 56–78) among the HCWs with a positive serology and HCWs with a negative serology, respectively (P = 0.71).

Lateral flow tests Presto® and NG-Test® could be performed from serum by 46 samples of the 72 HCWs including 36 negative and 8 positive for IgG detection by finger prick. Regarding the 8 samples with positive finger prick Presto® rapid test, IgG detection was positive for 6 of them with both Presto® and NG-Test® from serum, the 2 remaining samples not detected by serum rapid tests had low Abbott ratios (0.59 in the grey zone and 1.87). All the 36 samples with negative finger prick Presto® rapid test showed also negative IgG detection by both Presto® and NG-Test® except one positive with the Presto® test, having a grey zone ratio (0.58) with the Abbott assay.

Detailed demographic and clinical data by finger prick SARS-CoV-2 IgG detection are shown in Table 2. Among this population of HCW, the frequency of reported ageusia or anosmia was significantly higher in participants with SARS-CoV-2 antibodies (P = 0.006 and P = 0.029, respectively). HCW with and without SARS-CoV-2 IgG antibodies were furthermore comparable notably in terms of sex, age, frequency and duration of other reported symptoms.

Of the 72 HCW enrolled, 37 (52%) reported a prior naso-pharyngeal SARS-CoV-2 PCR test performed when they were symptomatic, consistent with the practice of only testing symptomatic HCW. SARS-CoV-2 PCR was positive for 2 of the 37. The median duration between symptoms onset and PCR sampling was 5 days (IQR = 2–19). Regarding the 35 with negative PCR, serology was negative for 31 and positive for 4 (3 with finger prick rapid test and one in the grey zone of the automated Abbott assay). The
two HCW with positive PCR had both positive IgG anti-SARS-CoV-2 whatever the technique used.

All the 35 sera of COBRA cohort collected in the pre-COVID-19 era were tested by Presto® test and were found negative, confirming the very good specificity of this lateral flow rapid test, as previously described [16].

### Table 1

| Patient, n | Delay between onset and serology test (days) | SARS-CoV-2 PCR | Finger prick Presto® Test | Serum Presto® Test | Serum NG-Test® | Abbott® CMIA ratio, |
|-----------|---------------------------------------------|----------------|--------------------------|--------------------|----------------|---------------------|
|           |                                             | IgG IgM         | IgG IgM                  | IgG IgM            | IgG IgM        | IgG IgM             |
| 1         | 75                                           | Neg Neg         | Pos Neg                  | Neg Neg            | Neg Neg       | 0.58                |
| 2         | 90                                           | ND Neg         | Neg Pos                  | Pos Neg            | Pos ND        | 0.02                |
| 3         | 83                                           | Neg Pos        | Neg Pos                  | Pos Neg            | Pos Neg      | 9.62                |
| 4         | 64                                           | Neg Pos        | Neg Pos                  | Pos Neg            | Pos Pos      | 1.02                |
| 5         | 73                                           | Neg Pos        | Pos Neg                  | Pos Pos            | Pos Pos      | 8.4                 |
| 6         | 64                                           | Pos Neg        | Pos Neg                  | Pos Neg            | Pos Pos      | 3.91                |
| 7         | 52                                           | Pos Neg        | Neg Neg                  | Pos Neg            | Neg Neg      | 0.59                |
| 8         | 64                                           | ND Pos         | Pos Neg                  | Neg Pos            | Pos Pos      | 5.98                |
| 9         | 44                                           | ND Pos         | Neg Pos                  | Pos Pos            | Pos Pos      | 4.37                |
| 10        | 30                                           | ND Pos         | Neg Pos                  | Pos Neg            | Pos Pos      | 3.74                |
| 11        | 80                                           | ND Pos         | Neg ND                   | ND ND              | ND ND        | 1.87                |
| 12        | 73                                           | ND Pos         | Neg Neg                  | Neg Neg            | Neg Neg      | 5.27                |

ND = not done; Neg = negative; Pos = positive.

For Abbott® CMIA results, values in the gray zone of the assay are indicated in orange.

### Table 2

| Demographics | IgG positive (n = 11) | IgG negative (n = 61) | P value |
|--------------|----------------------|----------------------|---------|
| Sex (n, %)   |                      |                      |         |
| Female       | 10 (90.9)            | 45 (73.8)            | 0.522   |
| Male         | 1 (9.1)              | 15 (24.6)            |         |
| Age (mean, SD, years) | 39.4 ± 13.2 | 42.9 ± 11.8 | 0.377   |
| Occupation (n, %) |                    |                      |         |
| Medical doctor | 6 (54.5)        | 34 (55.7)            | 0.487   |
| Nurse        | 2 (18.2)            | 18 (29.5)            |         |
| Other        | 3 (27.3)            | 9 (14.8)             |         |
| Place of activity (n, %) |              |                      |         |
| Hospital     | 9 (81.8)            | 34 (55.7)            | 0.355   |
| Urban        | 2 (18.2)            | 22 (36.1)            |         |
| Mixed        | 0 (0.0)             | 5 (8.2)              |         |
| Smoking (n, %) |                    |                      |         |
| Yes          | 0 (0.0)             | 8 (13.1)             | 0.344   |
| No           | 11 (100)            | 53 (86.9)            |         |
| Delay between onset of symptoms and inclusion (mean, SD, days) | 64.1 ± 16.4 | 66.0 ± 19.0 | 0.757   |
| Number of different symptoms per person (median, IQR) | 4 (3.5–5.5) | 4 (3.0–4.0) |          |
| Average duration of symptoms (mean, SD, days) | 12.1 ± 7.3 | 11.2 ± 11.2 | 0.799   |
| Cough (n, %) | 6 (54.5)             | 32 (52.5)            | 0.907   |
| Average duration (mean, SD, days) | 13.2 ± 9.4 (n = 6) | 9.3 ± 6.4 (n = 32) | 0.212   |
| Fever (n, %) | 5 (45.5)             | 26 (42.6)            | 0.862   |
| Average duration (mean, SD, days) | 1 (9.0) | 11 (18.0) | 0.677   |
| Chest pain (n, %) | 10.0 (n = 1) | 10.1 ± 7.2 (n = 11) | 0.989   |
| Average duration (mean, SD, days) | 15.8 ± 16.4 (n = 5) | 18.5 ± 16.3 (n = 2) | 0.852   |
| Anosmia (n, %) | 4 (36.4)              | 5 (8.2)              | 0.026   |
| Average duration (mean, SD, days) | 17.3 ± 18.6 (n = 4) | 7.2 ± 5.1 (n = 5) | 0.277   |
| Fatigue (n, %) | 11 (100)              | 53 (86.9)            | 0.246   |
| Average duration (mean, SD, days) | 12.2 ± 11.7 (n = 11) | 10.1 ± 7.5 (n = 53) | 0.449   |
| Headache (n, %) | 7 (63.6)             | 41 (67.2)            | 0.811   |
| Average duration (mean, SD, days) | 9.1 ± 1.5 (n = 7) | 7.9 ± 6.9 (n = 41) | 0.652   |
| Digestive disorders (n, %) | 3 (27.3)              | 12 (19.7)            | 0.687   |
| Average duration (mean, SD, days) | 6.0 ± 3.6 (n = 3) | 3.0 ± 1.7 (n = 12) | 0.046   |
| Myalgia (n, %) | 3 (27.3)              | 11 (18.0)            | 0.437   |
| Average duration (mean, SD, days) | 3.7 ± 2.9 (n = 3) | 7.4 ± 4.3 (n = 11) | 0.191   |
| Dyspnea (n, %) | 2 (18.2)              | 6 (9.8)              | 0.598   |
| Average duration (mean, SD, days) | 12.5 ± 3.5 (n = 2) | 11.2 ± 8.9 (n = 6) | 0.853   |

### 4. Discussion

Molecular testing of upper respiratory tract samples to detect SARS-CoV-2 RNA remains the reference screening test for Covid-19 infection diagnosis. Nevertheless, interest in serological assays to detect antibodies against SARS-CoV-2 is increasing. These indirect
tests are important to understand the kinetics of the humoral immune response against the virus.

We report in this study, assessing symptomatic HCW, that 15.3% of them had SARS-CoV-2 antibodies. This percentage is higher than that of the French general population found to be around 5%, with variations between territories, at the end of the first epidemic wave (Salje et al., 2020). We can hypothesize that this seroprevalence is under-estimated in our study since the median duration between symptoms onset and serology sampling is 68 days in median with a third quartile of 78 days, meaning that in some cases antibodies could have decreased and be no longer detected. Of the 11 HCW participants having a positive IgG result with a finger prick rapid test, only 1 had also a positive IgM result, corroborating the evolution kinetics of these markers; since that 2 months had passed between onset of symptoms and inclusion in the study.

In the present study of symptomatic HCWs, conducted after the first peak of COVID-19 epidemic in France, 45.5% and 36.4% of those with SARS-CoV-2 antibodies reported anosmia and ageusia respectively. As previously reported in other studies, ageusia and anosmia seemed to be very specific COVID-19 symptoms (Agyeman et al., 2020; Ibekwe et al., 2020; Lechien et al., 2020) and should be considered as a criterion for self-isolation, testing and contact tracing to prevent nosocomial transmission of disease. We found in the present study that other HCW's clinical symptoms were not correlated with the presence of SARS-CoV-2 antibodies two months later in median.

Among the 72 HCWs included in this study, half of them had a PCR test at the time of symptoms, with only 2 positive. A serological rapid test was IgG positive in 4 HCW with PCR negative, could be due to a PCR test performed at too early stage of symptoms, or to the quality of nasopharyngeal swab specimen. In addition, we can't exclude not-related SARS-CoV-2 symptoms at time of PCR and a more recent exposure to the virus. Interestingly, a study among Scottish Healthcare workers showed that 97.1% of patients who had previously been tested positive for SARS-CoV-2 by RT-PCR had positive antibodies, compared to 11.8% of individuals with a symptomatic illness who had been tested negative (Abo-Leyah et al., 2021), in similar range to what we observed. A recent meta-analysis based on 49 studies showed that the estimated overall seroprevalence of SARS-CoV-2 antibodies among HCWs was 8.7% (95% CI: 6.7–10.9) (Galanis et al., 2021). Thus, with 15.3% of seroprevalence among a group of symptomatic HCWs in the present study, we are in similar range.

During the first epidemic wave, in the Bichat hospital the overall PCR positivity rate was 30.7% and rose to 43.8% taking into account only HCW (personal communication).

The originality of the present study was to test the same rapid lateral flow test from capillary blood and from serum samples showing similar results except a discrepancy for one sample found to be negative for IgG detection at finger prick and positive from serum. Regarding the 2 rapid lateral flow tests tested, they confirmed good sensitivity and specificity as previously described (Charpentier et al., 2020) and results were concordant in all cases except one but with an IgG ratio in the grey zone of the automated Clia assay that could explain this discrepancy. This was important to confirm good performances of rapid serological tests in the specific population of symptomatic HCWs. A previous study evaluating the Covid-Presto® test rapid was performed on a negative panel of 120 pre-epidemic serum samples including 64 samples with a cross-reactivity panel, showed a specificity of 100% and of 98.3% for IgM and IgG, respectively, and a sensitivity for IgG for samples collected later than 10 days after symptoms of 97.1% (Charpentier et al., 2020).

The present study has several limitations. The study only recruits symptomatic HCW which lead to underestimating the prevalence of COVID-19 infection in this population. The relatively small sample size of HCWs included could have affected the study outcomes. Furthermore, a PCR test at time of symptoms was performed for only 52% of HCWs included.

Serological antibody assays are not useful in early diagnosis of SARS-CoV-2 infection. However, they may be complementary and have the potential for detecting past infection or showing the absence of previous infection by SARS-CoV-2.

In conclusion, in this study among symptomatic HCW during the first epidemic wave, about 15% of them had IgG anti-SARS-CoV-2 in a median of 2 months after symptoms, a higher seroprevalence that observed in the general population. Finger prick rapid lateral flow tests results were found to be highly concordant with CMIA automated assay. Obviously, HCWs are at high-risk of SARS-CoV-2 exposition, which makes them a priority in vaccination strategies; and there is a need to characterize their serological status against this virus; whether these antibodies or cellular immune response could protect against reinfection as well as knowing duration of natural protection need further studies.

Authors' contributions

CC, GP, NFH, DD and EB contributed to this study's concept. IL, BPC, DV, SLG and EB performed and interpreted capillary lateral rapid flow tests. HII, VMF, and NFH performed seric lateral rapid flow tests and automated immunoassay test. CC, GP, MA, DD, NFH and EB contributed to the analysis and interpretation of the data. CC, GP, HI, MA, DD, NFH and EB contributed to writing the manuscript. All authors contributed to the critical review of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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