



## Case Report

# Prevalence of SARS-CoV-2 Specific Igm and Igg Antibodies in A Cardiovascular Population

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### Abstract

**Introduction:** A great proportion of hospitalized Coronavirus Disease 2019 (COVID-19) patients have cardiovascular comorbidities. Immunity status for COVID-19 can be determined using serological testing, where the rapid serological test kits as Lateral Flow Immunoassays (LFIAs) are the most practical instruments.

**Aim:** The aim of this study was to determine the seroprevalence of SARS-CoV-2 in a cardiovascular population and compare it with the seropositivity of Stockholm's general population during the same period.

**Materials and Methods:** Consecutive patients were screened with nasopharynx PCR and LFIAs for SARS-CoV-2 during the admission for their planned cardiovascular interventions or surgeries. Complementary data were extracted from medical journals.

**Results:** A total of 175 consecutive patients with cardiovascular comorbidities were tested between May 25 and June 12, 2020. The median age was 64.0 years (interquartile range, 54.0-74.0; range 20-97 years) and 69.7% of the patients were male. Hypertension and electrophysiologic study/intervention were the most common comorbidity and planned intervention, respectively. A total of 12 patients had positive serology results, giving a seroprevalence of 6.9%. False positive IgM was found in 8 patients (4.6%).

**Conclusion:** The lower COVID-19 specific seroprevalence of this cohort compared to that of the general population of Stockholm could be due to self-quarantine or an acquired cellular rather than humoral immune response.

**Keywords:** Antibody; COVID-19; Cardiovascular population; Rapid serological test

### Introduction

The Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) was identified as the cause of a cluster of atypical pneumonia cases resembling viral pneumonia in late 2019 in Wuhan, Hubei province, central China [1,2]. It is responsible for the Coronavirus Disease 2019 (COVID-19), which WHO announced as a pandemic on 11 March 2020 [3]. COVID-19 diagnosis through Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) carries several limitations including false negative results, significantly lower sensitivity than chest CT and safety issues [4-7]. PCR should be used 1-7 days Post Symptom Onset (PSO) for highest detection rates but not later than 5. 5-8.0 days PSO as serology detection rate is higher [5,8]. Antibody responses against

COVID-19 can be measured by Lateral Flow Immunoassays (LFIAs). These Point-Of-Care Tests (POC-Ts) provide binary results within 15 minutes and are faster, safer and more practical diagnostic modalities. Furthermore, fingerstick peripheral blood has a consistency of 97.1-100.0% with serum/plasma of venous blood in terms of antibody detection [7,9].

LIFAs often use coronavirus' 2 main structural proteins, the Spike (S) and Nucleocapsid (N) proteins, as recombinant antigens [1,10]. Median seroconversion occur at 5-13 and 8-14 days PSO for SARS-CoV-2 specific IgM and IgG, respectively [5,6,8,11-14]. Comorbidities, most commonly hypertension and cardiovascular diseases, are present among 20-51% of hospitalized patients [15-18]. Elevated cardiac hypersensitive troponin I, raised NT-pro BNP, diabetes mellitus, cerebrovascular diseases, cardiovascular diseases, hypertension and age are associated with a more severe COVID-19 course [8,16-21]. Acute heart failure and myocarditis

and progression to a cardiogenic shock have been observed in COVID-19 patients [22-24]. Likewise, cardiac complications were seen during the SARS and MERS outbreaks [25,26]. The COVID-19 seroprevalence in a cardiovascular population and whether it differs from the general population remains unclear, even though they constitute a great proportion of hospitalized COVID-19 cases. The aim of this study was to determine the prevalence of SARS-CoV-2 specific antibodies among individuals with underlying cardiovascular diseases and compare it with the seropositivity of Stockholm's general population during the same period.

## Materials and Method

### Study Design and Participants

For this prospective single-center study, consecutive cardiovascular patients planned for elective cardiovascular interventions or surgeries between May 25 and June 12, 2020 were enrolled at Karolinska University Hospital in Stockholm, Sweden. Patients with positive antibody results were followed-up >3 months after the initial screening with new NPH PCR, LFIA and data regarding persistent and/or new symptoms since the screening timepoint. Controls were randomly chosen among the negative cases and were followed-up with LFIA and symptom questionnaire. This study was approved by the National Ethics committee (2020-02025). Written informed consent was obtained from every enrolled patient.

### Real-Time RT-PCR on Nasopharynx Swab Specimen

The protocol for collecting NPH swab was followed as provided by the manufacturer Xpert® Xpress SARS-CoV-2.

### Serology - Combined Antibody Test

A POC-T using colloidal gold-immunochromatographic assay for detection of IgM and/or IgG was used. This LFIA was designed and manufactured by Livzon Diagnostics Inc (Guangdong, China), approved by Chinese FDA for SARS-CoV-2. This test uses N-S recombinant protein as capture. Manufacturer's instructions were followed and the samples were checked within 15 minutes for the binary readout. In presence of antibodies, the SARS-CoV-2 antigen binds to the individual's SARS-CoV-2 specific IgG and/or IgM antibodies as blood sample flows through the device's cartridge. There are maximum 3 detection bands on the combined IgM-IgG cassettes, one quality control line (C line) and two test lines for IgM and IgG (M and G lines, respectively).

Results are positive when red/pink line(s) appear at C line in combination with presence of M and/or G lines depending on the antibody status of the patient. When negative, only the single C line appears red/pink. The test was redone in cases without

color reaction at C line as the test is invalid. Laboratories already faced high volume analysis burden and this cohort was regarded as non-COVID-19 or asymptomatic/mild-symptomatic COVID-19 individuals planned for cardiovascular interventions/surgeries rather than a confirmed COVID-19 cohort, thereby serology quantification was not conducted parallel to the LFIA.

### Result Interpretation

LFIA with positive IgG, isolated or in combination with positive IgM, were considered as "positive" cases. Isolated positive IgM in absence of positive NPH PCR, symptoms during the recent 5 months and/or epidemiologic history of COVID-19 was regarded as a false positive result.

### Statistical Analysis

Continuous variables were presented using Mean (SD), median, interquartile range (IQR) and range. Categorical variables were presented as numbers (percentages).

### Complementary Data Collection

Complementary data were collected from medical journals including demographic data, comorbidities of interest (cardiovascular, cerebrovascular, respiratory, nephrological and neurological) and ejection fraction from echocardiography studies.

## Results

### Demographics and Baseline Clinical Characteristics of the Patients

The majority of the patients (n=122, 69.7%) were males. The mean age of the participants was 62.2 years (SD, 14.7) and the median age was 64.0 years (IQR, 54.0 - 74.0; range, 20-97). Majority of the cohort (141 patients, 80.6%) were >50 years old. The mean BMI of the cohort was 26.9 kg/m<sup>2</sup> (SD, 4.5). The comorbidities and the type of planned interventions/surgeries are summarized in Table 1. The most prevalent comorbidity was hypertension (88 patients, 50.3%). Recent echocardiographic study results were available for 154 of the patients, where majority (129 patients, 83.8%) had normal left ventricular ejection fraction (LVEF >50%) and 7.8% had LVEF of <40%. The majority of the patients were planned for electrophysiologic study/intervention, followed by coronary angiography (with or without percutaneous coronary intervention). Other interventions/surgeries in order of frequency were minimally invasive repair of mitral and pulmonalis valves (2.3%), coronary artery bypass grafting (CABG; 1.1%), heart catheterization (1.1%), transvenous lead extraction (1.1%), stress/transesophageal echocardiography (1.1%), alcohol septal ablation (0.6%), aortic valve reparation and CABG (0.6%), minimally invasive ASD closure (0.6%) and reconstructive surgery of sternal wound after sternotomy (0.6%).

| <b>Comorbidities</b>   | <b>Total (%)</b> |
|--|------------------|
| Hypertension   | 88 (50.3)        |
| Atrial fibrillation  | 77 (44.0)        |
| Hyperlipidemia   | 56 (32.0)        |
| IHD  | 42 (24.0)        |
| Heart failure  | 34 (19.4)        |
| DM type 2  | 29 (16.5)        |
| Aortic valve disease   | 27 (15.4)        |
| GUCH   | 17 (9.7)         |
| Intermittent claudication  | 15 (8.5)         |
| Cardiomyopathy   | 14 (8.0)         |
| Implanted CIED   | 14 (8.0)         |
| Mitral valve disease   | 14 (8.0)         |
| Stroke   | 14 (8.0)         |
| COPD   | 9 ((5.1)         |
| CKD  | 6 (3.4)          |
| <b>Planned intervention/surgery</b>  | <b>Total (%)</b> |
| Electrophysiology  | 69 (39.4)        |
| Coronary angiography   | 41 (23.4)        |
| Vascular surgery   | 14 (8.0)         |
| PFO/ASD-closure  | 12 (6.9)         |
| Electrical cardioversion   | 9 (5.1)          |
| Open heart valve surgery   | 5 (2.9)          |
| TAVI   | 4 (2.2)          |
| Others   | 16 (9.1)         |
| Cardiovascular patients without planned intervention/surgery   | 5 (2.9)          |
| <b>Abbreviations:</b> CIED: Cardiac implantable electronic device; CKD: Chronic kidney disease; COPD: Chronic obstructive pulmonary disease; DM: Diabetes Mellitus; GUCH: Grown up congenital heart disease; IHD: Ischemic heart disease; PFO/ASD: Patent foramen ovale/Atrial septal defect; TAVI: Transcatheter aortic valve implantation. |                  |

**Table 1:** Comorbidities and the type of planned cardiovascular intervention/surgery.

### Nucleic Acid and Serology Results at Screening

A total of 7 patients (4.0%) had positive NPH PCR, where 6 of them were both positive for IgM and IgG and 1 was positive only for IgM. Overview of the test results are presented by Table 2. Of the 175 patients, serology assay results of 12 patients (6.9%) were regarded as positive and were thus seropositive. Among these, 9 patients (75.0%) had positive IgM in combination with positive NPH PCR, symptom recent 5 months and/or COVID-19 epidemiology. A total of 8 patients (66.7%) were positive for both IgM and IgG and 11 patients (91.7%) had only positive IgG, indicating a transition to the longer-lasting IgG response. Out of the 175 patients, 8 (4.6%) had isolated positive IgM and were thereby regarded as false positive cases.

|                            | <b>NPH PCR</b> | <b>Total positive IgM cases</b> | <b>False positive IgM cases</b> | <b>Total IgG positive cases</b> | <b>Positive IgM and IgG</b> |
|----------------------------|----------------|---------------------------------|---------------------------------|---------------------------------|-----------------------------|
| <b>Number of cases (%)</b> | 7 (4.0)        | 17 (9.7)                        | 8 (4.6)                         | 11 (6.3)                        | 8 (4.6)                     |

**Table 2:** Distribution of positive PCR and serology results.

### Reported Symptoms by the Positive Cases

Out of the 12 positive patients, 4 were asymptomatic during the recent 5 months at the screening time. Among the 8 symptomatic patients, fever (58.3%) was the dominant symptom followed by non-productive cough. The other symptoms are presented by Table 3.

| Symptom            | Frequency (%) |
|--------------------|---------------|
| Fever              | 7 (58.3)      |
| Cough              | 6 (50.0)      |
| Anosmia/hypogeusia | 4 (33.3)      |
| Rhinorrhea         | 4 (33.3)      |
| Myalgia            | 3 (25.0)      |
| Fatigue            | 2 (16.7)      |
| Sore throat        | 2 (16.7)      |
| Diarrhea           | 1 (8.3)       |
| Dyspnea            | 1 (8.3)       |
| Asymptomatic       | 4 (33.3)      |

**Table 3:** Frequency of symptoms reported by positive patients.

### Follow-up

Out of the 12 positive cases, 9 patients carried out the follow-up tests but 3 patients declined a follow-up due to the risk of COVID-19 transmission. None of the 9 patients tested positive for NPH PCR. 8 of the patients had similar antibody results as at the screening time, while 1 patient had positive IgM and IgG at the screening but had only positive IgM at the follow-up. One of the patients still had breathing difficulties, anosmia and hypogeusia. A total of 20 negative cases were followed-up as controls, where none had positive antibody results and none reported symptoms since the screening.

### Discussion

There are currently limited data on SARS-CoV-2 seroprevalence among cardiovascular patients. We screened consecutive patients of this subpopulation to address this knowledge gap. Seropositivity was defined by having 1) positive IgM together with positive NPH PCR, epidemiology and/or a symptomatic period, 2) positive IgG or 3) positive IgM and IgG, giving a seroprevalence of 6.9% in this cardiovascular cohort. A total seroprevalence of 4.04% was found among 28,792 healthcare workers in Denmark mid-April, with undoubtedly higher exposure to SARS-CoV-2 than our self-isolated cohort, also using the Livzon kit [28]. This lower seroprevalence could be explained by the higher extent of COVID-19 transmission across Sweden than Denmark at the beginning of April [29]. The public health agency of Sweden reported a seroprevalence of 9.3-11.5% in samples from Stockholm's outpatient clinics during weeks 22-24, the same period as this cohort was screened [27].

Considering seroprevalence of Stockholm's general population during the same period, the time that has lapsed since COVID-19 outbreak in Sweden and knowing that seroconversion often occurs within 2 weeks PSO, a higher seroprevalence than 6.9% would be expected [5,6,8,11-14,27]. One possible explanation could be that a great proportion of our cohort self-isolated during the pandemic because of risk factors as high age and cardiovascular comorbidity [17,18,21]. Self-isolation may also explain the lower seroprevalence of the 65-95 years old group during weeks 22-24 than the younger inhabitants of Stockholm [27]. At the follow-up, all the negative controls had negative serology results and seropositivity remained in all positive cases. However, 4.6% of the positive IgM results were regarded as false positive and 1 positive case at the screening (positive IgM and IgG) had an isolated positive IgM at the >3 months follow-up.

Livzon reported a sensitivity and specificity of 90.6% and 99.2%, respectively. Livzon's accuracy has been validated to a sensitivity of 80.0-96.8% and a specificity of 95.0-100.0% [7,13,28]. Interestingly, false positive IgM in SARS-CoV-2 is found in 61% of individuals with mid-to-high levels of rheumatoid factor-IgM and antibody kits had a lower specificity in positive than negative RF-IgM sera (38.9% versus 100.0%) [30]. Cross-reactivities with N protein of SARS-CoV and S2 protein of MERS-CoV can potentially occur [5,14]. However, cross-reactivity with human coronaviruses (229E, HKU1, NL63, OC43) is limited, potentially due to a lower N gene sequence homology [5,11].

SARS-CoV-2 specific T lymphocytes demonstrate memory phenotype, and might be the key cells responsible for recovery of COVID-19 in cases with very low neutralizing antibody levels [31,32]. In case of SARS; IgG titers were undetectable after 2 years, while SARS-CoV specific memory T-cells persist up to 17 years after infection in 15 SARS recovered patients and even show cross-reactivity with SARS-CoV-2 peptides [33,34]. SARS-CoV-2 neutralizing antibodies and S protein-specific B cells were undetectable in a young convalescent patient with COVID-19 pneumonia 3 months PSO [35]. However, SARS-CoV-2 specific memory T cells show robust responses in seronegative individuals in convalescent phase after asymptomatic/mild COVID-19 [31]. These findings suggest that T cells could provide multispecific and long-lasting acquired humoral immunity. Testing consecutive cases allowed us to study a heterogenous group rather than selected individuals.

Several limitations of this study need to be addressed. A multicenter study using a larger cohort would provide a more comprehensive picture of the cardiovascular population's seroprevalence and simultaneously minimizing the inevitable selection bias accompanied with a single center study. Lateral flow tests were not confirmed with quantitative titer measurement for

verification of the results. Furthermore, sensitivity and specificity analyses for the LFIA were not conducted in this study.

## Conclusion

The seroprevalence of SARS-CoV-2 in this cardiovascular cohort was lower than that of Stockholm's general population. This lower seroprevalence could be due to self-isolation of this cohort or development of a cellular rather than a humoral immunity against SARS-CoV-2.

## Conflict of Interest

The authors do not have any conflict of interest to declare.

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