

## DATA ON ANTI-SARS-COV-2 ANTIBODIES (IGM/IGG) IN COVID-19 PATIENTS

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**Abstract:** SARS-CoV-2 is an enveloped RNA virus capable of suffering mutations when passing from animals to humans, mutations which will allow for longer persistence in circulation but possibly also greater infectivity.

If the disease is detected, the identification of viral RNA in pathological products harvested from the respiratory tract by molecular tests (RT-PCR) is the absolute recommendation. Serological tests have a role in estimating the extent of the population's immunization.

Due to the high risk of intra-community transmission, it is essential to implement a surveillance program of all possible virus transmission routes.

The purpose of the study was in line with the current epidemiological situation, to evaluate a molecular tool for improving the control of the spread of SARS-CoV-2, for risk assessment and for the development of a protocol for the epidemiological management of the COVID-19 pandemic.

An advantage is the access to a considerable number of cases - Bucharest ranked 1<sup>st</sup> place in the number of confirmed cases of COVID-19 on 30.09.2021.

**Keywords:** COVID-19, ELISA, SARS-CoV-2, antibody detection, pandemic control.

### INTRODUCTION

During the pandemic, family members as well as healthcare and military staff are affected economically (decline of the economy, increase of unemployment rates, of prices) and socially (through isolation, disruption of education etc.). The immunization process threshold of more than 80% of the population takes time to reach. Finding complementary measures to keep the evolution of the pandemic under control and prevent a possible re-emergence of the disease is more approachable at this stage. Among these, identifying and monitoring the virus presence and determining the degree of immunization would contribute in reducing the pandemic consequences [1-15].

It should not be excluded that there have been cases of re-infection with SARS-CoV-2 and a worrying

ratio (almost 1:8 of these patients) developed a serious form of disease and died [16].

In the current context of the evolution of the COVID-19 pandemic, it is important to know the natural/acquired level of antibody coverage of the population in parallel with the vaccination coverage and the effectiveness SARS-CoV-2 vaccines in Romania.

### MATERIALS AND METHODS

All procedures performed in this study were in accordance with the Declaration of Helsinki. The Institute's Ethics Committee approved the Protocol for the study of the research project within the work Internal Plan through notice no. CE 82/2021.

Written consent has been requested from each patient included in the study group after informing

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them of how laboratory testing will be conducted and regarding the possible adverse reactions which may occur throughout the performance of the medical service. The test data used in the study complied with the condition of anonymity. The volunteer participants who were included in our study were unvaccinated people according to the purpose of the study.

The name coding was performed as a unique identification code for each participant, in order to ensure confidentiality and anonymity of personal and medical data.

The activities covered by this study were: a) the collection, sampling and interpretation of results from biological samples and b) the collection and correlation of clinical data with experimental data.

All patients in the studied group were subjected to a mandatory investigation protocol consisting of medical history and laboratory investigations. Prior to collecting the biological samples and conducting the medical examinations, a medical history was performed, which reported information on the health status (chronic conditions, acute conditions, treatments) of the study patients.

Screening, patient inclusion in the study group and medical procedures are described in Figure 1.

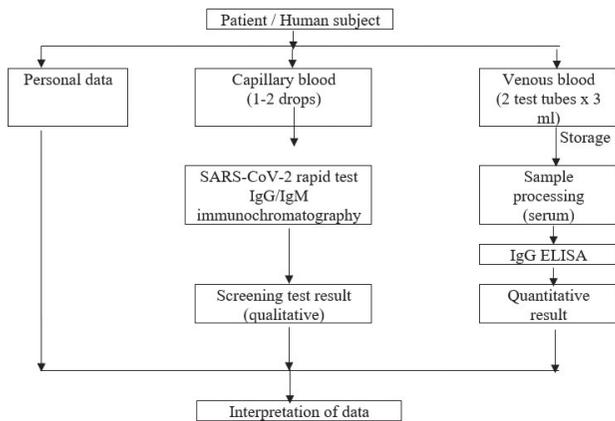


Figure 1. The flow-chart of investigations.

All patients were tested for SARS-CoV-2 antibodies with rapid tests and the ELISA method.

The control group (negative control) consisted of 12 people who had not experienced COVID-19 infection and who had tested negative at the time of collection. The positive control group consisted of 3 vaccinated people.

a) In order to perform the immunoenzymatic method, colorimetric ELISA (from venous blood) it was used:

- anti-SARS-CoV-2 QuantiVac ELISA (IgG) kits, IVD and CE kits for the quantitative detection of IgG anti-SARS-CoV-2 antibodies (anti spike S1) (the wells of the microplate were coated with the S1 subunit of the Spike protein specific to SARS-CoV-2, expressed in HEK 293 cells) and,

- anti-SARS-CoV-2 NCP ELISA IgG kits, IVD and CE kits for the quantitative detection of IgG SARS-CoV-2 NCP antibodies (the wells of the microplate were coated with the modified nucleocapsid protein specific to SARS-CoV-2 (with the removal of non-specific epitopes). Reading the results was performed at 450 nm radiation.

b) as well as COVID-19 rapid test kits (capillary blood), rapid immunochromatographic tests for the qualitative detection of IgG and IgM anti-SARS-CoV-2 antibodies. The cassettes show 3 membrane-specific regions: a region corresponding to the test validation control line, a region lined with anti-SARS-CoV-2 IgM antigen and a region lined with anti-SARS-CoV-2 IgG antigen. These tests consist of two components, an IgG component and an IgM component.

Sample collection and interpretation of the results were performed in accordance with the working protocols from the detection kit and those provided in the approved working procedure.

In particular, the following diagnostic and statistical data were followed for the study: age; gender (female/male); psychological factors (chronic stress, divorce, death, traumatic event, other psycho-emotional causes - conflicts, unemployment etc.); the presence of personal physiological history (pregnancy, confinement after birth); current acute conditions (fever, myalgia, arthralgia, rhinorrhea, impaired general condition, cough, breathing difficulty); personal pathological history focused on identifying association with one of the following conditions: a) Autoimmune diseases (systemic lupus erythematosus/rheumatoid arthritis/ulcerohaemorrhagic rectocolitis/Crohn's disease/Addison's disease/psoriasis/dermatomyositis /multiple sclerosis/sarcoidosis/ autoimmune vasculitis/ autoimmune thrombocytopenia etc.); b) Allergies; c) Chronic hepatitis (B/C), d) HIV, other viral infections, e) Diabetes mellitus (1/2); (f) Malignant haemopathies (leukemia, lymphomas, multiple myeloma); g) Other neoplasms; and medication administered: a) immunosuppressants/ immunomodulators/ corticosteroids etc.; b) antidiabetic, antiretroviral, antithyroid agents; chemo-/radiotherapy.

**RESULTS**

For the implementation of the scientific project, a prospective study was carried out on a statistical group of 72 volunteers, who visited the Medical Office of “Cantacuzino” NMMIRD during October and November 2021.

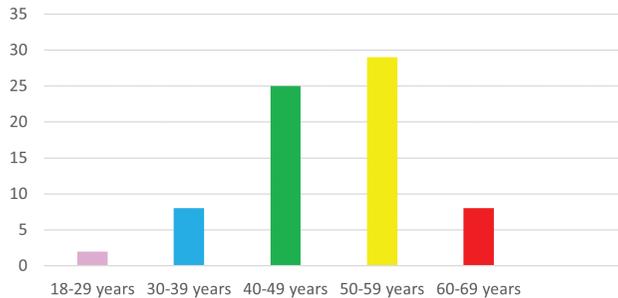
Of these, 69 were unvaccinated and 3 were vaccinated (the latter being included for the positive control of testing).

Of the 69 who were unvaccinated, 22 were male and 47 female. The distribution by age group was as follows: 18-29 = 2; 30-39 = 8; 40-49 = 23; 50-59 = 28 și 60 – 69 = 8.

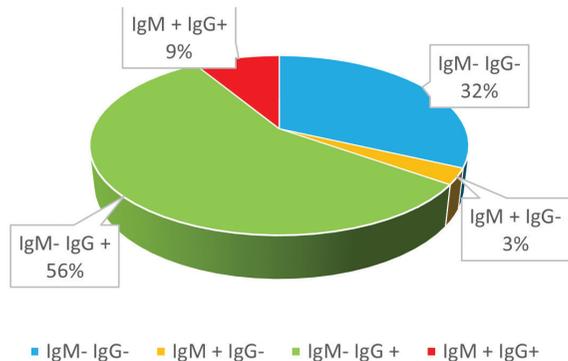
Thirty-three of them had a positive SARS-CoV-2 test in the past, and one patient had 2 positive tests at an interval of about 14 months.

All those infected with SARS-CoV-2 reported clinical symptomatology specific to COVID-19 and 17 complained of post-COVID symptoms. Of the 32 patients who had symptoms associated with SARS-CoV-2, 43.75% (n = 14) had two or more comorbidities; allergies, viral hepatitis and Hashimoto’s thyroiditis were the most common comorbidities in the studied group. Post-COVID symptoms included hypertension (HTN), increased heart rate (> 100), headache, fatigability, cough, breathing difficulty, intercostal neuralgia.

In rapid testing, all processed samples with



**Figure 2.** Distribution of patients by age group.



**Figure 3.** Distribution (%) of IgM/IgG rapid test results.

validated results were compliant for examination. 8 people (2 with positive PCR test) had IgM = +, of which 2 = ++, while 6 simultaneously showed IgM with IgG, indicating infection in the acute phase of the disease / infection in asymptomatic individuals.

Forty-three patients showed IgG, of which 9 = +, 6 = ++, 10 = +++, and the remaining 18 = ++++ (intensity above the control one). One of the people included in this latter category was a 30-year-old pregnant patient (23 weeks), tested positive in March current year, who, at rapid testing, showed very high anti-spike S1 IgG values.

Of these 43 people with a positive IgG test, 6 had both IgM and IgG bands present.

Thus, the presence of one or both specific antibodies (IgM/IgG) signaled infection with SARS-CoV-2 in 45 of the study patients.

There is a correlation between the level and persistence of the antibody titre and the duration and intensity of clinical symptomatology, respectively, but the analysis, processing, interpretation and correlation of medical data obtained during the study is in full progress.

Rapid IgG/IgM anti-SARS-CoV-2 (capillary blood) testing was complemented by ELISA serological testing.

For the interpretation of the results, we used a myCurvetFit computer program (<https://mycurvetfit.com>), using the commands: FitMethod → Linear → Point-to-Point, RU/ml on the abscissa, and OD on the ordinate. The calibrators and the + and - controls were recorded, after which the 72 results for the respective wells were read. The values obtained were listed in tables for further analysis of the data.

The interpretation of the results was carried out according to the manufacturer’s recommendations (Euroimmun, Germany), as follows: IgG-antispikes (RU/ml) = positive values ≥ 11 and IgG-anti NCP = positive values ≥ 1.1.

Recombinant Anti Human SARS-CoV-2 IgG Spike S1 += 45.

SARS-CoV-2 IgG anti modified nucleocapsid protein += 23.

11 patients requested and received results of the rapid testing for the detection of SARS-CoV-2 specific antibodies and 12 volunteers the results of anti-spike S1 IgG testing.

**DISCUSSION**

In our documentation there were few references to clinical studies in which the Romanian experience on

antibody analysis of people infected with COVID-19 were published, especially in relationship with clinical symptomatology.

The identification and monitoring of the virus presence, but also the evaluation of the degree of immunization may be useful tools for alerting or preventing the re-emergence of the pandemic, in order to reduce the impact on the quality of life, and on the health insurance system.

As in the course of the implementation of this project, we observed the necessity of performing immunological and cell immunity research in both unvaccinated and vaccinated people, it is desirable to extend the study, in the current context of the pandemic.

**In conclusion**, the study performed at the end of 2021 did not accumulate enough data, to attest the protective level of antibodies in unvaccinated people.

The rapid IgG/IgM anti-SARS-CoV-2 (capillary blood) test is a screening, and not a diagnostic method. A negative result did not exclude infection with SARS-CoV-2. A positive result was suggestive for recent infection, but clinical measures should not be based on a single laboratory finding. After analyzing the data obtained so far, we could not establish the persistence of these post-infection antibodies. Therefore, we recommend continuing the study by monitoring them at an interval of 2-3 months.

During the course of this internal project, some patients infected and recovered from COVID-19, although having had negative tests at the beginning of the study, reported the presence of symptoms such as HTN, vertigo, fatigability, headache, cutaneous rash etc., clinical manifestations which they did not experience prior to infection. That is why, after recovery, patients who have experienced COVID-19 need health monitoring to assess the complications of SARS-CoV-2 infection, and they still need medical supervision.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

#### **Acknowledgement**

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