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Research Article

Immune Response Variation in Administration of IgG Lysates

Samorindo Peci[1]*, Ulrich Hertmann[2], Federica Peci[3] and Rosjana Pica[3]

[1]*Ce.Ri.Fo.S, Milan, Italy*

[2]*Vitazell Laboratory, Chemnitz, Germany*

[3]*Istituto San Celestino, Milan, Italy*

***Corresponding Author:** Samorindo Peci, Ce.Ri.Fo.S, Milan, Italy.

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Abstract

SARS-CoV2, spread at the end of 2019, is causing billions of deaths. By the end of August 2020, more than 1,600,000 total cases of COVID-19 have been confirmed in 103 countries.

In laboratories and hospitals, researchers are working hard in search for a vaccine that can limit the contagiousness of the new Coronavirus. In this way, our research group hypothesized the use of autologous fragmented IgG to raise antibody response in patients with COVID-19.

Starting from a study carried out on hospitalized and non-hospitalized patients who contracted SARS-CoV2 between February and April 2020, we analyzed the antibody response of COVID-19 patients and developed an autovaccine, using the antibody lysates obtained from IgG taken from the serum of COVID-19 patients.

Our study aims to lay the foundations for more furthered studies on the use of autologous fragmented IgG to increment antibody coverage in COVID-19 and non-COVID-19 patients.

Keywords: *COVID-19; IgG Lysates; Antibody Response; Autologous IgG; SARS-CoV2; ACE2*

Abbreviations

COVID: Corona Virus Disease; RNA: Ribonucleic Acid; ACE2: Angiotensin-Converting Enzyme 2; IgA: Immunoglobulins A; IgM: Immunoglobulins M; IgG: Immunoglobulins G; ICU: Intensive Care Unit; N-IgM: Immunoglobulins M of Protein N; N-igG: Immunoglobulins G of Protein N; S-IgM: Immunoglobulins M of Protein S; S-igG: Immunoglobulins G of Protein S; Ag: Antigen; Ab: Antibody; IMS: Immunomagnetic Separation; RT-PCR: Reverse Transcription-Polymerase Chain Reaction

Introduction

The current COVID-19 pandemic, spread from China at the end of December 2019, has SARS-CoV2 as its protagonist; a virus belonging to the Coronaviridae family, which has a single molecule of RNA as its genome.

To understand the nature of SARS-CoV2 infection it is necessary to know its structure. SARS-CoV2 has a multifunctional molecular mechanism associated with an envelope with 3 structural glycoproteins: Protein M (membrane protein), Protein E (envelope protein), Protein S (Spike that directly mediates infection) and protein N (viral RNA binding) [1]. The mechanism of transmission of the disease depends on the Spike protein (Shown in figure 1).

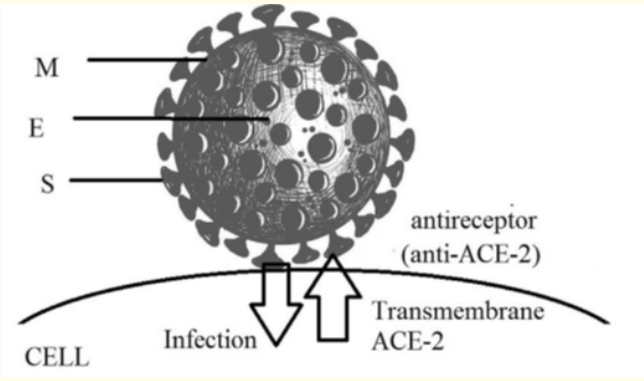


Figure 1: *By Sohail A., 2020 [2].*

Viral replication of viral "spike proteins" occurs in SARS-CoV2 infection; spikes serve as tiny picks that allow the Coronavirus to intercept the ACE2 (Angiotensin-converting enzyme 2) receptors on the surface of the human respiratory system cells to penetrate them and multiply [3].

To fight the entry and replication of SARS-CoV2 in target cells (which express the ACE2 receptor), clinical trials are underway using various strategies: from drugs that inhibit ACE2-mediated endocytosis of the virus; to monoclonal antibodies directed against the protein S of the virus (which binds the ACE2 receptor); and to polyclonal antibodies obtained from the plasma of convalescent patients [4]. Neutralizing antibodies could block viral entry by preventing protein S from binding to the host cell (e.g. ACE2) or by preventing the conformational changes that protein S undergoes to mediate membrane fusion. Neutralizing antibodies may also mimic receptor binding and trigger conformational changes in protein S before it binds to the ACE2 receptor [5].

In this context, the availability of soluble recombinant human ACE2 protein could be useful as a new biological therapy to fight or limit the progression of infection caused by Coronaviruses using ACE2 as a receptor [3].

Once the virus has attacked the ACE2 receptor, the infectious process begins.

Brief notes on epidemiology of SARS-CoV2

At the end of August 2020, more than 1,600,000 total cases of COVID-19 have been confirmed in 103 countries. Epidemiological data are still being processed [Data from 1 week before publication process: 29th October 2020].

So far, a greater sensitivity to the disease has emerged in people in adulthood or over the age of 60 an important role is also played by the presence of comorbidities such as respiratory or cardiovascular diseases, arterial hypertension, diabetes, leukemia and other hematological tumors related to the storm cytokine, and the risk related both to the deprivation index linked to the territory, and to pathologies linked to occupational exposure [6,7].

Severe SARS-CoV2 respiratory syndromes seem to spare children, according to literature. Probably children and adolescents are susceptible to infection, even if less than adults, but they have a much more favorable clinical course [8].

Immunoglobulin trend in COVID-19 patients

Acquired immunity represents a sophisticated defense system, consisting of mechanisms that are induced and stimulated by exposure

to the antigen. In this way, extremely specific immune responses are generated for the different molecules whose intensity and effectiveness are increased by repeated exposures.

The first time the body is infected or comes into contact with a foreign substance (antigen), the immune system recognizes the microorganism or substance as "non-self" and stimulates the plasma cells to produce specific immunoglobulins, able to bind to the foreign agent and neutralize it. In the event of subsequent exposures, the immune system is able to remember the antigen encountered and therefore to activate a faster production of specific antibodies; in the case of microorganisms, this memory mechanism helps prevent reinfections [9].

Immunoglobulins M (IgM) are produced at the body's first response to a new infection or to a new foreign antigen, providing short-term protection. The IgM concentration increases for a few weeks and then decreases as IgG production begins.

Immunoglobulins G (IgG) are produced during the first infection or upon exposure to foreign antigens, they increase a few weeks after contact, and then decrease and stabilize. IgG are responsible for the "secondary immune response", which occurs if there has been a previous encounter of the organism with certain antigens. The body retains the memory of the different IgG, which can therefore be reproduced at each exposure to the same antigen. IgG are responsible for the long-term protection against microorganisms. The immune mechanism underlying vaccines therefore consists in creating the memory towards a microorganism to which the subject has not yet been exposed, exposing the person to the alive but attenuated microorganism or to an antigen capable of stimulating the recognition of the microorganism.

The antibody response to a viral infection is illustrated in figure 2.

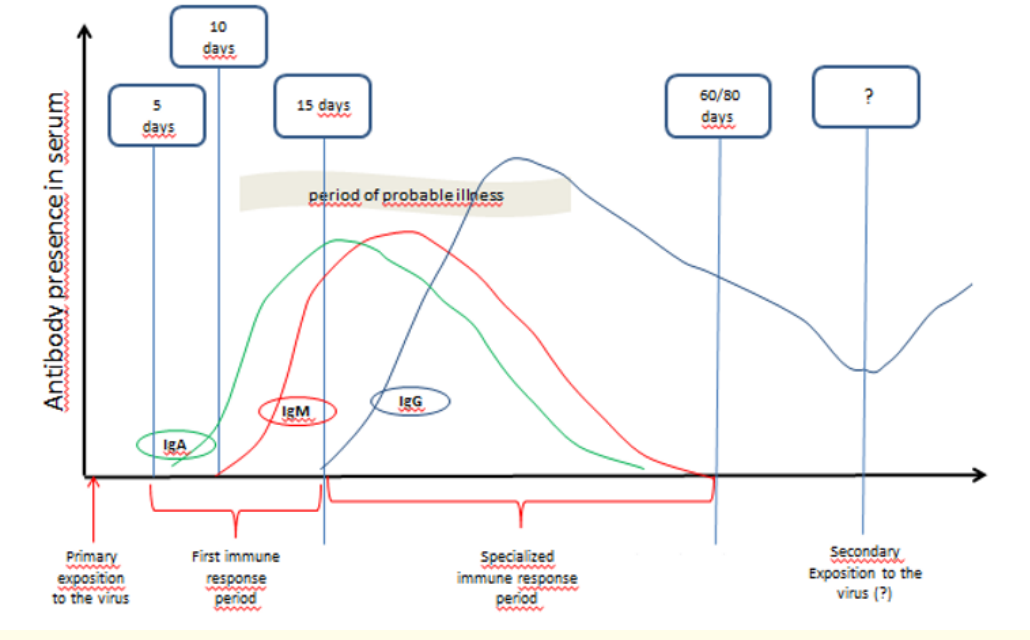


Figure 2: *By Margiotti, M., 2020 [10]. Antibody trend of IgA, IgM and IgG in the blood. Immunoglobulins A (IgA) provide protection against mucosal infections (respiratory tract, upper and lower airways, gastrointestinal tract, stomach and intestines), in fact they are the first to be produced in case of respiratory infections. IgMs are produced upon first exposure to a new antigen and represent the "primary immune response". IgG is produced later and represents the "secondary immune response". The presence of specific IgG for antigens of a given virus in the serum indicates that the organism has come into contact with the microorganism in a previous infection; if IgM are present, however, the infection is currently in the acute phase.*

As for SARS-CoV2 infection, studies are underway to better understand the trend of immunoglobulins in affected patients. Interesting data emerged from the studies already carried out.

Research carried out by Hoffman this year has shown that IgM and IgG are detectable in some patients from the 9th day, while in other patients' seroconversion seems to occur later. Interestingly, all IgM positive samples were also IgG positive. Generally, IgM is produced first, and then IgG is produced, but SARS-CoV studies suggest that IgM and IgG often develop around the same time [11].

Tuaillon's research team observed seroconversion between IgM and IgG 5 to 12 days after the onset of symptoms [12].

Sun, 2020, systematically analyzes the kinetics of the IgG and IgM antibody response to both SARS-CoV2 N and S proteins in the first 4 weeks after symptom onset in ICU and non-ICU (non-ICU) patients [13]. The study showed that seropositive rates of N-IgM, N-IgG, S-IgM and S-IgG antibody responses in non-ICU patients gradually increased within 1 - 3 weeks of initiation. N-IgM and S-IgM peaked in the second week, while N-IgG and S-IgG antibodies continued to rise in the third week. In the third week after symptom onset, seropositive rates for N-IgG and S-IgG reached 100%. In contrast, seropositive rates for N-IgM and S-IgM remained the same as in some patients they began to decline as a result of the conversion from IgM to IgG, which can help generate more effective antibodies that can inhibit the virus infection. Non-ICU patients tended to have faster and higher IgM to IgG class transition than ICU patients. It has been recognized that S-specific antibodies can block protein S binding to the cellular hACE2 receptor which mediates SARS-CoV2 binding and entry into target cells.

Zhang 2020's research team analyzed the possible relationships between the antibody response to COVID-19 and disease progression through antibody analysis in a longitudinal study [14]. All patients examined in this study presented with mild symptoms and were subjected to serological tests for the analysis of antibodies. 58 patients (51.79%) tested positive for both IgM and IgG antibody isotypes, 7 patients (6.25%) tested negative for both isotypes, 1 patient (0.89%) tested positive for IgM only and 46 patients (41.07%) tested positive for IgG only. IgM antibodies were detected within a week of the onset of the disease, persisting for about a month and gradually decrease. IgG antibodies were detected about 10 days after the onset of the disease but it has been shown that they persist longer. According to the data provided in this study, IgG antibodies persist for at least 40 - 50 days from the disease onset, although they have not been analyzed for longer periods.

To better understand the antibody response caused by SARS-CoV2, we have to cite the work of Ma 2020 [16], which drew a median of the data collected from 87 patients with COVID-19, examining the serum in different time frames from the first symptomatic manifestation of the disease. It has been found that IgA increases from the 4th to the 25th day, with a peak around 16 - 20 days and begins to decline from the 31st day from the onset of the disease (Figure 4). IgG increase 15 days after the onset of symptoms, reaches a peak around the 21st - 25th day and remains stable even around the 31st - 41st day [16].

This suggest that IgG are stronger even in late stages.

How immunization occurs

The fundamental reaction underlying immunological phenomena is the specific union of an antibody with its homologous antigen.

The interaction between the epitope of the antigen (Ag) and the domains of the variable regions of the antibody (Ab) has as its characteristic the specificity, i.e. the ability of a single combinatorial site of Ab to react with a single determinant antigenic or the ability of a population of antibody molecules to react with a single Ag.

Antibodies have two functional ends: Fab portions interact with antigens, and their Fc domains interact with the adaptive and innate immune systems, including natural-killer cells, phagocytes and complement. Fc antibody regions may be critical for the *in vivo* efficacy of passive immunization. Monoclonal antibodies, when developed for clinical applications, can be used for several properties, including the following: their neutralizing activity, the epitopes they target; and the antibody effect functions conferred by their Fc regions [5].

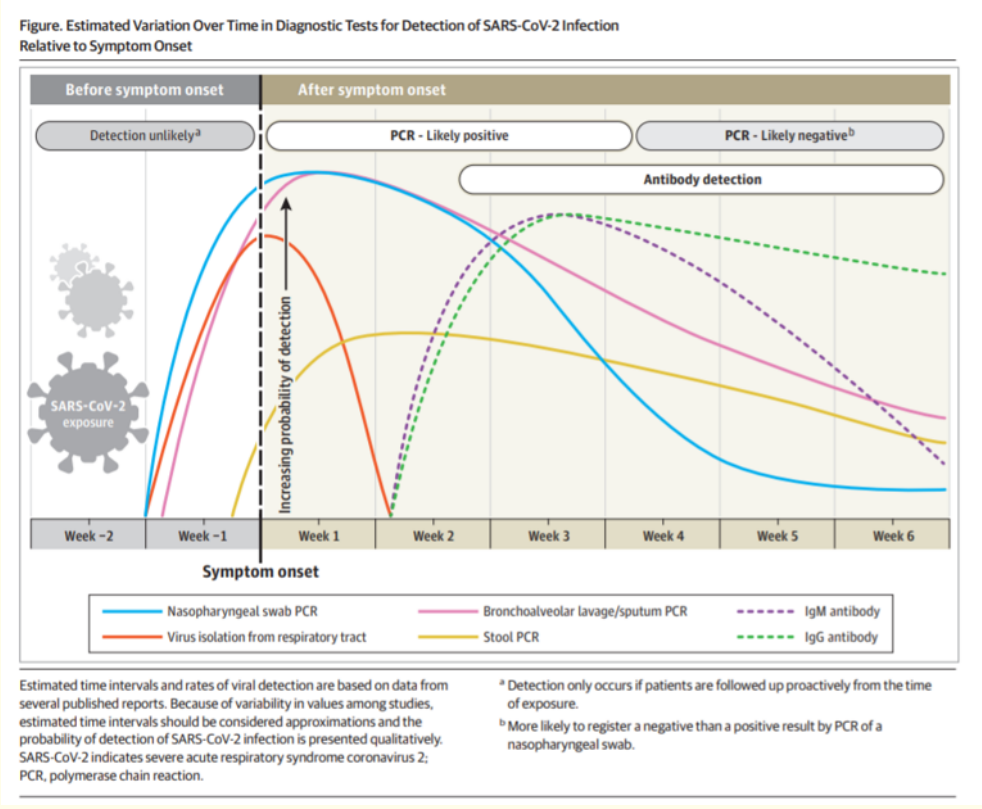


Figure 3: *By Sethuraman, N., 2020 [15]. The figure is a schematic and indicative representation of the timing for the detection of SARS-CoV2 in the human body and its contagion capacity, and of the immune response capable of progressively guaranteeing a lower risk of viral circulation at the population level. Note the possible presence of healthy carriers - asymptomatic capable of infecting, regardless of the presence of a serum conversion, where there is no attention to social distancing, hygiene practices, and the use of personal protective equipment. The identification of asymptomatic/pre-symptomatic subjects capable of being the cause of contagion by positive RT-PCR can more likely take place as a follow-up to contact tracing, or for misdirection of the entire population concerned, for example, to within an outbreak/red zone, or because it is exposed to occupational risk, or because it comes into contact with fragile groups (for example operators within the extended care unit).*

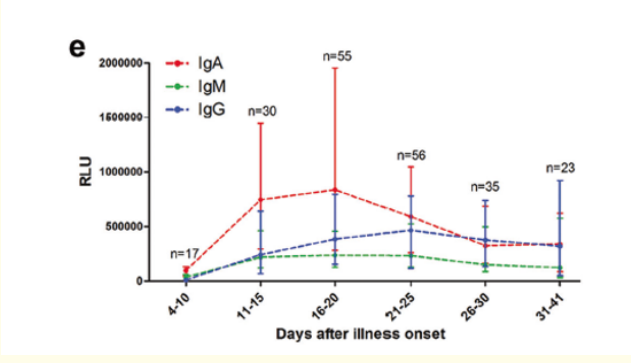


Figure 4: *By Ma, H., 2020 [16].*

Once the link between Ag and Ab has taken place, the body acquires the ability to fight infection.

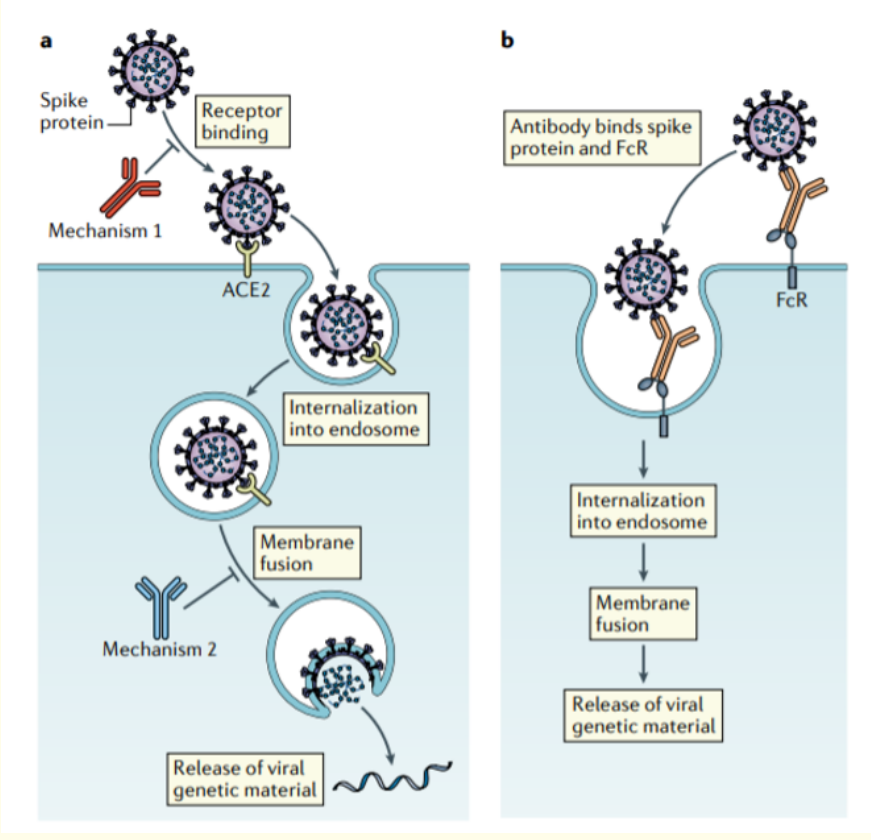


Figure 5: *By Abraham, J., 2020 [5]. | Potential mechanisms of coronavirus antibody neutralization and antibody enhancement of infection. Figure a: Mechanism 1: neutralizing antibodies could block viral infection by binding to the viral spike protein and preventing it from interacting with the cellular receptor angiotensin-converting enzyme 2 (ACE2). Mechanism 2: neutralizing antibodies could bind to the viral spike protein and block the conformational changes that the spike protein must undergo to facilitate fusion of the viral and host cell membranes. Figure b: Antibodies could enhance viral entry into immune cells by binding to the viral spike protein with their Fab portion and to Fc receptors (FcRs) with their Fc domain.*

The creation of a generic or specific immunity state for a given antigen takes place through the use of vaccines (active immunization) or with the administration of antibodies (passive immunization).

Many vaccines are currently being tested with studies on primates, in which they are inducing neutralizing antibodies to SARS-CoV2.

Our research group aims at the second type of immunization: the construction of "autovaccine" consisting of fragments of autologous IgG antibodies.

Materials and Methods

Patients

In this regard, 80 COVID-19 patients were recruited between March and April 2020. Among these 80 patients, we focused on the 20 in which a drop in IgG was found during the course of the infection. The date of infection is the first SARS-CoV2 positive swab.

Patients characteristic are shown in table 1.

Patient	Nationality	Sex	Age	Day of infection	Hospitalized	1st measurement	IgG	2nd measurement	IgG	Drugging	3rd measurement	IgG
1	German	M	64	26/02	Yes	18/05	4,2	30/06	3,8	Yes	09/09	5,3
2	German	F	60	25/02	No	18/05	2,5	30/06	2,3	Yes	09/09	4,3
3	French	F	72	30/03	Yes	20/05	3,8	02/07	2,7	No	10/09	2,6
4	Italian	M	71	12/04	Yes	22/05	4,1	03/07	4,1	Yes	09/09	5,3
5	French	M	70	28/03	Yes	20/05	4,3	02/07	4,2	Yes	10/09	6,1
6	German	M	69	20/03	No	21/05	4,8	30/06	3,9	Yes	09/09	4,1
7	German	F	65	01/04	Yes	20/05	3,9	30/06	3,8	No	09/09	3,6
8	German	F	78	21/03	Yes	20/05	4,6	30/06	4,1	Yes	09/09	4,9
9	French	M	82	28/02	Yes	19/05	5,1	02/07	3,9	No	10/09	3,2
10	Turk	F	80	27/03	Yes	19/05	6,2	30/06	5,9	Yes	09/09	6,5
11	French	F	74	03/04	No	20/05	3,9	02/07	3,8	No	10/09	3,0
12	German	M	73	01/04	No	18/05	3,7	30/06	3,3	Yes	09/09	3,9
13	French	F	70	20/03	Yes	20/05	4,2	02/07	4,1	No	10/09	3,1
14	German	M	69	12/03	Yes	20/05	4,7	01/07	4,0	No	09/09	3,0
15	Italian	F	68	14/03	Yes	20/05	3,9	03/07	3,2	Yes	10/09	4,8
16	French	F	66	02/04	Yes	21/05	3,8	02/07	3,7	Yes	10/09	5,9
17	German	F	60	30/03	Yes	21/05	4,4	30/06	4,2	Yes	09/09	6,2
18	Italian	M	78	22/03	Yes	18/05	3,9	03/07	3,6	Yes	10/09	4,3
19	Italian	M	88	18/03	Yes	19/05	4,6	03/07	4,1	Yes	10/09	4,8
20	French	F	57	03/03	Yes	18/05	6,1	02/07	5,2	No	10/09	3,0

***Table 1**: Characteristic of patient analyzed. There are patients of different Countries, different ages, different periods of infection. We made 3 measurement of plasmatic IgG level: the 1st measurement was carried out between 18 and 22 May, the 2nd measurement about 45 days after the first, the 3rd measurement was carried out about 70 days after the second one.*

Three antibody measurements were carried out: the first measurement took place between 18 and 22 May 2020 (Day 0), the second approximately 45 days after the first analysis (Day 45). Following the second measurement, patients were given a sublingual dose of fragmented IgG in decimal dilutions for 40 days (Table 2). The third measurement was taken approximately 70 days after the second control measurement (Day 70). The first 20 - 30 days of this time were used to complete the dilutions of the fragmented IgG; the third measurement therefore took place on horseback with the interruption of the autovaccine administration.

The measurements were made at the Vitazell Laboratory in Chemnitz (Germany).

Immunomagnetic separation (IMS)

The technique used for the extraction of antigens is immunomagnetic separation.

Immunomagnetic separation (IMS) allows the isolation of cells, proteins and nucleic acids within a cell culture or body fluid through the specific capture of biomolecules through the attachment of small magnetized particles, spheres, containing antibodies. These spheres are coated to bind to targeted biomolecules, gently separated and go through multiple wash cycles to obtain target molecules bonded to the magnetic spheres, which differ according to the strength of the magnetic field.

This is a very valid and used technique in immunology, because it guarantees the purity of the sample. IMS does not harm the sample because it does not use solvents and solutions that can contaminate and analytically influence the sample. Moreover, being an automated method, it also has the advantage of being simple to apply.

Let us briefly review the method with which the IgG fragments were obtained.

Patient serum containing the Ag for COVID-19 is placed in a test tube, inserted in a support that generates a magnetic field. Subsequently, a complex formed by the antibody with a biomagnetic tag is placed in the test tube. More precisely, microspheres consisting of nuclei containing iron are inserted and covered by a thin layer of a polymer that allows the absorption of biomolecules, in our case the immunoglobulins G (IgG). The antibodies that coat the paramagnetic spheres bind to the antigens present on the cell surface, causing the antigen/antibody reaction and facilitating the separation of the cells attached to the spheres. The separation process is created by a magnet, placed on the side of the tube. The cells bound to the biomagnetic tag separate thanks to the strength of the magnetic field. Two phases are created in the test tube: the phase containing our magnetized cells, placed on the side of the tube in contact with the magnet, and the supernatant containing the non-magnetized cell residues.

Once the supernatant is removed, the cells are eluted. A magnetic bead removal reagent is then applied to have an enzymatic release of the beads ensuring the purity of the target cells.

Subsequently, the IgG extract is subjected to centrifugation for 18 ‘at 10,000 rpm. IgG fragments are obtained thank to the presence of small metal marbles, that fragment immunoglobulins during centrifugation. Fragmented IgG are further diluted following the decimal dilution according to the Jacques Benveniste method, as shown in table 2.

	Time	Dilution
1st dose	Days 0 - 10	10^{10}
2nd dose	Days 10 - 20	10^{8}
3rd dose	Days 20 - 30	10^{6}
4th dose	Days 30 - 40	10^{4}

Table 2*: Doses of fragmented IgG, given to patients for 40 days. Each dose is at a higher concentration than the previous one.*

Each dose of fragmented IgG was administered to patients for 10 consecutive days. After 10 days, the more concentrated dose was taken. The administration lasted for 40 days.

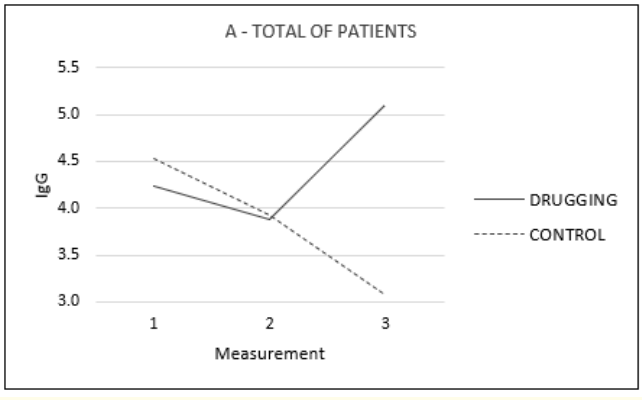
Results

The analyzed data, provided by the Vitazell Laboratory of Chemnitz in Germany, comes from 20 patients in whom a drop in IgG was found during the second antibody measurement.

The results were processed by dividing the patients into macro groups and calculating the average of the data found. We remind you that day 70 corresponds on average to the end of the autovaccine administration for 40 days.

As a first analysis, we compared the data of all the patients who received the autologous dose of fragmented IgG with the data of all the patients who were not administered.

From the graph it can be seen that the controls underwent a decrease in the plasma IgG value, while the patients to whom the IgG fragments were administered developed an increase in antibody response equal to 64.52% compared to the controls (IgG drugging = 5.1/IgG control = 3.1) (Figure 6 and 7).



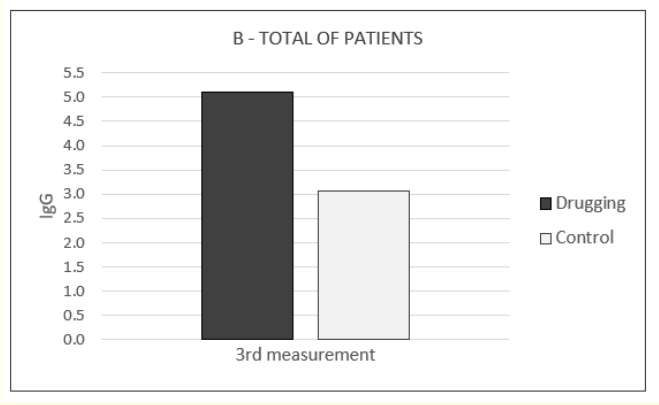


Figure 6: *IgG pattern in all patients. A: Measurement in drugging and control per day 0, day 45 and day 70. B: Difference between drugging and control in measurement per day 70.*

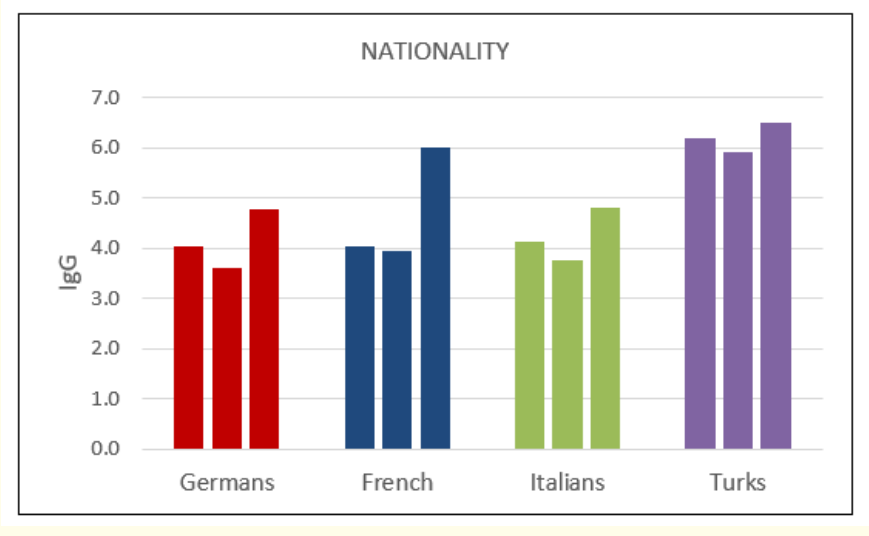
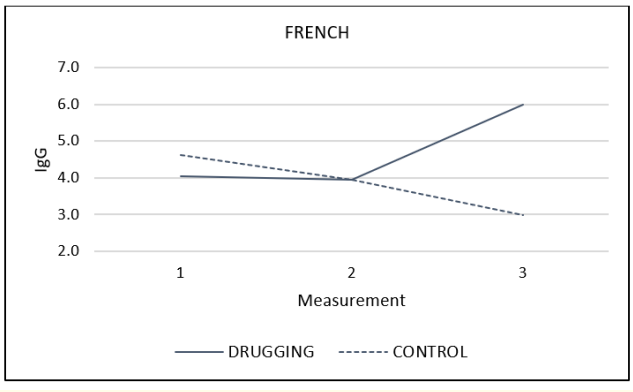


Figure 7: *IgG pattern in patients of different nationalities (Germans, French, Italians, Turks). Measurement per day 0, day 45 and day 70.*

Regarding German and French patients, not all of them received the fragmented IgG dose. In particular, 6 out of 8 German patients were administrated with fragmented IgG (drugging) and 2 patients were not (control), while among 7 French patients 2 received a dose of oral IgG fragments (drugging) and 5 did not (control).

We have thus analyzed the IgG values in the German and French patients, comparing them with the average of the control measurements.

The clear finding is that in both populations, the quantity of plasma IgG in the controls tends to decrease with each passing day. On the other hand, the increase in plasma IgG concentration in patients who have been administered an autologous dose of antibody is relevant (Figure 8).



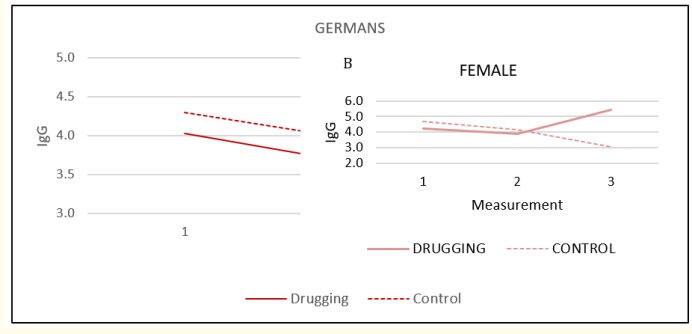


Figure 8: *IgG pattern in German and French patients. Differences between drugging and control in measurement per day 0 (1), day 45 (2) and day 70 (3).*

Comparing the third measurement between controls and non-controls, the increase in the immune response due to the administration of fragmented IgG is evident (Figure 9). The greatest increase is found in the French, with an IgG value of 6.0 which is twice that of the controls (IgG = 3.0).

Another analysis carried out concerns the sex of the patients. 7 out of 9 men received the dose of fragmented IgG, while in the group of females it was administered to 6 out of 12. In both men and women there is an increase in the immune response in patients who received the IgG fragments, compared to the drop in IgG in the controls, corresponding to -36.7% in men and -34% in women (Figure 10A).

Comparing the third measurement in the two populations, we detected increase in IgG in patients who received the dose. The best response is found in women, in which there is a 74% increase in IgG concentration compared to controls, 12.5% more than men (Figure 10B).

Our data also focused on hospitalized patient and out-of-hospital patients. For both clusters we have the control group to which the administration of fragmented autologous IgG was not carried out.

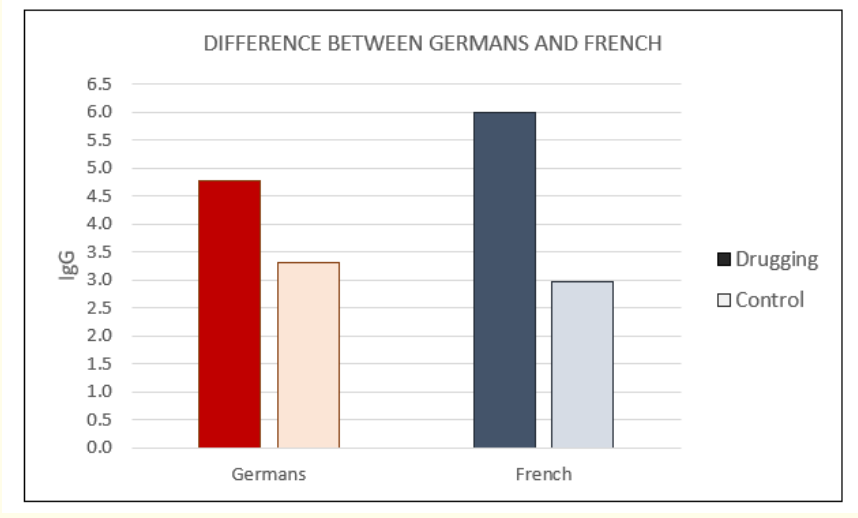
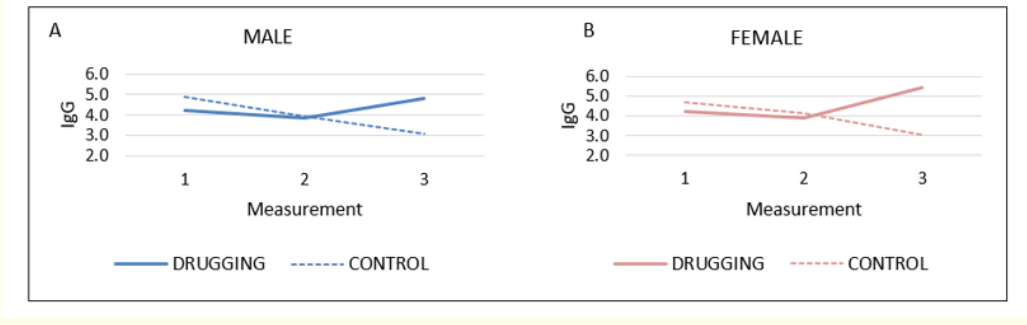


Figure 9: *Comparison between drugging and control in German and French patients after 70 days (3rd measurement).*



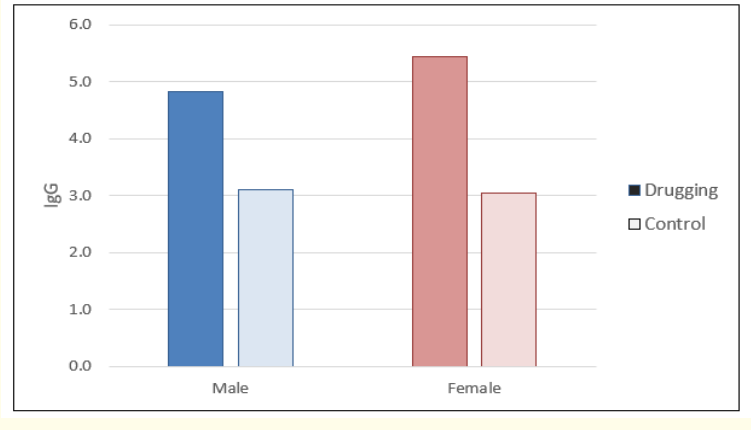


Figure 10: *Comparison between drugging and control in male and female patients. Measurement per day 0 (1), day 45 (2) and day 70 (3). B. Difference between male and female, comparing drugging and control after 70 days (3rd measurement).*

The data is clear: in both groups there is an increase in IgG in patients undergoing autologous administration (Figure 11).

Therefore, to monitor the infectious trend and the consequent immune response in subjects affected by SARS-CoV2, we divided the patient data by month of infection.

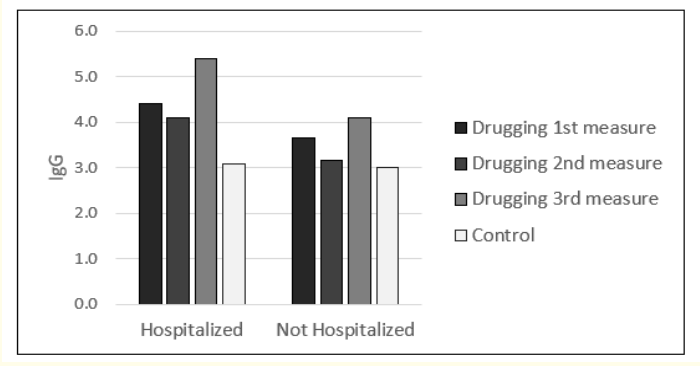


Figure 11: *Difference between hospitalized and not hospitalized patients. Measurement in drugging per day 0 (1st), day 45 (2nd) and day 70 (3rd) and control in day 70.*

We observed a decrease in IgG in controls (-37.3% for infected patients in February, -38.3% for patients who contracted COVID-19 in March). An increased antibody response is noted in patients administered with fragmented IgG. The greatest response was seen in patients infected in March (Figure 12 and 13).

To investigate whether the different immune response may depend on the age of the subjects analyzed, we divided the patients into 3 ranges according to their age: the first group ranges from 60 to 69 years, the second group from 70 to 79 years and the third group

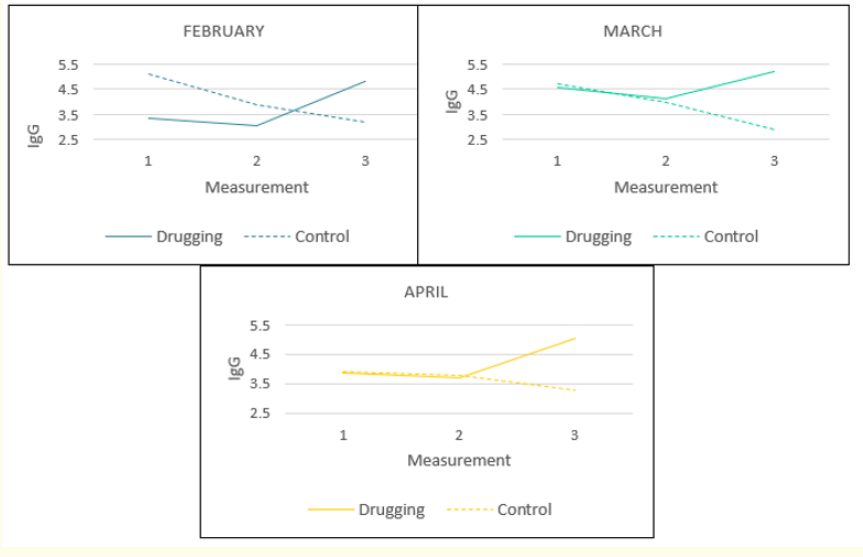


Figure 12: *Comparison between drugging and control in patients infected in different months (February, March, April). Measurement per day 0 (1), day 45 (2) and day 70 (3).*

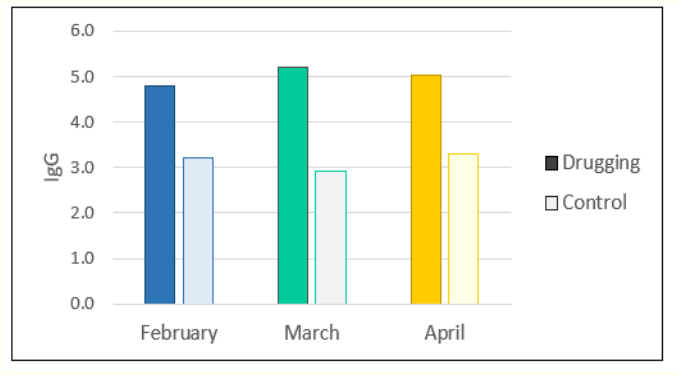
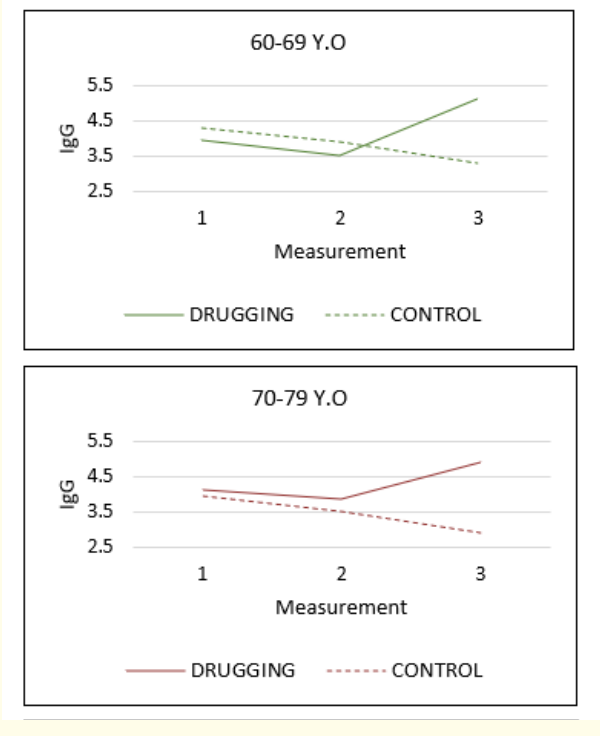


Figure 13: *Difference between patients infected in February, in March and in April, comparing drugging and control after 70 days (3rd measurement).*

includes patients from 80 to 89 years. A control group was provided for all three.

As in the previous analyzes, a decrease in IgG in the controls was shown in all three groups compared to an increase in the immune response in patients who were administered an oral dose of IgG. In particular, in patients over the age of 80, the decrease in IgG reached 37.3% (Figure 14).

An interesting information can be seen when comparing the third measurement of all groups. Patients with a major antibody response



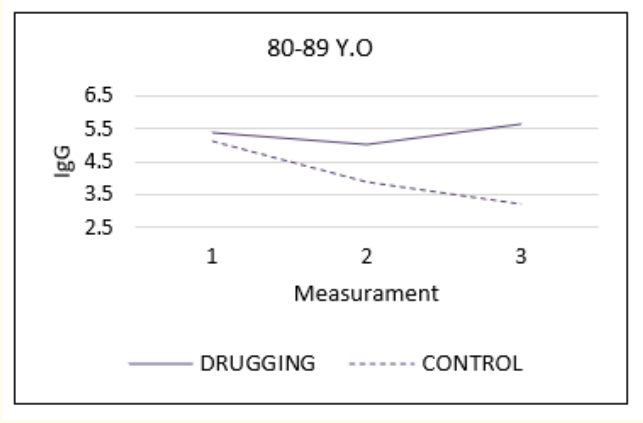


Figure 14: *Comparison between drugging and control in patients of different ages (60-69 y.o., 70-79 y.o., 80-89 y.o.). Measurement per day 0 (1), day 45 (2) and day 70 (3).*

belong to the 80-89 y.o age group (Figure 15).

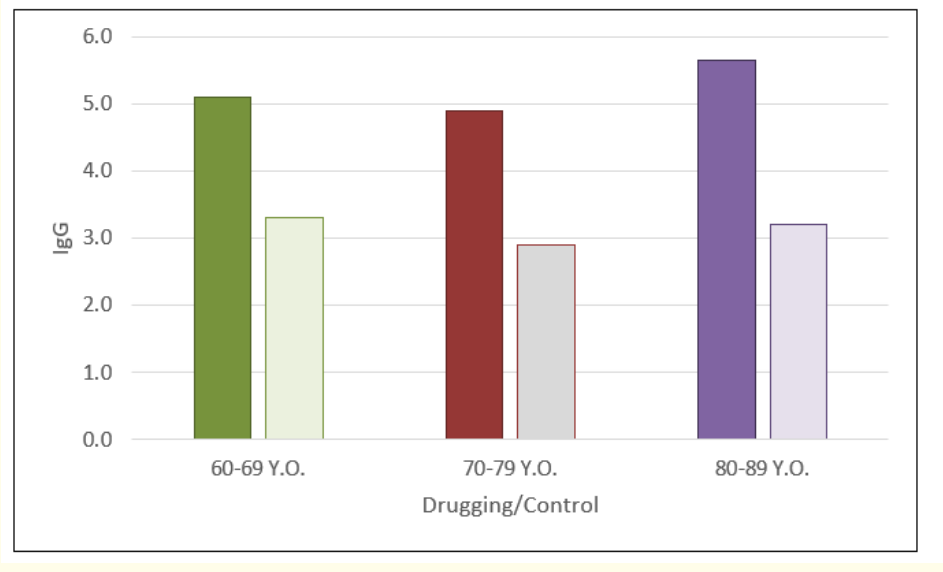


Figure 15: *Difference between patient of different age, comparing drugging and control after 70 days (3rd measurement).*

The results obtained from the analyzes of the various groups show in any case a real increase in the antibody response after the administration cycle of fragmented IgG.

The compliance ascertained from the anamnestic point of view in patients who were given the autovaccine doses is 95%. The remaining 5% of patients admitted that they had not been constant in taking fragmented IgG and that they had skipped the administration in a few days.

Discussion

The compliance ascertained from the anamnestic point of view in patients who were given the autovaccine doses is 95%. The remaining 5% of patients admitted that they had not been constant in taking fragmented IgG and that they had skipped the administration in a few days.

The study carried out by our research group shows the possibility of increasing the amount of plasma IgG in patients with COVID-19 who, about 100 days after infection, suffer a decrease in antibody response. The data shown is relevant and noteworthy.

Following the study carried out by Seow, J., 2020 [17], our group found a decrease in immunoglobulin level in patients who were not given a dose of autologous IgG fragments. On average, the plasma IgG level decreased over time by 31.1%. Patients over the age of 60 had a decline of more than 23%, in particular patients in the age group between 60 and 69 y.o. showed a decrease in IgG of 23.3%; in patients aged 70 - 79 y.o. a decrease of 27% was highlighted; while patients over 80 years of age experienced a 37.3% reduction in IgG. Among men and women, in our study it was the male population that showed a higher IgG lowering, equal to 36.7%.

It is therefore clear that with the passing of the months, the immune response to SARS-CoV2 tends to decrease.

In our study we have shown how the sublingual administration of autologous IgG fragments, obtained with the method described in the Materials and Methods paragraph, potentiates the immune response against SARS-CoV2. This could avoid reinfection in subjects who are debilitated as a result of the infection and with a weakened immune system.

The sublingual administration of autologous IgG fragments may involve not only patients who have already contracted COVID-19, but it could be useful to immunize those who have not yet contracted the virus.

The method by which the doses were created follows the Low-Dose principle. In fact, the concentration of the IgG fragments is in decimal dilution according to the Benveniste method (Table 2). The dilutions carried out make it possible to administer these fragments even in patients who have not been infected with SARS-Cov2, allowing on the one hand the immunization of that slice of the population that has not come into contact with the Coronavirus and on the other hand to maintain the antibody coverage in those who have faced and overcome the infection.

Our research group would also like to focus attention on the dose of autologous or autovaccine IgG fragments. Its administration has been shown to increase the antibody response and enhance the immune system against SARS-CoV2. The prefix "auto" indicates that the fragments that are provided to patients derive from extraction of autologous IgG fragments. It is therefore the same IgG of the patients to be processed and administered to them.

In our study, the administration of the first cycle of autovaccine doses elicited a strong immune response, which could be further strengthened after the administration of a second cycle.

It should be emphasized that the dose is administered orally and not by cutaneous injection, which makes the administration less invasive for patients and more easily acceptable by the population, being able to expand coverage. Furthermore, this type of vaccine is very versatile and is particularly suitable for subsequent administration. Therefore, the administration can easily be repeated after months to keep the IgG level against SARS-Cov2 high.

Conclusion

In conclusion, our study showed that, as a result of a decrease in plasma IgG values in subjects affected by COVID-19 that lasts over weeks, the administration of autologous IgG fragments led to the implementation of the immune response in all the subjects analyzed with an average increase in IgG equal to 64.52% compared to the controls, laying the basis for further studies in this regard.

The aspect that should not be underestimated is how an autologous preparation that is easy to administer and store can be useful in maintaining high antibody coverage of the infected.

The administration of fragmented IgG could also be used in first instance in family setting, guaranteeing antibody coverage in people who have had an infected person within their family and who therefore, even if they have not been infected, have somehow come into contact with the virus.

We are facing an RNA virus and to date we don't know if these antibodies generate immunization. However, we can see that autologous IgG fragments cause a stimulation with a consequent increase in immunological production; we cannot expect anything other than an immunization that can prevent a possible mutation from a vaccine. We believe that a pilot study on the administration of IgG fragments to the family stock with which the infection was shared - without developing antibodies - is useful, starting from studies of the administration of the plasma of recovered patients. We believe it's useful to discuss this very delicate issue, with many ethical questions such as speeding up the approval times for vaccines and possible administration to those who are cured, on which we could intervene with this method.

Conflict of Interest

Authors declare that there is no conflict of interest.

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