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Monitoring and detecting SARS-CoV-2 in Albanian Clinical Practice – The efficiency of CLIA serological assay, Real-Time PCR analysis and Biochemical analysis

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Abstract

SARS – CoV-2 is a pathogenic coronavirus which continuously evolves as changes in the genetic code occur during the replication of the genome. To monitor and detect this infection a combination of 3 parameters is used in Albanian Clinical Practice. Real-Time PCR analysis is the main standard for the identification of SARS-CoV-2- infection. Because of the limits in its utilization for large-scale screening, serological assays have been used for detecting SARS-CoV-2 presence, accompanied by the monitoring level of White blood cells, Red blood cells and CRP (C-reactive Protein) check. This study aims to choose and highlight the best methodology and its efficiency in COVID-19 diagnostics. Nasopharyngeal samples were collected from 1198 patients which were analyzed after with RT-PCR. After 90 of these patients tested positive for SARS-CoV-2 virus blood samples were collected from them to perform biochemical analysis. Only 55 of them were subjected to CLIA serological assay. As a result, 49 (89.1%) patients were positive only for IgG, 4 (7.3%) patients were positive for both antibodies IgG and IgM and only 2(3.6%) patients were negative for both antibodies, based on serological results. The level of CRP varied from X- to 25.09 mg/L ad the most affected group of immunological cells were monocytes, lymphocytes, neutrophils along with red blood cells, hematocrit and hemoglobin. However, both molecular and biochemical assays had better performance 8-10 days after symptoms appearance, meanwhile, the serological assay was more predictable at least 10 days after symptoms appearance. In conclusion, the three methodologies used separately are limited in usefulness when diagnosing SARS-CoV-2 infection but a combination of them is the most effective way to diagnose this virus.

Keywords: SARS-CoV-2; Covid-19; CLIA serological assay; RT-PCR; biochemical analysis

1. Introduction

Coronaviruses are sizable, enveloped viruses characterized by linear positive-strand RNA genomes that primarily lead to respiratory infections in humans (Mizumoto et al., 2019; Hartley

et al., 2020). Since December 2019, a global outbreak of the novel coronavirus, designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has occurred, with its impact being felt in Albania since March 2020. As this virus is known for causing significant respiratory infections and it is transmitted rapidly through interpersonal contact (Price *et al.*, 2021; Dinnes *et al.*, 2020), it is crucial a prompt diagnosis to mitigate transmission and disrupt the chain of infection.

Numerous approaches exist for the diagnosis of SARS-CoV-2, often overlapping in their methodologies. Molecular techniques are employed to identify the genetic material of SARS-CoV-2, relying on the specificity of nucleotide base pairing in homologous strands (Yang & Rothman, 2004; Dinnes *et al.*, 2020). The Real-time Reverse Transcription PCR method is regarded as the gold standard for SARS-CoV-2 diagnosis due to its high sensitivity (Goudouris, 2021; Shen *et al.*, 2020). However, this method is not without its challenges; conducting swab tests for the entire population in Albania is complicated by the limited number of certified laboratories capable of viral RNA analysis and the relatively lengthy turnaround time for results. Consequently, there remains a pressing need for the development of novel and rapid diagnostic assays for COVID-19.

Serological tests for the diagnosis of COVID-19 identify the presence of IgG, IgM, and IgA antibodies in serum. These tests are instrumental in determining both current and past infections by tracking the progression of the disease (Jacofsky *et al.*, 2020). Chemiluminescent immunoassays (CLIA) are employed to detect antigen-antibody complexes, with the analytical reaction being marked by a luminescent molecule (Mohit *et al.*, 2021). In this process, serum IgA, IgM, and IgG are bound to magnetic particles that are coated with SARS-CoV-2 antigens, such as the nucleocapsid (N) or receptor-binding domain (RBD). The chemiluminescent immunoassay is an automated procedure, that facilitating the analysis of a substantial number of samples. The steps involved in serological analysis are relatively straightforward and do not require prior preparation (Machado *et al.*, 2021).

Both molecular and serological analyses exhibit limited efficacy when conducted within the initial week of infection, as the virus remains in its incubation phase. Consequently, there are insufficient viral RNA copies for detection through RT-PCR, and SARS-CoV-2 antibodies have not yet been produced (Jarvis & Kelley, 2020; Rode *et al.*, 2021). Symptoms typically manifest around five days post-exposure to the virus, marking this period as the window phase. Optimal timing for real-time RT-PCR testing occurs approximately one week after symptom onset or two weeks following exposure, during which the viral load is significantly elevated (Loeffelholz & Tang, 2020; Falzone *et al.*, 2021). For serological assays aimed at detecting antibodies, the ideal timeframe is roughly four weeks post-exposure to the virus (Falzone *et al.*, 2021).

Antigen tests, biochemical analysis and imagery techniques can be also used to diagnose and monitor the patient. Antigen analysis discovers the presence of a specific viral antigen, like S protein and N protein (Nguyen *et al.*, 2020). Various number of laboratory parameters can evaluate the acuteness of the disease, like C protein (CRP), procalcitonin (PCT) and D-dimer (Letelier *et al.*, 2021; Eastin & Eastin 2020). Numerous biochemical assays exhibit significant alterations in individuals diagnosed with COVID-19. In patients presenting with severe manifestations, there was a notable increase in levels of D-dimer, creatinine, blood urea, RBC and neutrophils, accompanied by a decrease in lymphocyte counts (Parlakpinar *et al.*, 2020). Furthermore, elevated D-dimer levels were observed in 70% of patients experiencing severe symptoms and in those who succumbed to the illness (Li *et al.*, 2020).

Among radiologic techniques, chest X-Rays (CRX) and computerized tomography (CT) are the most efficient to diagnose pneumonia caused by COVID-19 (Li & Xia 2020).

This research work aims to evaluate the molecular, serological assays, and biochemical analysis employed for the diagnosis of SARS-CoV-2, as well as to assess the significance of each diagnostic method within the context of our country.

2. Materials and Methods

The study was conducted at Genius laboratory in Tirana, during March-June 2021. 1198 nasopharyngeal samples were analyzed with RT-PCR to identify the positive cases with SARS-CoV-2. Nasopharyngeal swabs were obtained from the nasopharyngeal cavity of the patients.

The alphaPrepTM kit (Viral DNA/RNA Extraction Kit Model: VRD-B096V)) was used to extract RNA from nasopharyngeal samples, which contained lyophilized buffer proteinase K and a PCR plate with 96 wells filled previously with buffer and magnetic beads. The PCR plates were placed in the Automated Nucleic Acid Extraction System NC-15 PLUS HanwoolTPC Co., Ltd, which consists in automatically extracting RNA.

2.1. *Molecular analysis*

For real time RT-PCR analysis, GeneFinder™ Covid-19 PlusReal*Amp* Kit was used which detects the three genes of SARS-COV2-, RdRp, E and N. This kit contains a mixture of Tris HCl, MgCl2, dNTPs, reverse transcriptase enzyme and Taq DNA polymerase, and a mixture of probes and the negative and positive controls.

Table 1. Cyclation conditions of real-time RT-PCR

	Phases	Temperature	Time	Cycles
1	Reverse transcription	50 °C	20 min	1
2	Pre-denaturation	95°C	5 min	1
3	Denaturation	95°C	15 sec	45
	Bonding	58°C	60 sec	

Table 2. Fluorescent dye for target genes

	Phases	Temperature	Time	Cycles
1	Reverse transcription	50 °C	20 min	1
2	Pre-denaturation	95°C	5 min	1
3	Denaturation	95°C	15 sec	45

15 µl of the master mix prepared, RNA sample was extracted from each patient and the negative and positive control were added to each well of the PCR plate. The stripes were centrifuged in a micro-centrifuge and then placed in the Applied Biosystems® QuantStudio™ 7 Flex Real-Time PCR System thermocycler.

Real Time RT-PCR analysis was performed according to the cycling program of QuantStudio-Real Time PCR software v1.3. Cycling conditions are shown in Table 1 and the fluorescent dye for target genes is shown in Table 2. Samples were considered positive at molecular screening if all three genes were detected.

2.2. *Serological assay*

CLIA immunoassay was used as a serological technique to analyze the patient's blood serum. Blood samples were collected in CLIA Activator standard microtubes. They were centrifuged

for ten minutes at 4000 rpm and then the serum supernatant was collected. The kit used to detect IgM was MAGLUMI TM 2019-nCOV IgM (CLIA) Snibe Diagnostics. This kit has magnetic nanoparticles coated with antihuman monoclonal IgM antibodies and recombinant 2019-nCOV antibodies tagged with ABEI, an activated ester which is widely used as a chemiluminescent tag in monoclonal antibody mixtures for immunological tests. MAGLUMI SARS-CoV-2 S-RBD IgG (CLIA) Snibe Diagnostics kit was used to detect IgM, with nanoparticles conjugated with the antigen SARS-CoV-2 S-RBD and with IgG antihuman antibodies. The serologic test was performed in the automated MAGLUM 4000 plus SNIBE Diagnostics analyzer (Shenzhen New Industries Biomedical Engineering Co., Ltd, Shenzen, China). Starters 1 & 2 were also used, to generate the chemiluminescent signal for detection. The light signal is measured by RLU, which is proportional to the concentration of the sample antibodies. According to the instructions of the kit, for antibody values lower than or equal to 1Au/ml the results are positive. If IgM and IgG value is lower than 1Au/ml the result is considered negative or not detectable.

2.3. B*iochemical analysis*

Venous blood (4.5 mL) was collected and subsequently transferred into a gel tube. The tubes were permitted to remain undisturbed at room temperature for a duration of 30 minutes, after which they underwent centrifugation at 3500 rpm for 10 minutes to separate the serum. The assays for red blood cell functionality and white blood cell counts were conducted following the manufacturer's guidelines.

Levels of C-reactive protein and D-dimer among infected patients were calculated based on particle enhanced immuno-turbidimetric.

2.4. *Statistical analysis*

The data processing and the descriptive statistics were analyzed with the SPSS program. Chi-square, Mann-Whitney and Kruskal Wallis statistical tests were performed.

3. Results

A majority of the cases presented with symptoms resembling influenza, including fever, cough, and mild muscle pain, but not all of them were hospitalized. All patients fortunately were subsequently discharged safely after demonstrating a recovery of their clinical symptoms.

3.1. *RT-PCR results*

An amplification graphic with Ct values of the genes is obtained for each patient that had a real-time RT-PCR test done. Out of the 1198 samples analyzed with real-time RT-PCR, 90 (7.5%) of them were positive for SARS-CoV-2 and 1108 (92.5%) patients were negative.

Table 3. Distribution of positive cases based on gender

Real-Time RT-PCR result		
Gender	Positive frequency (%)	Negative frequency (%)
Female	47 (52.2%)	637 (57.5%)
Male	43 (47.8%)	471 (42.5%)
Total	90 (100%)	1108 (100%)

Out of 1198 patients that underwent the real-time RT-PCR test, 684 were females and 514 were males. Of 90 individuals that resulted positive, 47 (52.2%) of them were females

and 43 (47.8%) were male. It resulted that there is no significant change in the number of affected females and males (Chi-square $X^2(1)=0.943$, p=0.331).

3.2. *Serological test results*

Out of 90 patients that resulted positive from RT-PCR, 55 of them did the serologic test after two weeks. It resulted that 49 patients (89.1%) were positive only for IgG, 4(7.3%) were positive for both antibodies and only 2(3.6%) were negative for IgM and IgG.

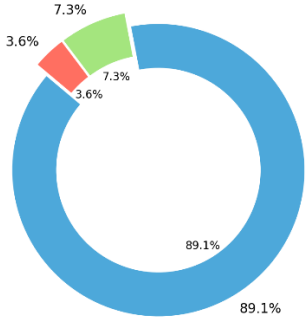

Figure 1. Presence of the patient's antibodies

Thus, four patients tested positive for IgM, meanwhile, these were not detected these antibodies for the other 51 patients.

Detection of both antibodies in this case shows that these positive patients for SARS-CoV-2 are still in the active phase of infection. Jacofsky *et al.*, 2020, noticed that when infection continues the body produces both antibodies actively. The combination of molecular and serologic assays shows that routine diagnosis for COVID-19 is very important. Molecular analysis confirms the virus's presence and the serologic test plays an important role in prognosis and disease progression (Wu *et al.*, 2020).

Also, concerning IgG, 53 patients were positive and two patients were negative for this antibody. Most of the patients who were confirmed positive from real-time RT-PCR, resulted positive only for IgG after two weeks and this result shows that they are in the late stages of infection or in the recovery period.

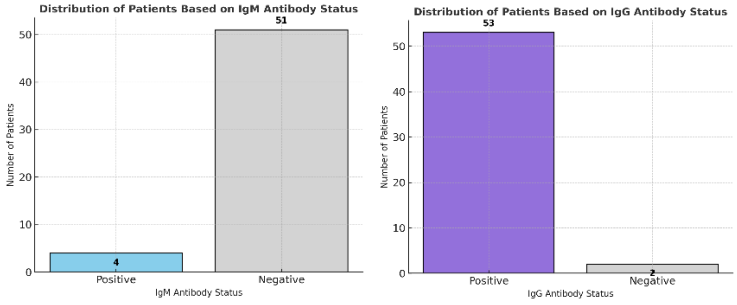

Figure 2. IgM and IgG positivity levels

Only two patients, in this study, had neither antibodies in their system. One of the reasons might be that some people produce antibodies three weeks after infection or even later. This shows that serologic tests cannot be used as a primary assay without previously verifying them

using RT-PCR because it is not a method that detects the presence of the virus right away. According to our study, one out of 16 infected people had no IgG antibodies and this is a disadvantage to serologic tests (Petersen *et al.*, 2020).

3.3. *Serological analysis according to age and gender*

The results of our study showed that out of 55 patients, 28 were female and 27 were male. Four females tested positive for IgM levels and IgG was detected in only one male and female patient. According to Hossain *et al.*, 2021 and Lai *et al.*, 2020, seroprevalence of IgG and IgM in females and males is the same, thus indicating that gender is not a decisive factor in disease progression (Hossain *et al.*, 2021; Lai *et al.*, 2020).

Average IgG levels in females resulted from 43.1643 Au/ml with a standard deviation of 32.78776 Au/ml and in males it was 47.0259 Au/ml with a standard deviation 43.660952 Au/ml. Even though in figure 3 it seems that males have a higher level of antibodies, it results that there was no significant change in the antibody levels between the two genders (Mann Whitney U=364.500, p=0.820). This result is similar to another study (Luo *et al.*, 2021). Males might have higher levels of antibodies because infected males with SARS-CoV-2 have increased inflammatory responses that might cause the body to produce more B cells, therefore they produce more antibodies (Korte *et al.*, 2020).

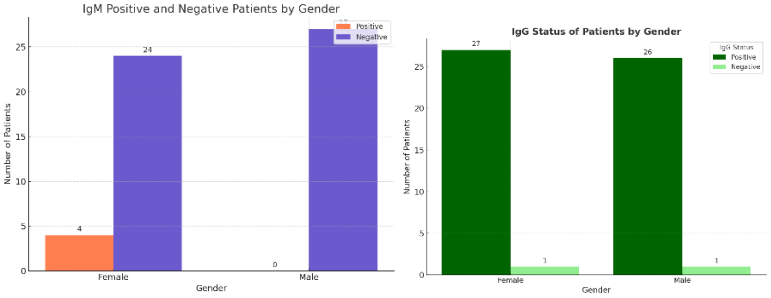

Figure 3. IgM and IgG seropositivity based on gender

The average age of 55 individuals that were submitted to the serologic test was 43 ± 16.997 years old, meanwhile, the average age of individuals that were submitted to the test was 31-35 years old. Age frequencies are shown in the graph below (figure. 4). IgM antibodies were detected in three patients who were 20-30 years old and in one 79-year-old patient. All of the patients between the age range 20-30, 31-45, 46-59 were positive for IgG. Only two patients age 77 & 87 tested negative for this antibody. Another reason is immunosuppression or age factor (CDC, 2020; Rode *et al*, 2021). The immune system is weak an old age and antibodies require more time to differentiate (Bajaj *et al.*, 2020).

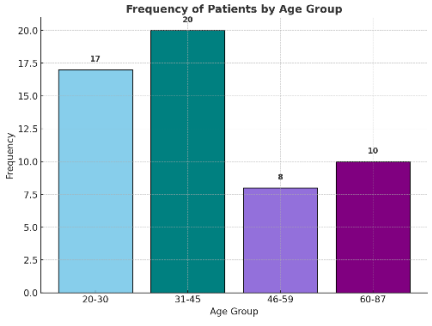

Figure 4. Age groups frequencies

IgM levels had no significant changes in different age groups (Kruskall Wallis $X(3)^2$=2.348, p=0.503). IgG levels had significant changes between age groups where the lowest level was detected in the group ages 60-87 years old (Kruskall Wallis $X^2(3)$=9.586, p=0.022). However, it should be taken into consideration that there were fewer patients in the age range 46-59 and 60-87 years old.

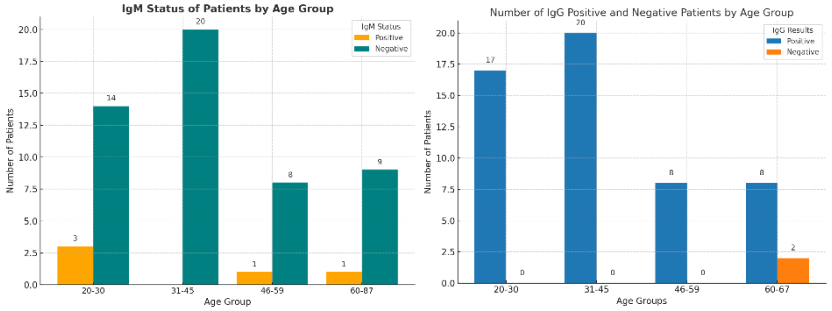

Figure 5. IgG and IgM seropositivity based on age

According to Luo *et al.*, 2021, in a study where the dynamic level change of IgG and IgM were evaluated, it resulted that during the first 30 days, antibody levels were lower in older group ages and higher in younger group ages. The high level of antibodies at young ages during the first stages of the infection might play an important role in preventing the disease from aggravating. However, in other studies, it is noticed that older ages produce higher antibody levels in the later stages of infection compared to younger ages (Klein *et al.*, 2020; Luo *et al.*, 2021). This was also noticed in our study where most of the patients who were confirmed positive with real-time RT-PCR, after two weeks had created IgG in their serum and this shows a late stage of infection or the stage of healing.

3.4. *Biochemical characteristics of patients with Covid-19*

Out of 1198 individuals in total, only 90 patients tested positive for the presence of SARS-CoV-2. All of them were subject to biochemical analysis to monitor their blood parameters as well as prevent possible complications from Covid-19. Table 4 presents a summary of the mean values of biochemical index outcomes for patients diagnosed with COVID-19.

Table 4. Biochemical characteristics of 90 positive patients with Covid-19

Complete Blood Count (CBC)		
Analysis	*Results (ascending values)*	*Reference Values*
White blood cells and WBC morphology		
White Blood Cells (WBC)*	4.5 - 11.11	5 - 10 x10^3/uL > 18 year
Neutrophils NEU%	41.1 - 69.6	50 - 62 % > 18 year
Lymphocytes LYM%	19.2 - 43.9	25 - 40 % > 18 year
Monocytes MONO%	5.5 - 15.4	3 - 7 % > 18 year
Basophils BASO%	0.2 – 0.8	< 1 % > 6 year
Eosinophils EOS%	0.6 – 16.5	< 3 % > 2 week
Neutrophils NEU#	2.01 – 7.74	2 - 7.5 x10^3/uL
Lymphocytes LYM#	1.15 – 2.24	0.5 - 4 x10^3/uL
Monocytes MONO#	0.37 – 1.13	0.2 - 0.8 x10^3/uL
Basophils BASO#	0.01 – 0.06	< 0.1 x10^3/uL

Eosinophils EOS#	0.03 – 1.04	< 0.4 x10^3/uL
Red cells and RBC line		
Red Blood Cells (RBC)*	3.81 – 5.75	4.5 - 5.5 x10^/µL > 18 year
Hematocrit (HCT)*	34 – 46.3	42 - 52 % > 18 year
Hemoglobin (HGB)*	11.2 – 17.3	14 - 17.4 g/dL > 18 year
MCV*	77.3 – 89.9	84 - 96 fL > 18 year
MCH*	23.9 – 31.4	28 - 34 pg > 18 year
MCHC*	30.9 – 36.4	32 - 36 g/dL > 6 year
Eritrosedimentation		
Eritrosedimentation (ESR)	5 – 76	< 20 mm/h > 50 year
Coagulation Control		
Analysis	***Results***	***Reference Values***
D-dimer	0.42 – 1.51	< 0.5 ug/mL
Proteins Check		
Analysis	***Results***	***Reference Values***
Proteina C-Reaktive (CRP)*	3.19 – 25.09	< 5 mg/L

The results from this study show that the main biochemical indexes affected by SARS-CoV-2 were white blood cells (WBC), red blood cells and erythrocyte sedimentation rate (ESR)????. Among white blood cells, not all parameters had significant changes, but the most significant were the WBC number which varied from 4.5-11.11x10^3/ul, neutrophils from 41.1-69.6%, lymphocytes with a decreased level ranging from 19.2-43.9% and increase in monocytes number 5.5-15.4%. Among the red blood cells line, the RBC number showed a decrease in their number which ranged from 3.81-5.75 /ul, a decrease in hematocrit and haemoglobin levels which varied from 34-46.3 % and 11.2-17.3 g/dl respectively. Eritrosedimentation was elevated in almost all patients from 5-76 mm/h. Meanwhile, the blood analysis panel was associated with two of the most important parameters, the coagulation control, D-dimer and C-reactive protein.

As it was mainly expected for the patients who tested positive for SARS-CoV-2 virus, C-reactive protein was elevated in 95% of the cases, ranging from 3.19 – 25.09 mg/L. The 5% cases which had lower values of CrP were probably analyzed in the beginning of symptoms because their complete blood count was as well within ranges of normal.

COVID-19 patients exhibit D-dimer levels upon admission that are, on average, double those observed in individuals with community-acquired pneumonia (Yu *et al.*, 2020). Even in our study, there is an increase of D-dimer levels which varies from 0.42 – 1.51 ug/ml. Although the patients presented to the laboratory for testing did not have symptoms of acquired pneumonia yet, none of them had performed radiological techniques to prove the blood results.

4. Discussion

This study highlights the importance of combining various methodologies to detect and prevent an infectious disease such as Covid-19. Molecular methods are the golden standard for diagnosing SARS-CoV-2 because they are very specific and detect the genetic material of the virus directly in its active phase of infection (Goudouris, 2021).

Real-time RT-PCR analysis is capable of identifying infection only in the active phase of SARS-CoV-2, meanwhile, serologic tests are capable of showing an infection which has already passed (Krajewski *et al.*, 2020). The viral load can be under the detection threshold, which is why this analysis can produce false negative results (La Marca *et al.*, 2020). The best time to obtain samples for analysis is approximately one week after symptoms start or two weeks after first contact with the virus because at this period the viral load is very high in the upper respiratory tract (Loeffelholz &Tang, 2020; Falzone *et al.*, 2021). During this period

false negative results are minimal. After the viral charge has reached its peak, it starts to reduce and symptoms begin to relieve as well. Still, during this period, the probability of a false negative result increases. (Jarvis & Kelley, 2020).

Quantitative analysis for IgM and IgG plays an important role in the diagnosis, evaluation and prognosis of COVID-19 (Hou *et al.*, 2020). According to our study, one out of 16 infected people had no IgG antibodies and this is a disadvantage to serologic tests (Petersen *et al.*, 2020). The serologic assay is more valuable as a complementary test to RT-PCR, which is why it is important in diagnostics (Zhao *et al.*, 2020). According to Milani *et al.*, 2020 individuals with aggravated symptoms of the virus, develop a stronger immune response and both antibodies are at high concentration in these patients. Ghaffari *et al.*, 2021 state that knowing the dynamic of the immune response is crucial to formulate diagnosing and treatment strategies. IgM studies are important because it is the first class of immunoglobulins that are produced when our organism is exposed to a pathogen. IgM stability is much lower than that of IgG in the serum (Qu *et al.*, 2020). In comparison to IgM, IgG is an antibody which is very specific in neutralizing pathogens, which is why its study is very important. Usually, IgG is produced in the later stages of an infection and it plays an important role in creating long-term immune memory, even though it is not known yet the stability of these antibodies after the infection with SARS-CoV-2 (Nguyen *et al.*, 2020; Winter & Hegde, 2020). Gluck *et al.*, 2020 noticed in their study that IgG was detectable in 90% of individuals, 30 weeks after the appearance of symptoms and IgG levels remained relatively stable for at least six months. Quantitative detection of IgG has a great impact on research to understand if the created antibodies protect us from future infections and how long will this immunity last (Dan *et al.*, 2021).

Combining molecular analysis with serologic tests is very important in routine diagnosis of COVID-19. Molecular analysis confirms the virus's presence and the serologic test plays an important part in evaluating the progression and prognosis of the infection (Wu *et al.*, 2020). Two patients, in our study, had not created any antibodies for SARS-CoV-2. This indicates that serologic tests cannot be used alone without real-time RT-PCR confirmation because it is not a diagnostic method that detects the presence of the virus. Furthermore, Peterson *et al.*, 2020 noticed in their study, that in 1 out of 16 people infected, IgG antibodies were not present and this is a disadvantage for serologic tests. The serologic test is very valuable as a complementary analysis and a great help for real-time RT-PCR, which is why routine application of the serologic test in diagnosis and clinical management is important (Zhao *et al.*, 2020). In this study, eventhough there is a small number of samples, all of the patients who did the serologic test resulted positive for SARS-Cov-2. Thus, these results emphasize the importance of serologic tests for epidemiologic studies because they limit the spreading of SARS-CoV-2, evaluating the real prevalence of the infection in the entire population. Real-time RT-PCR gives false negative results for patients that are in the convalescence period and for asymptomatic patients that are characterized by a low viral charge (Borges *et al.*, 2020).

Molecular analysis and serological tests are associated with biochemical analysis in order to a better monitoring of Covid 19 patients in Albania. C-reactive protein and D-dimer levels were evaluated among other biochemical indicators. As expected in SARS-CoV-2 positive patients, CRP levels were elevated even in this study, meanwhile none of the patients whose tested positive for Covid-19 in our study did not have an exceeding level above 2.0 g/ml.

C-reactive protein (CRP) is a plasma protein produced by the liver, with its synthesis stimulated by various inflammatory mediators, including interleukin-6 (IL-6). Although it is a non-specific marker, CRP is utilized in clinical settings as a biomarker for a range of inflammatory conditions, and elevated levels of this protein correlate with increased disease severity (Li *et al.*,2020). The plasma levels of these proteins, specifically albumin and C-reactive protein

(CRP), are altered by a minimum of 25% during the acute phase in reaction to specific cytokines generated during various inflammatory processes that involve some extent of tissue injury (Zhoe et al., 2020) (Mehta *et al.*, 2020).

Research conducted by Hayıroğlu *et al.*, 2020 has indicated that fluctuations in D-dimer levels from baseline at admission correlate with clinical outcomes in patients diagnosed with COVID-19. As a surrogate marker for thrombotic burden, D-dimer measurements may serve as a valuable tool in informing treatment strategies. Findings from Zhang *et al.*, 2020 suggest that an admission D-dimer level exceeding 2.0 g/mL is linked to a heightened risk of mortality.

The evidence gathered from this study indicates that numerous biochemical markers are modified in patients with COVID-19, and these alterations have been linked to the severity of the illness, as well as, in certain instances, to patient prognosis. By integrating laboratory parameters with additional clinical information, such as molecular analysis RT-PCR and serological assays as next-phase monitoring, it may be feasible to classify patients in the early phases of the disease.

It must be emphasized that the study does not include patients with complications, diabetic or coronary and heart disease patients. Other biochemical markers have been used for these patients and scientific data will be published soon.

5. Conclusions

Based on the result of this study, females and males are equally affected by SARS-CoV-2 and ages 60-87 have the highest rate of infection. Levels of IgM and IgG have no significant change from females to males and IgG levels are lower at ages 60-87 years old. The most affected markers from biochemical panel analysis, were white blood cell numbers, monocytes, neutrophils, and lymphocytes number following decrease in red blood cell number, hematocrit and hemoglobin levels. The level of D-dimer was elevated in most of the patients as well as an obvious increase in the level of C-reactive protein.

RT-PCR method is effective in diagnosing the early stage SARS-CoV-2 in its active phase of infection and it cannot be replaced by serologic tests. Meanwhile, CLIA is an indirect method that detects IgG and IgM. Serologic tests cannot be used to confirm SARS-CoV-2. Thus, the application of serologic methods is clinically important in helping RT-PCR. Nonetheless, molecular and serological techniques need support from biochemical indicators to monitor and treat the disease. These tests help monitor patients that have resulted positive from RT-PCR, to see if patients are healed or if they are in the active phase of infection.

This categorization of clinical assays could facilitate the identification of individuals at risk of developing critical conditions, thereby enhancing their clinical management and enabling the pursuit of appropriate therapeutic interventions.

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Conflict of interests

The authors affirm that they have no competing interests to disclose.

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