Microbiology - Infectious diseases including COVID 19

T182

USEFULNESS OF A D-DIMER CUT-OFF TO ESTIMATE THE HIGH RISK OF PULMONARY THROMBOEMBOLISM IN COVID-19 PATIENTS

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BACKGROUND-AIM

Pneumonia caused by SARS-CoV-2 (COVID-19) is associated with inflammatory hyper-response and alterations in the coagulation profile. D-dimer elevation is frequent and values above 1 μg/mL have been associated with increased severity and in-hospital mortality. Previous retrospective studies have shown a high prevalence of pulmonary thromboembolism (PTE) in patients with COVID-19, although in these studies diagnostic tests were performed only when there was clinical suspicion of PTE.

METHODS

A prospective study was performed between April 6th and 17th 2020, in a tertiary hospital. All confirmed cases of COVID-19 pneumonia with D-dimer > 1 μg/mL (ACL TOP 700, Instrumentation Laboratory, US; DDU units with a HemosIL© HS reagent) had a computed tomography angiography (AngioTAC) to investigate the presence of PTE. The sociodemographic and clinical characteristics, laboratory data and radiological results obtained by AngioTAC (pulmonary arterial occlusion index) were analyzed and compared between the PTE group and the non-PTE group, a statistical significance of 5% was considered.

RESULTS

Thirty consecutive patients (11 women) were included. PTE was diagnosed in 15 patients (50%). In patients with PTE, thrombus was located mainly in segmental arteries (86%) and bilaterally (60%). Although patients with PTE were significantly older median [interquartile range, IQR] of 67 [63-73] vs. 57 [48-69] years, $p=0.048$), they showed no sociodemographic differences or risk factors for thromboembolic disease. D-dimer, platelets, and C-reactive protein were significantly higher in the PTE group. A positive correlation was detected between D-dimer levels and the magnitude of arterial occlusion of PTE ($r=0.795$; $p<0.0001$).

CONCLUSIONS

Patients with COVID-19 pneumonia and D-dimer levels > 1 μg/mL had a high prevalence of PTE, regardless of clinical suspicion. These findings could contribute to improve the prognosis of patients with COVID-19 pneumonia by anticipating the initiation of anticoagulant treatment once the PTE is confirmed and before the appearance of compatible clinical symptoms.

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USEFULNESS OF ANGIOTENSIN-CONVERTING ENZYME LEVELS FOR PREDICTING PULMONARY THROMBOEMBOLISM IN COVID-19 PATIENTS

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BACKGROUND-AIM

Decreased angiotensin-converting enzyme (ACE) expression and ACE-2 overexpression have been demonstrated in bronchoalveolar cells of COVID-19 patients, leading to increased angiotensin production(1-9). Angiotensin has a sensitizing effect on bradykinin receptors, a peptide with vasodilator effect that is degraded by ACE. SARS-CoV-2 infection would trigger a bradykinin storm with a local vascular effect in the lung, which could be the cause of thrombotic phenomena associated with COVID-19. The aim of this study was to determine the possible predictive role of ACE levels in patients with COVID-19 pneumonia for risk of developing pulmonary thromboembolism (PTE).

METHODS

A prospective study performed in April 2020 in a tertiary hospital. Hospitalized patients with COVID-19 pneumonia and a D-Dimer value >0.250 µg/mL (ACL TOP 700,Instrumentation Laboratory,US; DDU; HemosIL© HS) were consecutively included. Serum levels of ACE, hs-Troponin I and NT-proBNP were measured using the Architect ci16200 (Abbott,US). Computed tomography with pulmonary angiography was performed to investigate the presence of PTE. Epidemiological and clinical variables, laboratory data and pulmonary artery occlusion index were analyzed comparing the PTE and non-PTE groups. A statistical significance of 5% was considered.

RESULTS

Thirty patients (37% women) with a median age of 64 years (p25-p75: 48-71) were included. PTE was diagnosed in 8 patients (27%). No differences by age or sex were found between groups. Elevated levels of ultrasensitive Troponin I, D-dimer and NT-proBNP were found in patients with PTE [12.5 vs 3.1 ng/L; 2.43 vs 1.35 µg/mL; 568 vs 114 pg/mL (p=0.018), respectively]. A positive correlation was detected between PTE and D-Dimer (r=0.795; p<0.0001) and negative with ACE (r=-0.339; p=0.011). Lower ACE levels were found in the PTE group (26.3 [21.9-31.0] vs. 31.5 [26.8-58.1] U/L; p=0.032). For an ACE cutoff point < 26 U/L (Chi-square 6.355) an OR=10.20[95%CI:1.55-67.22] (p=0.0158) was obtained for PTE.

CONCLUSIONS

In hospitalized patients with COVID-19 pneumonia, D-Dimer levels above 2 µg/mL have a high prevalence of PTE, regardless of clinical suspicion. ACE levels below 26 U/L may indicate increased risk of PTE, and their determination may improve the stratification and prognosis of these patients.

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ROLE OF CALPROTECTIN IN PREDICTION OF SEVERE RESPIRATORY FAILURE IN COVID-19 PATIENTS: A MULTICENTER PROSPECTIVE STUDY IN SPAIN (CALCOV STUDY)

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BACKGROUND-AIM

Severe COVID-19 is characterized by a dysregulated immune response in which neutrophils and macrophages play a critical role. Early detection of the phagocyte activation can be used to identify COVID-19 patients at high risk for progression to severe disease, including respiratory failure. Calprotectin reflects neutrophil activation and is involved in the self-amplifying thrombo-inflammatory storm in severe COVID-19. The aim of the study was to evaluate the role of serum calprotectin in early prediction of severe respiratory failure in COVID-19 patients.

METHODS

Design: multicenter prospective observational study enrolling consecutive adult COVID-19 patients admitted to four Spanish hospitals. Upon arrival to ED, blood samples were collected for laboratory tests, including serum calprotectin, which was measured by a particle enhanced turbidimetric immunoassay (PETIA) (Gentian AS, Norway) on a Cobas c501 analyzer (Roche Diagnostics).
Outcome: the primary outcome of the study was severe respiratory failure requiring invasive mechanical ventilation (IMV).

RESULTS

Study population included 395 patients, 57 (14.4%) requiring IMV. In these patients, median serum calprotectin levels were significantly higher (3.73 mg/L (IQR: 2.91-8.10) vs. 2.63 mg/L (IQR: 1.70-4.07); $p<0.001$). ROC AUC of calprotectin to predict the need for IMV was 0.723 (95% CI: 0.652-0.790); optimal cutoff, according to Youden Index was 2.98 mg/L (sensitivity: 73.7%, specificity: 60.4). As criteria to rule-out the endpoint, a cut-off point of 2.23 mg/L, with a sensitivity of 86.0 % and negative predictive value of 94.7%, was suggested. In multivariate analysis, serum calprotectin was an independent predictor of IMV (OR: 1.161; 95% CI: 1.018-1.324; $p = 0.026$).

CONCLUSIONS

Our data show that serum calprotectin level serve as a valuable predictor of patients in need of invasive ventilatory support. An early identification of patients at risk of severe respiratory failure, requiring critical care management is of great importance. The assessment of calprotectin, in combination with other biomarkers and clinical data should be used for optimal triage and care of COVID-19 patients.

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RISK FACTORS FOR SEVERITY OF SARS-COV-2 INFECTION

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BACKGROUND-AIM

SARS-CoV-2 is the coronavirus responsible for the COVID-19 disease that can cause a severe condition characterized by bilateral pneumonia and severe acute respiratory syndrome.

METHODS

Descriptive and retrospective study carried out at Virgen de Valme University Hospital (Seville, Spain) that included hospitalized patients with SARS-CoV-2 infection. The 197 patients included were classified into two groups: 127 mild patients and 70 severe patients (admission to intensive care unit and/or death). A multivariate analysis was performed using logistic regression with the SPSS Statistics 25.0 program to identify risk factors for severity of SARS-CoV-2 infection.

RESULTS

The parameters at the time of hospital admission with the greatest association with the severity of SARS-CoV-2 infection are: age ≥ 70 years (OR: 2.277; p = .044), leukocytes ≥ 9.5 x 109/L (OR: 6.577; p < .001), CRP ≥ 90 mg/L (OR: 2.277; p = 0.008), GGT ≥ 30 U/L (OR: 2.440; p = 0.36), and PCT ≥ 0.5 ng/mL (OR: 11.590; p < .001).
The AUC obtained by the ROC curve was: age (0.642), leukocytes (0.708), CRP (0.708), GGT (0.632), and PCT (0.844). The cut-off points with the highest Youden index were: age ≥ 67 years (sensitivity 75.7% and specificity 52.0%), leukocytes ≥ 9.44 x 109/L (sensitivity 47.1% and specificity 91.3%), CRP ≥ 90.5 mg/L (sensitivity 67.1% and specificity 70.1%), GGT ≥ 28 U/L (sensitivity 75.7% and specificity 47.2%), and PCT ≥ 0.19 ng/mL (sensitivity 70.0% and specificity 87.4%).

CONCLUSIONS

This study has identified risk factors for the progression of SARS-CoV-2 infection in hospitalized patients. Specifically, advanced age (≥ 70 years), increased levels of leukocytes (≥ 9.5 x 109/L), and high concentrations of CRP (≥ 90 mg/L), GGT (≥ 30 U/L), and PCT (≥ 0.5 ng/mL) at the time of hospital admission are associated with a greater probability of presenting a severe form of COVID-19.
These laboratory parameters are frequently used in clinical practice and they are available in all hospitals, allowing quick and easy analytical results in automated analyzers.
Definitely, these prognostic factors can be used to predict the course of COVID-19 in the initial stages, allowing the adaptation of health care to the prognostic estimate of the disease.

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STRUVITE CRYSTALS IN THE URINE AND THEIR RELATIONSHIP WITH PROTEUS MIRABILIS

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BACKGROUND-AIM

Ureolytic germs, especially Proteus mirabilis, are among the main etiological agents of urinary tract infection (UTI). These germs release large amounts of urease that hydrolyzes urea and alkalizes urine, helping ions to precipitate and form struvite (magnesium ammonium phosphate) crystals.

METHODS

A descriptive and retrospective study was carried out for one year (January - December 2020). All urine from patients with clinical suspicion of UTI and with a joint request for urinalysis and urine culture were analyzed. The data obtained were statistically analyzed using the MedCalc v.18.9 program.

RESULTS

The study included 15,748 urine samples, of which 2,948 (18.7%) had a positive culture. Of these, in 3.2% (93/2 948) more than 100,000 CFU/mL of Proteus mirabilis were isolated. Of the positive cultures for this uropathogen, struvite crystals were observed in 32.3% (30/93).

Analyzing all the samples that presented struvite crystals, 53.6% (30/56) were urine with Proteus mirabilis, in 19.6% (11/56) other uropathogenic bacteria were isolated and 26.8% (15/56) remaining presented negative or mixed culture.

Regarding the biochemical parameters of the urine with Proteus mirabilis, 27/30 (90.0%) had a pH ≥ 8 and 25/30 (83.3%) a concentration greater than or equal to 500 leukocytes per field.

The sensitivity and specificity of the presence of struvite crystals in the urinary sediment for the diagnosis of a UTI due to Proteus mirabilis is 32.26% and 99.84% respectively, with a positive predictive value of 95.7% and a negative predictive of 93.0%.

CONCLUSIONS

Proteus mirabilis is the ureolytic bacteria most frequently associated with the formation of abundant struvite crystals in urine. The biochemical parameters most frequently related to the presence of this bacterium in urine are alkaline pH and the presence of abundant leukocytes.

The presence of struvite crystals makes it possible to guide the diagnosis of UTI due to Proteus mirabilis, before the urine culture, to select the most appropriate empirical antibiotic treatment, avoiding possible therapeutic failure and the appearance of serious complications in patients.

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IDENTIFICATION OF VOLATILE BIOMARKERS OF INFECTION BY LISTERIA MONOCYTOGENES

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BACKGROUND-AIM

Listeria monocytogenes (LM) is a facultative anaerobic bacterium that causes listeriosis. Volatile organic compounds (VOCs) have been evaluated in recent years as diagnostic compounds for a multitude of diseases.

METHODS

7 strains of LM and two isolates of Staphylococcus aureus (SA) and Enterococcus faecalis (EF) were analyzed. A 0.5 McFarland standard was prepared in 10 ml of saline. 100 µL of this standard was added to 5 mL of thioglycollate broth and incubated at 37°C for 48 hours. A liquid-liquid extraction was carried out with 5 mL of dichloromethane and 31 VOCs were analyzed using a gas chromatograph coupled to a mass spectrometer.

RESULTS

The main VOCs that made it possible to differentiate LM from the other bacteria analyzed were: butanol, 3-methyl-1-butanol, 2,3-butanediol, acetic, isobutyric, butanoic, and isovaleric acids, mercaptoacetone, 3-(methylthio)-1-propanol, pyrazine, and acetoin.

CONCLUSIONS

The bacteria catabolize glucose through the glycolytic pathway to pyruvate and later, through the acid-mixed fermentation pathway, they generate acetic acid and acetoin, which is produced more prominently in SA. Acetoin, due to the action of acetoin reductase, gives rise to 2,3-butanediol which is only detected in SA, so it seems to indicate that LM does not have this enzyme in its metabolism.

Volatile isoacids are primary products of LM growth and, especially, isovaleric acid is the one that best allows us to differentiate this bacterium. Short-chain fatty acids, such as acetic and butanoic acid, are associated with anaerobic metabolism.

LM requires supplemental sulfur amino acids to grow and the thioglycollate medium used contains L-cystine, which is why the sulfur aromatic compounds detected are consumed.

Linear sulfur compounds, such as 3-(methylthio)-1-propanol, are associated with desulfurization reactions of sodium thioglycolate by the action of listeriolysin O present in LM. Furthermore, mercaptoacetone, which is detected in the thioglycolate medium, is consumed by SA and EF, but produced by LM.

In short, the VOCs that allow better differentiation of LM and, therefore, can be proposed as biomarkers of infection are butanol, isovaleric acid, mercaptoacetone, and 3-(methylthio)-1-propanol.

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PREDICTORS OF DEATH IN PATIENTS WITH COVID-19 ADMITTED TO THE ICU

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BACKGROUND-AIM

SARS-CoV-2 is the betacoronavirus responsible for the infectious disease COVID-19. A high percentage of patients require admission to the Intensive Care Unit (ICU) due to the rapid progression to a critical condition.

METHODS

A descriptive study including patients with SARS-CoV-2 infection admitted to the ICU between August 2020 and January 2021. The patients were classified as recovered and deceased based on the outcome of their stay in ICU. The data were processed with the SPSS Statistics 25.0 program and multivariate analysis was performed using logistic regression.

RESULTS

Eighty-two patients (73.2% men and 26.8% women) were included, of which 34.1% recovered while 65.9% died. There are statistically significant differences between the two groups in age, hematological parameters (leukocytes, neutrophils, and red blood cells), and biochemical parameters (urea and potassium).
Among the interventions carried out in the ICU stand out orotracheal intubation, performed in 86.6% of patients, the administration of vasoactive amines (81.7%), and placement in the prone position (79.3%). During their admission to the ICU, 36.6% of the patients developed bacteremia or fungemia, while 52.4% developed pneumonia associated with mechanical ventilation.
In the multivariate analysis, the parameters at the time of admission to the ICU with the greatest association with the death of the patients were an age greater than 63 years ($p = 0.012$), neutrophils > 10.2x109/L ($p = 0.026$), urea > 7.2 mmol/L ($p = 0.037$), and red blood cells ≤ 4.7x1012/L ($p = 0.058$).

CONCLUSIONS

Patients admitted to the ICU require respiratory support measures due to the great advance of the SARS-CoV-2 infection and in a large percentage of cases, they died.
This study has identified predictors of mortality in patients admitted to the ICU as a consequence of SARS-CoV-2 infection. Specifically, advanced age, increased levels of neutrophils, an elevated urea concentration, and decreased levels of red blood cells at the time of admission to the ICU are associated with a higher probability of death in these patients.
These markers can be used to predict the course of COVID-19 in critical stages, allowing clinicians to anticipate the possible outcome of the disease.

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T189

VITAMIN D DEFICIENCY AND COVID-19 OUTCOME. RESEARCH IN PATIENTS FROM A SPANISH MEDITERRANEAN REGION

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BACKGROUND-AIM

SARS-CoV-2 infection represents a challenge for world's health systems, for which the identification of modifiable factors could help improve outcomes. Ecological studies have reported inverse correlations between vitamin D status and COVID-19 incidence and mortality in European countries. On the other hand, several prevalence studies have shown that a large part of the Spanish population has a vitamin D deficiency. We hypothesized the existence of a vitamin D deficiency in patients admitted for COVID-19, which could be associated with the severity and evolution of this illness.

METHODS

A retrospective observational study, including 404 patients attended from August 2020 to January 2021 at a tertiary hospital in the southeast Spanish on the Mediterranean coast. All patients had a positive PCR test for SARS-CoV-2. The included patients had a measurement of blood level vitamin D values on admission. For quantitative determination of the total serum levels of 25-hydroxy vitamin D (D2+D3), was used the LIAISON® 25 OH Vitamin D Assay. According Vitamin D levels, patients were classified as deficiency (≤20 ng/dl), insufficiency (21-29 ng/dl) or sufficiency (≥30 ng/dl).

RESULTS

We observed that 48.1% of the patients had insufficiency of vitamin D, and 24% had deficiency. Additionally, vitamin D levels appeared significantly lower among patients whose clinical outcome was death and in those with diabetes mellitus (DM). When calculating the Odds Ratio (OR), we found that OR = 2.72 (95% CI:1.33 - 5.59, z=2.746, p=0.006) for the risk of having a clinical outcome of death when presenting vitamin D values less than 30 ng/dl. In our study we also found significantly lower levels of vitamin D in diabetic patients. If we jointly consider having DM and having serum vitamin D deficiency, the calculated OR amounts to OR = 2.933 (95% CI:1.21 - 7.07, z=2.395, p=0.017). These results would mean considering vitamin D deficiency as a risk factor, especially in diabetics patients, and a screening of this vitamin could be considered in these patients, with a view to supplementation.

CONCLUSIONS

These results could suggest a possible role for hypovitaminosis D as a risk factor for the disease or its clinical development, especially in diabetic patients.

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COMPARISON OF NASAL CAVITY AND NASOPHARYNGEAL SWAB TO DETECT SARS-COV-2 WITH A RAPID ANTIGEN TEST

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BACKGROUND-AIM

Rapid antigen test (RAT) is supplementary to real-time PCR (RT-PCR) for diagnosis of COVID-19 and has the advantage of short turn-around time and low cost. Although nasopharyngeal swab is recommended to collect the specimen, it causes pain and discomfort, whereas nasal cavity is easier to collect the sample. We compared the performance of RAT between nasal cavity and nasopharyngeal specimen.

METHODS

COVID-19 confirmed 74 positive patients and 100 negative patients were enrolled for this study. Cycle threshold (Ct) value less than 30 and confirmation duration less than 7 days were considered as a recruiting criteria for COVID-19 positive cases. Nasal cavity specimen was collected after blowing the nose. Nasal cavity and nasopharyneal swab samples were used for RAT. The other nasopharyngeal swab was used for RT-PCR. Sensitivity and correlation between two sites were compared in regards with Ct value, days after symptom onset (DSO).

RESULTS

Sensitivity of STANDARD Q COVID-19 Ag test (Q Ag, SD Biosensor, Korea), based on immunochromatographic method was 77.5% for nasal cavity and 81.7% for nasopharyngeal swab specimens, respectively ($P=0.375$). Sensitivity was higher than 82.4% within 4 DSO. Mean Ct values of E gene were 18.4 and 27.5 for Q Ag positive and −negative, respectively for nasal cavity ($P<0.0001$). Kappa value was 0.784 between nasal cavity and nasopharyngeal specimens indicating a good agreement.

CONCLUSIONS

Q Ag revealed a good performance compared to RT-PCR, especially within 4 DSO and viral load seems a key element deciding the sensitivity of RAT. As there was no significant difference of sensitivity between nasal cavity and nasopharyngeal swab, nasal cavity should be considered as a primary sampling site of RAT for diagnosis of COVID-19.

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PERFORMANCE OF STANDARD Q COVID-19 AG HOME TEST TO DETECT SARS-COV-2 WITHIN FIVE DAYS OF DISEASE ONSET

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BACKGROUND-AIM

During the wide prevalence of COVID-19, the medical professionals often suffer through the lack of personnel due to the excessive number of impending tests for the disease. For handling such a situation, Rapid antigen home test (RAHT) is particularly useful because it can replace the work of labor force, supplementing the shortage of medical personnel. Contrasting to the rapid antigen test (RAT), RAHT does not require personal precaution equipment of medical personnel or visit to the screening center. Therefore, RAHT is handy, easy to use, and cheaper than real-time polymerase chain reaction (RT-PCR). Here we compared the performance of RAHT compared to RT-PCR to identify RAHT's diagnostic efficacy.

METHODS

COVID-19 confirmed 79 positive patients and 217 negative patients were enrolled for this study. Confirmation duration less than 5 days were considered as recruiting criteria for COVID-19 positive cases. Nasal cavity specimen was collected and tested for STANDARD Q COVID-19 Ag Home Test (SD Biosensor, Korea) by each participant. Nasopharyneal swab sample was collected for RT-PCR using STANDARD M nCoV Real-Time Detection kit, SD Biosensor. The sensitivity of RAHT was evaluated per each group stratified by the presence of a symptom onset, the duration between a symptom onset, a confirmation date, and the Ct values of RT-PCR. Comparison of RAHT sensitivity among the stratified groups were estimated based on Fisher's exact test.

RESULTS

Sensitivity of STANDARD Q COVID-19 Ag Home Test (SD Biosensor), compared to RT-PCR was 94.94% (75/79) (95% CI, 87.54%-98.60%) and specificity was 100%. Sensitivity was significantly higher in the symptomatic (98.00%) than asymptomatic (83.87%) patients ($p = 0.03$), whereas there was no difference in sensitivity between 0-2 days (17/17, 100%) and 3-5 days (32/33, 96.97%) after the symptom onset ($p = 1.00$). RAHT detected all 51 patients whose Ct values were ≤20, whereas 92% (23/25) for Ct values of 20-30. When Ct values were >30, it detected one out of three (33.33%).

CONCLUSIONS

RAHT had an outstanding sensitivity result for the COVID-19 confirmed cases, particularly for those who had symptoms. There was a hierarchical decrease in sensitivity respective to its Ct values. This finding indicates that RAHT is useful for screening the disease during the early phase of disease onset when the viral load is high and the disease is contagious.

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CEREBROSPINAL FLUID MULTIDISCIPLINARY MANAGEMENT IN MENINGOENCEPHALITIS. A CRYPTOCOCCOSIS CASE REPORT

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BACKGROUND-AIM

Cryptococcal (neoformans and gattii) meningoencephalitis is the most common condition in AIDS patients, especially in those with CD4 lymphocyte counts below 100/uL. In the days before antiretroviral therapy (ART), 10% of HIV patients debuted with neurological symptoms and between 30 and 50% developed neurological complications throughout the disease.

Cerebrospinal fluid (CSF) analysis by a multidisciplinary team is a key point for diagnosis due to its nonspecific clinic. The study involves biochemistry laboratory (cell count and glucose and proteins measurement), and microbiology laboratory (stain, capsular antigen detection, culture and nucleic acid amplification techniques)

METHODS

A CSF was received in the emergency laboratory from a 54-year-old uruguayan male with confusional state to establish differential diagnosis (encephalitis, carcinomatosis or metastasis)

Glucose (2 mg/dL), proteins (278 mg/dL; normal values (NV) 14-45 mg/dL) and adenosine deaminase (35 U/L; NV: <35U/L) were measured. Cell count was performed in a Fuchs-Rosenthal chamber (objective 40x) (340 red blood cells/Ul, 38 leukocytes/uL, 100% mononuclear) observing surprisingly some non-cellular rounded structures with an intense thick edge when Turk stain (methylene blue and acetic acid) was added.

The case was discussed with microbiology, who performed urgent gram and India ink stain, observing encapsulated structures suggestives of cryptococcus spp.

RESULTS

A capsular antigen detection test by agglutination and multiplex PCR (Biofire TORCH Biomerieux®) were performed, confirming the diagnosis of cryptococcus neoformans meningoencephalitis. In addition, the PCR was positive for varicella - zoster.

HIV study was expanded on suspicion, obtaining positive serology (total HIV antibodies +), viral load (258244 HIV copies/mL) and lymphocyte count (absolute CD4 lymphocytes 31/uL (NV: 723-1124/uL)) and hepatitis C serology (total HVC antibodies +).

Antifungal and antiviral treatment (liposomal Amphotericin B, Flucytosine and Acyclovir) was prescribed.

CONCLUSIONS

It is essential to have multidisciplinary management protocols for biological fluids, to accelerate the diagnosis of infectious diseases, improving the patient´s approach, especially on suspected meningoencephalitis.

It must be considered that COVID19 pandemic has seriously affected HIV prevention and early diagnosis programs, re-detecting some forgotten pathogens nowadays such as cryptococcus spp.

We are undoubtedly witnessing a new era in which molecular techniques for rapid diagnosis (just a few hours) will provide speed and accuracy. We must integrate them in our daily work.

Microbiology - Infectious diseases including COVID 19

T193

CLINICAL LABORATORY DATA FUSION WITH VITAL SIGNS ALLOWS A RAPID PREDICTION OF SEVERE COVID-19 DISEASE USING AN EASY APPLICABLE NOMOGRAM

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BACKGROUND-AIM

In order to adequately provide patient care and optimally manage the medical resources, prediction models for COVID-19 are needed to support medical decisions.

METHODS

COVID-19 patients admitted to our hospital during the first wave of SARS-COV-2 infection were stratified in two groups according to the severity of the disease. Severe patients were considered those who fit the criteria of admission in the intensive care unit or death. Retrospectively, we investigated the clinical and analytical parameters and developed and internally validated a multivariable logistic regression model and a nomogram in order to predict mortality or ICU (intensive care unit) admission. All analyses were performed with SPSS and R.

RESULTS

285 patients with confirmed COVID-19 infection were classified into severe (n=71) and non-severe (n=214) groups. Most laboratory data presented statistical differences between the two groups. In the multivariate analysis, low level of lymphocytes (HR: 0.05(95% CI: 0.008-0.297)) and increased LDH (HR: 1.007(95% CI: 1.002-1.013)) were predictors of poor outcome as same as increased respiratory rate (HR: 1.144(95% CI: 1.022-1.281)) and decreased O2 Saturation (HR: 0.777(95% CI: 0.623-0.969)) as vital signs. This final multivariate model showed good discriminatory power for severe COVID-19 disease (ROC curve AUC= 0.90 (95% CI: 0.85-0.94) and the developed nomogram allowed its easy use.

CONCLUSIONS

The nomogram performed with the combination between clinical variables and analytical data can be used to predict the severity of COVID-19. According to our study, the risk prediction model expressed as a nomogram shows the relevance of respiratory rate, O2-saturation, LDH and Lymphocytes in COVID-19 risk of fatal outcome or ICU admission.

Microbiology - Infectious diseases including COVID 19

T194

LEUCONOSTOC MESENTEROIDES: ¿ABSCESS AND SEPSIS? OR ¿SEPSIS AND ABSCESS?

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BACKGROUND-AIM

Leuconostoc spp. are Gram-positive coccobacilli intrinsically resistant to glycopeptides. It is considered an opportunistic pathogen in immunocompromised patients, intravascular device carriers and those on parenteral nutrition. Sporadic cases of bacteremia, (particularly catheter-associated bacteremia), endocarditis, meningitis and other infections (urinary, abdominal) have been reported.

METHODS

A 69-year-old male was admitted to the intensive care unit (ICU) as a result of septic shock secondary to acute pancreatitis and choleperitoneum secondary to endoscopic retrograde cholangiopancreatography (ERCP) being treated with vancomycin and meropenem due to Klebsiella spp. isolation in blood cultures. Owing to ERCP complications, surgery was required and parenteral nutrition was started.

Three days after surgery, he began with febrile syndrome, central catheter was removed and blood cultures were collected, resulting positive after 24 hours of incubation in BactAlert Biomerieux® system. Against expectations, Gram positive coccobacilli in chains were observed and identified by mass spectrometry (Maldi-TOF) as Leuconostoc mesenteroides.

The isolate was susceptible (disc broadcast) to amoxicillin-clavulanate, ampicillin, cefazolin, amikacin, gentamicin, erythromycin, clindamycin, teicoplanin, linezolid and penicillin G; and resistant to cefuroxime, cefotaxime and vancomycin. E-Test of amikacin (MIC: 0.064) and tigecycline (MIC: 0.064) was performed, adding amikacin to previous treatment.

RESULTS

Final diagnosis was bacteremia by Leuconostoc mesenteroides secondary to abdominal abscess.

With amikacin treatment, the patient did not present fever again, being discharged from ICU and admitted to digestive ward where he recovered from septic shock and multiorgan failure.

CONCLUSIONS

Leuconostoc mesenteroides is a rare microorganism which is not usually suspected in bacteremia although the majority reported cases are associated with it. Gastrointestinal colonization after surgery and vancomycin treatment has also been demonstrated, being difficult to establish the infectious focus in this case report.

Leuconostoc, despite of being gram-positive coccobacilli, shows intrinsic resistance to vancomycin. As a result it behaves as a superinfection in patients being treated with vancomycin.

Today, in vitro sensitivity cut-off points are not defined (both EUCAST and CLSI) hindering the correct treatment choice. Therefore, the experiences grouping, as this clinical case, could shed some light on the future and improve its correct treatment.

Microbiology - Infectious diseases including COVID 19

T195

CONCORDANCE BETWEEN INTERFERON-GAMMA RELEASE ASSAY (IGRA) AND TUBERCULIN SKIN TEST IN THE DIAGNOSIS OF LATENT TUBERCULOSIS AMONG IMMIGRANTS

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BACKGROUND-AIM

Tuberculosis (TB) is a major health problem in sub-Saharan Africa and other developing countries. Immigration from regions with a high incidence of tuberculosis has slowed the decline of TB in low-incidence regions. Most of the immigrant patients that we see in our hospital are from TB endemic areas. Many of them, may have had previous contact with TB and have a higher risk of latent tuberculosis infection (LTBI). Early detection LTBI is defined by a positive tuberculin skin test (TST) or a positive interferon gamma release assay (IGRA). The aim of this study is to analyze the concordance between both rapid methods for diagnosing LTBI and compare both rapid tests to determinate falses positives in foreign-born population.

METHODS

A retrospective study of 88 immigrant patients was carried on in our hospital to diagnostic LTBI between January 2019 to December 2020.The methods used were the TST and the Diasorin® IGRA Quantiferon-TB Gold Plus assay. IGRA test was used to confirm negative TST(< 5mm, falses negatives for immunosuppression) or positive up to 18 mm (possible falses positives for previous exposure to non-tuberculous mycobacteria or the BCG vaccine).Sociodemographic and clinical data were reviewed using the Modulab® and Ariadna® laboratory programs. Concordance was assessed with the Kappa coefficient(k).

RESULTS

A total of 88 people were included from: Senegal(29), Morocco(27), Guinea(18) and Mali(14). Median of age was 33.7 years and 71.6 % were males. Patients are divided into subgroups according to their country of origin and the concordance between the two tests varies according to the subgroup considered: patients from Guinea had the highest agreement between the two tests(k=0.667), followed by patients from Mali(k=0.429), Senegal(k=0.280) and Morocco(k=0.080). In general, the concordance is weak(k=0.327). Using IGRA test as a reference, we obtained 22.7 % false positives and 11.36% false negatives with TST.

CONCLUSIONS

The concordance between two tests is weak, which makes the use of IGRA necessary to diagnose these patients. According to the literature, the use of IGRA is recommended for TST up to 18 mm in the immigrant population. This was also confirmed in our study, which detected a high percentage of false positives if only this population was diagnosed with TST.

Microbiology - Infectious diseases including COVID 19

T196

EVALUATION THE IIPCR TECHNIQUE POCKIT™ CENTRAL ® AND IMPLEMENTATION OF A PROTOCOL FOR THE DIAGNOSIS OF SARS-COV-2

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BACKGROUND-AIM

During the COVID19 pandemic, numerous diagnostic techniques have been developed for SARS-CoV-2 virus, with real-time polymerase chain reaction (RT-PCR) as the gold standard. Rapid techniques like iiPCRPockit™ Central (Horiba®) have emerged, so the aim of the study was to evaluate this technique by comparing the results with the reference method used (RT-PCR Seegene®) for possible use in our hospital.

METHODS

We did a prospective study between March-April 2021 with 173 nasopharyngeal exudate samples from patients suspected COVID19 illness using both methods. The RT-PCR method quantitatively detects N, RdRP/S and E protein gene sequences with amplification cycles (Ct). The Pockit™Central® method is a qualitative isothermal PCR that detects the orf1ab region within 85 minutes; the amplification make a fluorescent signal before and after the reaction at 520nm and the ratio is used for interpretation.
According to the manufacturer, a ratio ≥1.15 is positive for SARS-CoV-2.

RESULTS

Of the 173 samples:
- 35 positive Pockit/ RT-PCR: ratio >1.2 and amplification of all three genes in RT-PCR.
- 120 negative Pockit/ RT-PCR: ratio <0.99 and no amplification in RT-PCR.
- 2 positive Pockit/negative RT-PCR: ratio >1.5 and no amplification in RT-PCR.
- 12 positive RT-PCR/negative Pockit: ratio <0.99 and amplification of one or two genes in RT-PCR with Ct>35.
- 4 inconclusive: 1 indeterminate Pockit/negative RT-PCR: ratio 1.14 and no amplification in RT-PCR;
3 inconclusive Pockit/RT-PCR negative: ratio<0.99 and amplification of a single gene with Ct>35.
We obtained sensitivity for Pockit of 74.4%, specificity 98.3%, positive predictive value 95% and negative predictive value 91%.

CONCLUSIONS

Given the results, we decided to establish our own cut-off. We considered negative samples with a ratio <0.99, positive >1.2 and indeterminate between 0.99-1.2 (confirmation by another method).
We observed that those discordant Pockit negative/RT-PCR positive results, all had Ct values >35 and were considered not infectious because of low viral load. Pockit positive/RT-PCR negative results could be due to across reactivity with others coronavirus.
As it has a high specificity it is very useful for rapid screening of patients. The technique was implemented with the new criteria established by this study.

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T197

ANTIBODY RESPONSES AND LABORATORY AB-NORMALITIES IN CORONAVIRUS DISEASE 2019 (COVID-19)

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BACKGROUND-AIM

The SARS-CoV-2 outbreak has rapidly progressed into a pandemic. The association between antibody responses and the COVID-19 disease severity remains poorly understood. However, recent studies suggest that the early appearance of IgM and IgG antibodies, and higher titters, are more frequent in the severe cases of SARS-CoV-2 infection.
Aim: To evaluate the humoral responses, and analytic features, of patients who died for COVID-19.

METHODS

This longitudinal study enrolled a total of 63 patients with laboratory-confirmed COVID-19 who had been discharged (control group) or had died by April 2020. EDTA-blood samples were collected on hospital admission, where IgA and IgG antibodies against SARS-CoV-2 were tested using enzyme linked immunosorbent assay (ELISA) kit. Demographic information, clinical classification and laboratory tests were also evaluated. For statistical analysis, Fisher #s or Student #s t tests were used to compare differences between survivors and non-survivors individuals as well as to identify predictive factors of severity.

RESULTS

Within the first week after symptom onset, virus-specific IgA were detected in 61.9% (39/63) of cases, 58% (18/31) in the non-survivor group and 65.6% (21/32) in the control group ($p=0.7$). The seroconversion rate for IgG was 39.6% (25/63), 41.9% (13/31) in the non-survivor group and 37.5% (12/32) in the control group ($p=0.4$). In addition, IgG levels were higher in the non-survivor group than in the survivors (2.8 vs 1.25; $p< 0.02$), and almost reaching statistical significance for IgA (56.4 vs 7.1; $p=0.05$).
Laboratory measures such as increased values of C-reactive protein (CRP), lactate dehydrogenase (LDH), gamma-glutamyl-transpeptidase (GGT), ferritin and D-dimer, along with diminished concentration of proteins, were found to predict worse prognosis. Regarding hematological parameters, neutrophilia was the most relevant abnormality in deceased patient.

CONCLUSIONS

High titter of antibodies was independently associated with worse overall survival in patients with severe COVID-19. This, together with several clinical and laboratory parameters may allow a better staging, monitoring and prediction of prognosis.

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T198

DEVELOPMENT OF SARS-COV-2 IGG ANTIBODY ASSAY USING LOCI TECHNOLOGY ON THE DIMENSION VISTA INTEGRATED CHEMISTRY SYSTEM*

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BACKGROUND-AIM

In response to the COVID-19 pandemic, Siemens Healthineers developed an immunoassay for the measurement of anti-SARS-CoV-2 IgG in patient serum and plasma (COV2G assay) using LOCI® technology on the Dimension Vista® Integrated Chemistry System. The Dimension Vista system incorporates multiple detection technologies that enable high-sensitivity immunoassays.

METHODS

The Dimension Vista COV2G assay is a homogeneous chemiluminescent immunoassay based on LOCI technology. The assay measures the anti-SARS-CoV-2 IgG antibody in both serum and plasma. A patient sample is incubated with dyed Chemibeads coated with the molecules of recombinant receptor-binding domain (RBD) of viral spike protein S1. Anti-RBD IgG antibodies in a seroconverted sample bind to RBD molecules on the Chemibeads to form an immunocomplex. Biotinylated anti-human IgG antibody molecules are added to bind the anti-RBD IgG antibody complexed with the Chemibeads. Streptavidin-coated Sensibeads containing a photosensitive dye are added to bind the biotin and form a bead-aggregated immunocomplex. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen in the Sensibeads, which diffuses into the Chemibeads and triggers a chemiluminescent reaction. The resulting signal is measured at 612 nm and is proportional to the concentration of the total IgG antibody activity in the sample. The cutoff calibrator with a value of 1000 qualitative index is traceable to the established SARS-CoV-2 cutoff standard for serum through patient sample concordance testing.

RESULTS

Time to first result is 16 minutes. The assay has a 14-day calibration interval and 30-day onboard stability. The assay requires 2 μL of serum or plasma and is linear up to 140,000 index units, with a limit of quantitation of 600 index units. Seroconversion sensitivity was assessed using 73 serial draws from 12 SARS-CoV-2 PCR-positive patients, and four samples drawn ≤ 7 post–PCR-positive were found negative. Diagnostic sensitivity was 61.1% (51.8 –69.5%, 95% CI, n = 113), 92.9% (81.0–97.5%, n = 42), and 100.00% (95.9–100.00%, n = 90) for samples drawn on days 0–7, 8–14, and ≥15 post PCR positive, respectively. Diagnostic specificity was measured using 1509 negative serum and plasma samples and was found to be 100.0% (99.7–100.0%, n = 1509). Precision was assessed using seven samples (two controls and five serum pools) at qualitative indices from 523 to 102,139 in accordance with CLS EP05-A3. Precision CVs ranged from 1.2 to 2.6%. Within-lab CVs ranged from 2.3 to 4.3%. No significant interference bias was observed in the presence of lipemia (1500 mg/dL INTRALIPID), bilirubin (40 mg/dL conjugated and unconjugated), hemoglobin (1000 mg/dL), total protein (18 g/dL), or biotin (1200 ng/mL). Patient samples containing common cross-reactants for serology assays (adenovirus, anti-influenza A and B, anti-hepatitis B virus, anti-respiratory syncytial virus antibody, anti-nuclear antibody, Bordetella pertussis IgG, cytomegalovirus IgG, Epstein-Barr virus, hepatitis B core antigen IgM, hepatitis B surface antigen, anti-hepatitis C virus antibody, HCV antibody, HIV antibody, , multiple myeloma, pneumonia, syphilis, and toxoplasma IgG) were also tested, and no false positives occurred.

CONCLUSIONS

The Dimension Vista COV2G assay demonstrates acceptable precision, accuracy, and turnaround time for the measurement of anti-SARS-CoV-2 IgG in patient serum and plasma.

This test has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for detecting he presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner. Product availability may vary by country and is subject to regulatory requirements.

Microbiology - Infectious diseases including COVID 19

T199

LONGITUDINAL EVALUATION OF THE ANTI-NUCLEOCAPSID IGG AFTER THE CONFIRMED COVID-19 IN NORTHERN CROATIAN POPULATION

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BACKGROUND-AIM

This study longitudinally evaluated the IgG response against the N-protein after the onset of COVID-19 infection. We determined the kinetics and magnitude of the antibody response against SARS-CoV-2 in confirmed COVID-19 patients who were the first infected with SARS-CoV-2 in Krapina-Zagorje county in northern Croatia.

METHODS

We studied 177 blood specimens from 51 patients who tested positive by PCR for COVID-19 and provided longitudinal blood samples over a duration of several months, allowing to evaluate the IgG response against the N-protein. SARS-CoV-2 IgG assay was interpreted as positive (ratio#1.4 S/C) or negative (ratio<1.4 S/C).

RESULTS

The majority of subjects (48/51) reported symptomatic disease. Among the 49 patients who underwent serological antibody testing at first time point (median: 47 days), 47/49 were positive for IgG 6.02 (0.24-10.54 S/C), while at sixth time point (median: 275 days) 4/16 patients were positive for IgG, 9/16 were in grey zone, and 3/16 were negative. Using Wilcoxon statistical analysis we found statistically significant decrease of SARS-CoV-2 N-protein IgG indices between the first and the sixth time point (median signal to cut-off ratio, S/C, 8.18 IQR #6.91, 9.51# to 0.94 IQR #0.56, 1.18#, P=0.001).

CONCLUSIONS

We clarified the kinetics and magnitude of the antibody response against SARS-CoV-2 in confirmed COVID-19 patients. Our results provide critical evidence that N-protein IgG response persists in the majority of patients for at least six to eight months after COVID-19 infection.

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T200

ASSOCIATION BETWEEN SARS-COV-2 ANTI-S1 RBD IGG TITERS AND BINDING INHIBITION BY NEUTRALIZING ANTIBODIES OF RECOMBINANT S1 RBD TO BIOTINYLATED ACE2 RECEPTORS

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BACKGROUND-AIM

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and vaccination trigger spike (S)-antigen antibody formation. As quantification of anti-S antibodies became possible in routine laboratories, questions about ‘protective titers’ and ‘neutralizing properties’ arose.

This study investigated the association between anti-S1 receptor binding domain (RBD) IgG titers and binding inhibition (IH%) of recombinant S1 RBD to biotinylated angiotensin-converting enzyme 2 by neutralizing antibodies. In addition, the possibility to calculate IH% from anti-S1 RBD IgG titers was examined.

METHODS

Serum from 22 individuals was collected before first dose (t0), before second dose (t0 + 4-12 weeks) and one week after the second dose of SARS-CoV-2 vaccination. On all 66 samples, anti-S1 RBD IgG titers (sCOVG assay, Atellica® IM, Siemens) and IH% using a surrogate virus neutralization assay (NeutraLISA®, Euroimmun) were measured.

RESULTS

Median anti-S1 RBD IgG titers were significantly higher in samples with a positive (≥35%) IH% result compared to samples with an intermediate (20-34%) or negative (<20%) IH% result, with titers being 3610 BAU/mL (95%CI[696-5452]), 75 BAU/mL (95%CI[52-98]) and 32 BAU/mL (95%CI[16-60]), respectively. Sensitivity/specificity of anti-S1 RBD IgG titers for a positive IH% result was 100%/18% using the Siemens defined cut-off for presence of IgG antibodies (≥ 21.8 BAU/mL) and was 93%/100% using the optimized cut-off by receiver operating characteristic (ROC) curve analysis (>107 BAU/mL). Using a semi-logarithmic regression analysis, a R2-value of 0.87 was observed with calculated IH% = -69 + [54 x log(anti-S1 RBD IgG titer in BAU/mL)]. Using this formula, 4 discordant interpretations (positive versus intermediate or negative versus intermediate) were observed when comparing measured and calculated IH% (weighted kappa 0.93 with 95%CI[0.86-1.00]).

CONCLUSIONS

All individuals with anti-S1 RBD IgG titers >107 BAU/mL had IH% results ≥35% and would be considered positive for neutralizing antibodies. Using a semi-logarithmic regression equation, IH% could be calculated from anti-S1 RBD IgG titers. Our lab will implement this formula to calculate IH% for a prospective study, searching for protective anti-S1 RBD IgG titers.

Microbiology - Infectious diseases including COVID 19

T201

IDENTIFICATION OF PATHOGENIC FUNGI BY CULTIVATION METHOD

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BACKGROUND-AIM

Fungi are pathogens that have the ability to invade all types of human and animal keratinized tissues. Due to the growing prevalence of fungal infections, their causing diseases have become a worldwide public health issue.

METHODS

Study was performed in SIA Central Laboratory and included samples taken in 2020. We have included 1572 microbiological tests. Clinical samples were taken from hair, mucosa, skin and nails. Material was subsequently tested for superficial mycoses by inoculation on selective media. Culture conditions: aerobic, 25°-30°C up to 4 weeks. Several additional tests were performed for some species (for example, urea for Trichophyton spp.).

RESULTS

Trichophyton rubrum (28%) was the most frequent causative agent of infection. Other dermatophytes (Epidermophyton floccosum) and different species of Trichophyton (Trichophyton mentagrophytes and others) were detected in 6,3%. Microsporum canis spp. was detected in 0,6%. Totally the dermatophytes as the causative agents of fungal infections were isolated in 34,9%. The other large group of pathogenic fungi were Candida albicans - 7,1% and other Candida spp. - 34,1%. Exophiala spp.- 0,8%. The moulds were isolated in the remaining 23,1%. Non dermatophytes - Scopulariopsis sp., Acremonium sp., Aspergillus sp., Geomyces pannorus -– were detected in 13%, .

CONCLUSIONS

Most frequent fungal infections were caused by dermatophytes T. rubrum and T. mentagrophytes. C. albicans and other Candida spp. were the most common yeasts. From non dermatophytes the most often isolated was Scopulariopsis sp., Acremonium sp., Aspergillus sp.,Geomyces pannorus.
The use of the cultivation method allows to detect larger range of pathogenic fungi, but the great disadvantages are the longer test time, subjectivity of a specialist and often the possibility only to identify fungi at genus level. Therefore, the use of molecular biological methods is preferrable. Mainly due to their considerable advantages – higher sensitivity, specificity and faster test time, as well as absence of subjectivity and human error factor.

Microbiology - Infectious diseases including COVID 19

T202

DETERMINATION OF SARS-COV-2 VARIANTS OF CONCERN USING A RT-QPCR: A 5-MONTH EXPERIENCE

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BACKGROUND-AIM

Variants of concern (VOC) of SARS-CoV-2 are present worldwide but their distribution varies from one region to another. The Next-Generation Sequencing (NGS) is the reference method for accurately identifying VOCs. However, the NGS is expensive, time-consuming and requires expertise for interpretation. In addition, a fairly high viral load is required to achieve good performance. In recent months, it has been possible to detect the most frequent mutations of the spike protein of SARS-CoV-2 using a RT-qPCR. We present here the observations of the evolution of SARS-CoV-2 epidemiology in the region of Brussels thanks to this technique.

METHODS

All SARS-CoV-2 positive samples analysed by one of the molecular methods used in our laboratory were selected for identification of variants using the Allplex Variant I and Variant II Assays (Seegene Technologies, Seoul, South Korea). Those 2 assays detect the following mutations: H69/V70del, N501Y, E484K (Variant I) and K417N, K417T, L452R and W152C (Variant II). Extraction of viral RNA was performed using the STARMag Viral DNA/RNA 200 C Kit (Seegene Technologies, Seoul, South Korea) on a STARLet platform (Hamilton Company, Reno, NV, USA). The complementary DNA synthesis and the amplification were performed with a CFX96 C1000 thermal cycler (Bio Rad Laboratories, Hercules, CA, USA).

RESULTS

94,624 molecular tests were performed in our lab between March 7, 2021 and August 23, 2021. After elimination of duplicates, 3,418 positive samples were tested for VOCs. At the beginning of March, the alpha variant accounted for 64% of the VOCs. The first VOC delta was detected in early May and increased over the weeks to reach 50% of VOCs by mid-June and 90.2% by the end of the observed period.

CONCLUSIONS

VOCs detection by RT-qPCR is an affordable alternative to NGS. It is faster (turnaround time <5 hours), about 7 times cheaper, and provides results for Ct>25. The Allplex Variant I and Variant II Assays are able to distinguish the alpha, beta, gamma, delta, delta+, epsilon, iota and B.1.525 variants. Routine use of this method allows monitoring of local epidemiology and provides additional information to clinicians. We showed that the delta variant had become the main VOC in the Brussels region by the middle of June.

Microbiology - Infectious diseases including COVID 19

T203

DEVELOPMENT OF SARS-COV-2 TOTAL ANTIBODY ASSAY USING LOCI TECHNOLOGY ON THE DIMENSION VISTA INTEGRATED CHEMISTRY SYSTEM*

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BACKGROUND-AIM

In response to the COVID-19 pandemic, Siemens Healthineers developed a qualitative assay for the measurement of total antibodies (IgG and IgM) against SARS-CoV-2 (COV2T assay) using LOCI® technology on the Dimension Vista® Integrated Chemistry System. The Dimension Vista system incorporates multiple detection technologies that enable high-sensitivity immunoassays.

METHODS

The Dimension Vista COV2T assay is a homogeneous chemiluminescent immunoassay based on LOCI technology. The assay measures the total antibody activity against SARS-CoV-2 in both serum and plasma. A patient sample is incubated with dyed Chemibeads coated with the molecules of recombinant receptor-binding domain (RBD) of viral spike protein S1. Anti-RBD antibodies in a seroconverted sample bind to RBD molecules on Chemibeads to form an immunocomplex. Biotinylated RBD molecules are added to bind the unbound arms of the anti-RBD antibodies complexed with RBD on the Chemibeads. Streptavidin-coated Sensibeads containing a photosensitive dye are added to bind the biotin and form a bead-aggregated immunocomplex. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen in the Sensibeads, which diffuses into the Chemibeads and triggers a chemiluminescent reaction. The resulting signal is measured at 612 nm and is proportional to the concentration of the total antibody activity in the sample. The cutoff calibrator with a value of 1000 qualitative index is traceable to the established SARS-CoV-2 cutoff standard for serum through patient sample concordance testing.

RESULTS

Time to first result is 16 minutes. The assay has a 14-day calibration interval and 30-day onboard stability. The assay requires 10 µL of serum or plasma and generates a positive result if anti-SARS-CoV-2 antibody is detected. Seroconversion sensitivity was assessed using 62 serial draws from nine SARS-CoV-2 PCR-positive patients, and only one sample drawn on day 4 post–PCR-positive was found negative. Diagnostic sensitivity was 66.67% (57.32–75.29% 95% CI, n = 96), 97.37% (94.72–99.53%, n =38), and 100.00% (95.83–100.00%, n = 72) for samples drawn on days 0–6, 7–13, and >13 post–PCR-positive, respectively. Diagnostic specificity was measured using 1529 negative serum and plasma samples and was found to be 99.80% (99.67–99.93%, n = 1529). Precision was assessed using four samples (two controls and two serum pools) at qualitative indices from 0 to 2596 in accordance with CLS EP05-A3. Precision CVs ranged from 1.0 to 2.5%. Within-lab CVs ranged from 2.3 to 3.7%. No significant interference bias (≤10%) was observed in the presence of lipemia (1500 mg/dL INTRALIPID), bilirubin (40 mg/dL conjugated and unconjugated), hemoglobin (1000 mg/dL), or biotin (1200 ng/mL). Patient samples containing common cross-reactants for serology assays (anti-influenza A and B, anti-HBV, anti-nuclear antibody, hepatitis B core antigen IgM, hepatitis B surface antigen, anti-hepatitis C virus antibody, anti-respiratory syncytial virus antibody, and toxoplasma IgG) were also tested, and no false positives occurred.

CONCLUSIONS

The Dimension Vista COV2T assay demonstrates acceptable precision, accuracy, and turnaround time for the measurement of anti-SARS-CoV-2 total antibodies in patient serum and plasma.
This test has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for detecting he presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner. Product availability may vary by country and is subject to regulatory requirements.

Microbiology - Infectious diseases including COVID 19

T204

DEVELOPMENT OF SARS-COV-2 IGG ANTIBODY ASSAY USING LOCI TECHNOLOGY ON THE DIMENSION EXL INTEGRATED CHEMISTRY SYSTEM*

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[1] *Siemens Healthcare Diagnostics Inc., Newark, DE, U.S.*

BACKGROUND-AIM

In response to the COVID-19 pandemic, Siemens Healthineers developed an immunoassay for the measurement of anti-SARS-CoV-2 IgG in patient serum and plasma (CV2G assay) using LOCI® technology on the Dimension® EXL™ Integrated Chemistry System. The Dimension EXL system incorporates multiple detection technologies that enable high-sensitivity immunoassays.

METHODS

The Dimension EXL CV2G assay is a homogeneous chemiluminescent immunoassay based on LOCI technology. The assay measures the anti-SARS-Cov-2 IgG antibody in both serum and plasma. A patient sample is incubated with dyed Chemibeads coated with the molecules of recombinant receptor-binding domain (RBD) of viral spike protein S1. Anti-RBD IgG antibodies in a seroconverted sample bind to RBD molecules on the Chemibeads to form an immunocomplex. Biotinylated anti-human IgG antibody molecules are added to bind the bound anti-RBD IgG antibodies complexed with the Chemibeads. Streptavidin-coated Sensibeads containing a photosensitive dye are added to bind the biotin and form a bead-aggregated immunocomplex. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen in the Sensibeads, which diffuses into the Chemibeads and triggers a chemiluminescent reaction. The resulting signal is measured at 612 nm and is proportional to the concentration of the total antibody activity in the sample. The cutoff calibrator with a value of 1000 qualitative index is traceable to the established SARS-CoV-2 cutoff standard for serum through patient sample concordance testing.

RESULTS

Time to first result is 16 minutes. The assay has a 14-day calibration interval and 30-day onboard stability. The assay requires 2 µL of serum or plasma and is linear up to 140,000 index units, with a limit of quantitation of 610 index units. Seroconversion sensitivity was assessed using 73 serial draws from 12 SARS-CoV-2 PCR-positive patients, and four samples drawn ≤ day 7 post–PCR-positive were found negative. Diagnostic sensitivity was 61.9% (52.7-70.4%, 95% CI, n = 113), 92.9% (81.0-97.5%, n = 42), and 100.00% (95.9–100.00%, n =90) for samples drawn on days 0–7, 8–14, and ≥15 post–PCR-positive, respectively. Diagnostic specificity was measured using 1509 negative serum and plasma samples and was found to be 100.0% (99.7–100.0%, n = 1509).Precision was assessed using seven samples (two controls and five serum pools) at qualitative indices from 570 to 108,126 in accordance with CLS EP05-A3. Precision CVs ranged from 0.97 to 3.34%. Within-lab CVs ranged from 2.86 to 6.15%. No significant interference bias was observed in the presence of lipemia (1500 mg/dL INTRALIPID), bilirubin (40 mg/dL conjugated and unconjugated), hemoglobin (1000 mg/dL), total protein (18 g/dL), or biotin (1200 ng/mL). Patient samples containing common cross-reactants for serology assays (adenovirus, anti-influenza A and B, anti-hepatitis B virus, anti-respiratory syncytial virus antibody, anti-nuclear antibody, Bordetella pertussis IgG, cytomegalovirus IgG, Epstein-Barr virus, hepatitis B core antigen IgM, hepatitis B surface antigen, anti-hepatitis C virus antibody, HCV antibody, HIV antibody, multiple myeloma, pneumonia, syphilis, and toxoplasma IgG) were also tested, and no false positives occurred.

CONCLUSIONS

The Dimension EXL CV2G assay demonstrates acceptable precision, accuracy, and turnaround time for the measurement of anti-SARS-CoV-2 total antibodies in patient serum and plasma.
This test has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for detecting he presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner. Product availability may vary by country and is subject to regulatory requirements.

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T205

DEVELOPMENT OF SARS-COV-2 TOTAL ANTIBODY ASSAY USING LOCI TECHNOLOGY ON THE DIMENSION EXL INTEGRATED CHEMISTRY SYSTEM*

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[1] Siemens Healthcare Diagnostics Inc., Newark, DE, U.S.

BACKGROUND-AIM

In response to the COVID-19 pandemic, Siemens Healthineers developed a qualitative assay for the measurement of total antibodies (IgG and IgM) against SARS-CoV-2 (CV2T assay) using LOCI® technology on the Dimension® EXL™ Integrated Chemistry System. The Dimension EXL system incorporates multiple detection technologies that enable high-sensitivity immunoassays.

METHODS

The Dimension EXL CV2T assay is a homogeneous chemiluminescent immunoassay based on LOCI technology. The assay measures the total antibody activity against SARS-CoV-2 in both serum and plasma. A patient sample is incubated with dyed Chemibeads coated with the molecules of recombinant receptor-binding domain (RBD) of viral spike protein S1. Anti-RBD antibodies in a seroconverted sample bind to RBD molecules on the Chemibeads to form an immunocomplex. Biotinylated RBD molecules are added to bind the unbound arms of the anti-RBD antibodies complexed with RBD on the Chemibeads. Streptavidin-coated Sensibeads containing a photosensitive dye are added to bind the biotin and form a bead-aggregated immunocomplex. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen in the Sensibeads, which diffuses into the Chemibeads and triggers a chemiluminescent reaction. The resulting signal is measured at 612 nm and is proportional to the concentration of the total antibody activity in the sample. The cutoff calibrator with a value of 1000 qualitative index is traceable to the established SARS-CoV-2 cutoff standard for serum through patient sample concordance testing.

RESULTS

Time to first result is 16 minutes. The assay has a 14-day calibration interval and 30-day onboard stability. The assay requires 10 µL of serum or plasma and generates a positive result if anti-SARS-CoV-2 antibody is detected. Seroconversion sensitivity was assessed using 62 serial draws from nine SARS-CoV-2 PCR-positive patients, and only one sample drawn on day 4 post–PCR-positive was found negative. Diagnostic sensitivity was 68.75% (59.65–77.15% 95% CI, n = 96), 97.37% (94.72–99.53%, n = 38), and 100.00% (95.83–100.00%, n = 72) for samples drawn on days 0–6, 7–13, and >13 post–PCR-positive, respectively. Diagnostic specificity was measured using 1527 negative serum and plasma samples and was found to be 99.87% (99.77–99.96%, n = 1529). Reproducibility was assessed using four samples (two controls and two serum pools) at qualitative indices of 38.4 to 2360 in accordance with CLS EP05-A3. Precision CVs ranged from 2.2 to 2.7%. Within-lab CVs ranged from 3.0 to 3.9%. No significant interference bias was observed in the presence of lipemia (1500 mg/dL INTRALIPID), bilirubin (40 mg/dL conjugated and unconjugated), hemoglobin (1000 mg/dL), or biotin (1200 ng/mL). Patient samples containing common cross-reactants for serology assays (anti-influenza A and B, anti-HBV, anti-nuclear antibody, hepatitis B core antigen IgM, hepatitis B surface antigen, anti-hepatitis C virus antibody, anti-respiratory syncytial virus antibody, and toxoplasma IgG) were also tested and no false positives occurred.

CONCLUSIONS

The Dimension EXL CV2T assay demonstrates acceptable precision, accuracy, and turnaround time for the measurement of anti-SARS-CoV-2 total antibodies in patient serum and plasma.
This test has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for detecting the presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner. Product availability may vary by country and is subject to regulatory requirements.

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T206

EVALUATION OF SIMPLE AZTREONAM-AVIBACTAM SYNERGY TESTS FOR MULTI-DRUG RESISTANT CLINICAL ENTEROBACTERALES ISOLATES

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BACKGROUND-AIM

The spread of Carbapenem-resistant gram-negative organisms, namely carbapenemase-producers, confronts clinicians with limited therapeutic choices in some cases of infection. The monobactam Aztreonam (ATM) is an effective drug to treat Metallobetalactamase (MBL)-producing isolates, for which few or no other options remain. The novel carbapenemase inhibitor Avibactam (AVI) protects ATM against betalactamases and carbapenemases other than MBL. While ATM-AVI is undergoing phase 3 trials, the currently approved antibiotics Ceftazidime (CAZ)-AVI (CZA) and ATM can be administered in combination. However, commercially available tests for ATM-AVI synergy are not available.

METHODS

We tested 19 MBL-positive clinical Enterobacterales isolates for synergistic action of ATM+AVI using disk diffusion and gradient diffusion methods with suprapositioning of gradient strips. Minimal inhibitory concentrations (MICs) of 16/19 isolates were above the EUCAST breakpoint for susceptibility to ATM alone at standard dose. Disks containing 30 µg ATM and 10 µg CAZ + 4 µg AVI, respectively, were placed approximately 20 mm apart on Müller-Hinton (MH) agar after inoculation with bacterial suspension (McFarland 0.5). For determination of MICs for ATM plus AVI, first a CZA gradient strip (0.016-256 mg/l) was applied to inoculated MH agar, removed after 10 minutes, and subsequently an ATM gradient strip (0.016-256 mg/l) was placed at exactly the same position on the plate and left in place. Results were read after 16-18 h incubation at 35°C in ambient air.

RESULTS

Zone diameters and MICs were determined for all isolates. They were unchanged for 3 isolates that were susceptible to ATM alone. Oval zones of inhibition in the presence of CZA developed in all 16 isolates with ATM MICs above 1 mg/l and MICs for ATM decreased from ≥ 256 to ≤ 1 mg/l in 15 of these 16 MBL-positive isolates.

CONCLUSIONS

The disk diffusion method is a possible screening test for synergistic effects of ATM+AVI and can be confirmed by determining the resulting ATM MIC by suprapositioning gradient diffusion strips. The method is easily adaptable in routine microbiology laboratories and allows reporting of an urgently needed therapeutic option for clinicians struggling to treat patients with infections caused by MBL-producing Enterobacterales. As ongoing phase 3 studies on ATM+AVI may lead to its approval by authorities, more convenient methods for testing the fixed combination may become available.

Microbiology - Infectious diseases including COVID 19

T207

THE INTERIM RESULTS OF THE LONG-TERM MONITORING OF THE HUMORAL RESPONSE TO THE COVID-19 VACCINATION IN THE REAL WORLD SETTING.

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BACKGROUND-AIM

The methods employed in vaccine immunogenicity studies are not feasible in the real-world setting. Hence, we intended to assess the humoral response induced by a mRNA-based vaccine with a commercially available CLIA assay, over a 4-month course.

METHODS

The cohort included one hundred vaccinated subjects, 86% females and 14% males, and comprised COVID-19 convalescents (15%) and non-convalescents (85%). The mean age of the study group was 45 (23-74). The antibody concentrations were measured on day 10′, 20′, 30′, 60′, 90′ and 120′ after the first dose of the vaccine. The assay used, LIAISON® SARS-CoV-2 TrimericS IgG (DiaSorin Inc.), measures IgG antibodies recognizing trimeric spike (S) protein, which may be considered one of the correlates of immunity.

RESULTS

Over the course of the study, 100% of the participants developed and sustained a measurable concentration of anti-SARS-CoV-2 trimeric S IgG antibodies. The highest concentration, exceeding the quantification range of the test (>2080 BAU/ml) was reached by the majority of the subjects (67%) on day 30′.

A statistically significant increase in the mean antibody levels was observed between day 10′ and day 20′ (first dose administration), as well as between day 20′ and day 30′ (second dose). The concentration of the antibodies remained stable between day 30′ and day 90′ but was followed by a significant decrease between day 90′ and day 120′. However, the level of antibodies higher than 520 BAU/ml, correlated by the producer with the microneutralization titer of at least 1:80, was still observed in 83% of the subjects on day 120′.

COVID-19 convalescents developed higher antibody levels at all of the studied time points. Particularly pronounced was the difference noted on day 10′, when 80% of the convalescents and 0% of non-convalescents reached the upper quantification limit of the test (>2080 BAU/ml). The levels of antibodies in convalescents remained fairly stable between day 10′ and 120′. On the contrary, in non-convalescents a statistically significant increase was seen between days 10′- 20′ - 30′ and a significant decrease between day 90′ and 120′.

CONCLUSIONS

The observations presented above may guide the future vaccination strategies, namely identification of the non-responders and verification of the necessity of a second booster administration.

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T208

QUANTITATIVE SARS-COV-2 ANTI-SPIKE RESPONSES TO PFIZER-BIONTECH VACCINES IN HEALTHCARE WORKERS

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BACKGROUND-AIM

The immunization of healthcare workers (HCWs) plays a recognized key role in prevention in the COVID-19 pandemic. In Croatia, the vaccination campaign began at the end of December 2020.

METHODS

A cohort study involved 122 HCWs following two vaccine doses with the Pfizer-BioNTech vaccine. HCW were encouraged to attend for serological testing 3 weeks after first vaccination (Titer 1), at the same time as receiving second dose of vaccine. Thereafter, approximately 5 weeks after the first vaccination (Titer 2), the third follow up was 9 weeks after the first vaccination (Titer 3), and the fourth follow up was 13 weeks after the first dose of vaccine (Titer 4).

RESULTS

Among all vaccinated HCWs, 119 (97.5%) had detectable anti-SARS-CoV-2 spike IgG antibodies after first dose and among these, arithmetic mean was 6274.4 AU/mL. Those with previous infection developed substantially higher titers. In HCWs receiving a second Pfizer-BioNTech vaccine dose, antibodies were boosted in previously uninfected individuals with the highest levels of antibodies, but with some waning of responses from 5 to 13 weeks post-vaccination. The anti-SARS-CoV-2 S-protein levels increased between the first (Titer 1) and second (Titer 2) follow-up (median anti-spike IgG reading, AU/mL 1368.00 IQR [487.75, 6259.00] to 16216.00 IQR [10582.25, 22981.75], $P<0.001$). Further anti-SARS-CoV-2 S-protein levels decreased between the second (Titer 2) and third (Titer 3) follow-up (median anti-spike IgG reading, AU/mL, 16216 IQR [10582.25, 22981.75], to 6970 IQR [4171.00, 9651.25], $P<0.001$). Furthermore, anti-SARS-CoV-2 S-protein levels were significantly decreased between the third (Titer 3) and fourth (Titer 4) follow-up (median anti-spike IgG reading, AU/mL 6970.00 IQR [4171.00, 9651.25] to 3942.00 IQR [2264.25, 6571.75], $P<0.001$). Adverse effects after the vaccine were more frequent after the second dose, but no severe adverse effects were observed.

CONCLUSIONS

Three months after vaccination, we confirm a very high efficacy and a persistence
of anti-spike antibodies. Antibodies levels tend to decline at 3 months after the first vaccine dose, This decline occurs in both baseline seropositive and seronegative subjects,

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T210

HUMORAL IMMUNE RESPONSE TO TWO DOSES OF AZD1222/ COVISHEILD VACCINE AMONG HEALTH CARE WORKERS FROM A TEACHING HOSPITAL IN SRI LANKA

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BACKGROUND-AIM

Within one year of reporting the first case of SARS CoV-2 infection from China, several types of vaccines for COVID-19 were developed and used in mass immunisation campaigns worldwide, following successful completion of phase 3 trials. This study was conducted to assess the humoral immune response to the Covisheild vaccine against the COVID-19 infection employing the antibody development in the host using a cohort of health care workers as they are more exposed to the infection.

METHODS

To assess the Covisheild vaccine efficacy is based on neutralising antibody production, a descriptive cross-sectional study using 200 participants who received two doses of the Covisheild vaccine. The antibody response was assessed at 3-4 weeks after each vaccine dose and just before the 2nd dose using a two-site sandwiched immunoassays technique developed to detect IgG by Ortho Clinical diagnostics. Data were analysed using SPSS-22 for univariate and bivariate analysis of the study.

RESULTS

Out of 177 participants, 38% were doctors, 23.1% were nurses, 17.6% were laboratorians, the rest were healthcare helpers, and 60% were females. The mean age of the participants was 44.2 years (range 23-68, SD: 9.73). None of them had PCR-positive COVID-19 infection at the time of inclusion in the study, and 23.1% stated to have low risk, 56.9% intermediate risk, and the rest a high risk for COVID-19 exposure. Fever and myalgia were the commonest symptoms (60%) following the 1st dose, while none reported having any significant symptoms following the 2nd dose. IgG was measured in 177, after 1st dose, with a mean Ab index of 5.8 (SD: 4.3) with 82.5% overall seroconversion and highest seroconversion (86.9%) in the 40–55 year age group. Before the 2nd dose was given after 12-weeks from the 1st dose, 121 participants were assessed for IgG levels, and the mean Ab response was 4.0 (SD: 4.8) with the persistence of seroconversion only in 64.5% ($p=0.001$). Of the seronegative participants at the initial Ab assessment, 50% (n=4/8) in the <40 year age group achieved seroconversion by 12-weeks while only one achieved seroconversion among >=40 years (n=1/15), which was statistically significant ($p=0.33$). IgG was measured in 168 participants, after the 2nd dose, with a mean Ab index of 9.3 (SD: 4.6) with 96.4% overall seroconversion and highest seroconversion (98.9%) in the 40–55 year age group. The increase in seroconversion after 2nd dose was statistically significant ($p=0.000$).

CONCLUSIONS

A majority showed seroconversion irrespective of age and sex after a single dose of vaccine, and it increased significantly by the 2nd dose of the vaccine. The seroconversion rates were highest in middle-aged individuals while it was lowest in >60 years. Although a single dose of the vaccine induced an excellent Ab response, it significantly reduced after 12-weeks. Therefore, the long-term persistence of Ab response for the Covisheild vaccine needs to be assessed.

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T211

FOSB PROTEIN IS A NOVEL POOR PROGNOSIS BIOMARKER IN HOSPITALIZED COVID-19 PATIENTS

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BACKGROUND-AIM

The diagnosis and follow-up of COVID-19 patients represent a challenge for clinical laboratories. Several laboratory parameters have shown their usefulness in this task. The prognosis of these patients depends on the complications that appear during the disease. The objective of this study was to identify new serum prognostic biomarkers in hospitalized COVID-19 patients.

METHODS

Relative serum levels of 92 protein markers involved in biological response to organ damage were measured by proximity extension assay technology (Organ damaged panel, Olink) in 18 healthy volunteers (HV) and in 70 hospitalized COVID-19 patients that needed critical care therapy (CC; n:35) or not (non-CC; n:35) at admission. Clinical and laboratory data were collected after signed inform consent. Results were compared using the unpaired Student t test, one-way analysis of variance and Kruskal-Wallis test with Dun's post test when applicable. To assess the relationship between two continuous variables, the Spearman's rank correlation coefficient for nonparametrical data was used.

RESULTS

Among the 92 proteins; KIM1, RASSF, NCF2, FGR and FOSB were significantly increased in CC patients compared with the HV and non-CC groups. Univariant analysis identified statistically significant relationship between FOSB relative levels and several unspecific inflammation (C-reactive protein; r: 0.521, procalcitonin; r:0.467, ferritin; r:0.463 and leukocyte count; r:0.220), cellular damage (LDH; r:0.580), coagulative disorder (prothrombin time; r:-0.476 and D-dimer; r:0.532), cardiac (Troponin I; r:0.324) and hepatic damage (GOT; r:0.340 and total bilirrubin; r:0.222) markers. A significant correlation (r:0.629) was also noted between FOSB levels and hospitalization days in COVID patients. Finally, FOSB was significantly increased in COVID patients who developed deep venous thromboembolism or who required intensive oxygen therapy.

CONCLUSIONS

The FOSB protein is a promising poor prognostic biomarker in hospitalized COVID-19 patients that may be useful in the early identification of patients who will develop severe complications during their hospitalization.

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T212

ANALYSIS OF SARS-COV-2 CONVALESCENT SERUM USING A MULTIMARKER PANEL OF S1, S2, RBD AND NUCLEOCAPSID ANTIBODIES

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[1]*National Reference Laboratory*

BACKGROUND-AIM

The SARS-CoV-2 pandemic has placed the globe in uncertainty with respect to understanding the adaptive immune patterns and the potential for immune-protection against this virus through vaccinations and convalescent recovery. At this point we do not know what antigens should be measured for the immune response. It is known that nucleocapsid antigen (N) is not suited for detecting a response to vaccinations, yet it is sufficient for detecting an adaptive response to natural infection. It remains unanswered if there is benefit in measuring antibody responses to the ACE Receptor Binding Domain (RBD) the Spike 1 (S1) or Spike 2 (S2) proteins in the determining what constitutes a protective response in convalescence. We studied the antibody response to SARS-CoV-2 using the Roche quantitative IgG, IgM and Diasorin IgG S1, S2 antibody assays and the BIO-RAD BioPlex Panel Assay with separate markers for N, S1, S2 and RBP using convalescent serum.

METHODS

Thirty-five SARS-CoV-2 recovered individuals antibody responses were measured using the aforementioned assays according to manufacturer's instructions.

RESULTS

Of the 35 individuals 34 tested positive using Roche, 33 using Diasorin and 33 using BioRad. One individual was negative on all three systems. The Diasorin and BioPlex were 100% concordant with Roche being 97% concordant to the Diasorin and BioPlex. S2 antibody production was lower in titer than S1 and RBD in most cases. Eight of the positive individuals had undetectable S2. While nucleocapsid antibodies were absent in two positive individuals. One individual was negative by Roche and Diasorin as well as the BioPlex panel except for S2 antibodies.

CONCLUSIONS

The BioPlex panel demonstrated good correlation across the panel except for S2. This may be explained by the mechanism of infection at the ACE receptor. SARS-CoV-2 entry into cells requires binding of RBP mediated by S1 followed by a proteolytic cleavage and structural change to expose the S2 protein. S2 partial cleavage then permits S2 to facilitate the entry of the virus into the cell by directly interacting with the lipid membrane. The different antibodies generated and subsequently detected may in part be due to the extent of availability and exposure in vivo in a natural infection. It is unknown if the peptide sequences used in the assay represent the cleaved form of S1 or S2 or are representative peptide epitopes. Further, it was observed that one individual had what could be considered an atypical immune response with all assays negative with the exception of S2 on the BioPlex assay. Considering the recently developed neutralizing assays focus on the blocking of ACE receptor binding by the RBP it is interesting that this has been chosen to be the surrogate indicator of neutralizing blocking efficacy considering that antibody neutralization of S1 proteolytic cleavage and also antibody binding to exposed S2 protein to prevent cellular entry could also be -considered targets of neutralization antibodies. The value in multi=marker panels may eventually be in predicting immune response efficacy, the need for revaccination or understanding immune adaptation in reinfection among other potential uses.

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T213

SENSITIVITY OF TWO SARS-COV-2 ANTIGEN ASSAYS COMPARED WITH SARS-COV-2 RT-PCR

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BACKGROUND-AIM

Real time RT-PCR screening for SARS-CoV-2 is the recommended methodology for diagnosis of SARS CoV-2 virus. Early and rapid diagnosis of potentially contagious individuals permits disease containment through quarantine and tracking and tracing of contacts. Alternate methods that can potentially reduce costs, simplify analysis and improve turn around time in both symptomatic and asymptomatic individuals for mass screening purposed are desirable. Sensitive immunological assays to detect the presence of SARS-CoV2 antigen fit the criteria for improving mass screening. We have compared two antigen immunoassays directed against the nucleocapsid (N) protein to RT-PCR using nasopharyngeal swab transport medium to determine the relative sensitivity and create a proposed workflow.

METHODS

Seventy RT-PCR (Ord1b and N gene) positive specimens (DiaplexQ, SolGent) with varying cycle time (ct) values were tested using both the Diasorin quantitative and Roche qualitative antigen assays according to manufacturers' instructions. Nasopharyngeal swab transport media was used as the specimen type.

RESULTS

The sensitivity of the Diasorin and Roche antigen assays were 61% and 66% respectively. The total agreement between Roche and Diasorin was 23 out of 70 specimens Diasorin did not detect 11 specimens which were detected by Roche and conversely 2 specimens were not detected by Roche which were detected by Diasorin. The highest ct detected by Roche was 36 and for Diasorin was 32. Conclusions: Antigen assays while being less sensitive than RT-PCR do provide a result in a shorter period with lower technical requirements. The Roche assay in this study demonstrated higher sensitivity to detection when compared to Diasorin especially at higher ct values.

CONCLUSIONS

Mass screening is possible as the Diasorin assay has been approved by the FDA for both symptomatic and asymptomatic individuals. Antigen tests can potentially be a first line test with a reflex to PCR when the antigen test is negative.

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T214

ANTIBODY RESPONSE AFTER VACCINATION WITH SARS-COV-2 SPEAK BNT162B2 MRNA VACCINE OF HEALTHCARE PROFESSIONALS

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BACKGROUND-AIM

At the present time vaccination is the main strategy to control the SARS-CoV-2 pandemic. Pfizer/ BioNTech's SARS-CoV-2 Speak BNT162b2 mRNA vaccine was granted emergency FDA clearance for human use in December 2020. This vaccine uses the Spike S protein of coronavirus as antigen.
The aim of this study is evaluate antibody response of healthcare professionals after two doses of Pfizer-BioNTech vaccine, in addition to the relationship between the humoral response and age, sex and body mass index.

METHODS

It was selected all the healthcare professionals who had received two doses of Pfizer-BioNTech vaccine in our Laboratory before April of 2021. Serum samples were tested for the presence of specific antibodies against SARS-Cov-2 spike by electrochemiluminescence immunoassay with the equipment Cobas e601 by Roche Diagnostics. Antibody titers above 0.8 U/mL were considered positive. It was collected data of age, sex, and body mass index for all participants. Statistical analysis was realized by SPSS 21.0.

RESULTS

A total of 61 healthcare professionals was included in this study, 21 men and 40 women. Kolmogorov-Smirnov test was significant ($p<0.05$), it considered a non-parametric population. Median age of the participants was 51.3 years old [30.0-67.0]. Median body mass index of the participants was 24.6 kg/m2 [20.4-50.0]. All the professionals showed antibody response after vaccination (734.3 U/mL [28-2832]). Mann-Whitney-U test for independent samples showed no significant statistical differences in antibody distribution for age, sex and body mass index ($p>0.05$).

CONCLUSIONS

In conclusion, Pfizer-BioNTech vaccine was totally effective in all the participants of this study. Nevertheless, antibody response of healthcare professionals after two doses of Pfizer-BioNTech vaccine has not relationship with age, sex and body mass index. As main limitation of the study is the sample size, new studies with a bigger sample size would be necessary.

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T215

EXPANDING THE CLINICAL APPLICATION FIELD FOR TARGETED LIQUID CHROMATOGRAPHY MASS SPECTROMETRY METHODS - THE DEVELOPMENT OF A FLEXIBLE SARS-COV2 DETECTION METHOD AS A PROOF-OF-CONCEPT

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BACKGROUND-AIM

To prevent reoccurrence of outbreaks of the SARS-CoV-2 virus widespread diagnostic testing became part of the strategic plan. Tests based on reverse transcription polymerase chain reaction (RT-PCR) form the backbone. Despite the efficiency, automation and acceptable costs, reliance on one type of technology comes with caveats, e.g. recurring reagent and test shortages. We therefore developed an alternative diagnostic test that detects proteolytically digested SARS-CoV-2 proteins using liquid chromatography – mass spectrometry (LC-MS). As this test relies on proteins to detect viral load instead of RNA, it provides an orthogonal and complementary approach to RT-PCR, using other relatively inexpensive and widely available reagents and different instruments.

METHODS

An international community-based consortium named Cov-MS validated a generic and broadly applicable MRM template. Fifteen academic labs and several industrial partners combined their efforts and expertise to increase applicability, accessibility, sensitivity and robustness of LC-MS-based SARS-CoV-2 detection.

RESULTS

Based on in-house and public data 17 biomarker peptides from two SARS-CoV-2 proteins (Nucleocapsid and Spike) were selected as targets and incorporated in a dedicated 8-minute MRM assay. In addition, a stable isotopically labelled analogue for each peptide was included (QconCAT). Next, specific antibodies for peptide immunopurification (SISCAPA®) were introduced into the workflow, greatly reducing matrix effects. This improved the limit of detection down to 100 amol on column, irrespective of the sample storage and transport medium. A method comparison between this LC-MS-based assay and RT-PCR on approximately 300 nasopharyngeal swabs from patients with varying viral load, demonstrated that the LC-MS based assay complements the current diagnostic strategies.

In order for this assay to be future-prove and to increase its added value compared to a classic RT-PCR, a strategy to rapidly adapt the method for the incorporation of ever emerging variants of concern (VoC), and even other types of respiratory viruses (e.g. Influenza, RSV) was developed.

CONCLUSIONS

We proved the capabilities and strengths of the application of LC/MS-based techniques in viral screening and set a template for the rapid development of new viral assays.

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T216

ANALYSIS OF DEMOGRAPHIC AND BIOCHEMICAL PARAMETERS IN COVID PATIENTS ADMITTED TO ICU

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BACKGROUND-AIM

The exaggerated response of cells infected by SARS-CoV-2, generates macrophage activation and cytokine disruption, increasing serum levels and those of acute phase reactants, including ferritin.

These increases correlate with the severity of the pathology and increased risk in the appearance of acute respiratory distress syndrome, as well as an impact on multiple organs and worse prognostic.

Objective

- Determine if the presence of macrophage activation syndrome (MAS) is related to a higher mortality rate in COVID patients admitted to ICU.
- Evaluate other parameters like age, gender, ferritin and length of stay of deceased coronavirus patients versus survivors.

METHODS

50 patients admitted to ICU with coronavirus. Collect gender, age, ferritin on different days, presence of MAS and mortality rate.

MAS was considered according to the presence of fever, hyperferritinemia, increase in the ratio of neutrophils to lymphocytes, increase in lactate dehydrogenase, D-dimer and interleukin-6.

A descriptive study, normality tests (Kolgorov-Smirnov-Lilliefors) and intergroup comparisons of parameters using Chi2, risk assessment and logistical regression for death were made.

RESULTS

Distribution: 27 men (54%) and 24 women (46%)

18 deaths (36%), men representing higher mortality (n=13; 75%) and a risk of 2.215 (IC95%: 0.929-5.279) times superior to the death of women, which was no significant.

30 out of 50 patients, presented MAS (60%), without significant findings related to the deceased (13) versus the survivors (17). The absence of MAS (20), was associated to survival in 75% of the patients (15).

The median age for MAS was 67 years, compared to 77 for non-MAS, with 23 and 15 days admission respectively ($p<0.05$; Mann-Whitney U Test).

The median ages did not present differences (72.5 vs 73 years) relating to death or not; however, more admission days for the deceased (23.5) versus survivors (16) were found.

For ferritin no significant differences in the results from serial days of extraction were found in both deceased and survivors.

We could not calculate the logistical regression for death considering sex, age and ferritin, because we only found significance for sex.

CONCLUSIONS

1. Ferritin is a useful parameter to assess macrophage activation and an acute inflammatory process, but it is not useful as the sole prognostic factor to predict death.
2. The male sex has higher mortality than the female, but in this cohort it has not been possible to find significant differences between the two, probably due to having an insufficient sample size
3. Age was not differential for the outcome.
4. The admission length were significantly longer in MAS and in patients who died, indicating a greater severity in the overall diagnosis.
5. A larger sample would be required as well as the assessment of other risk parameters to be able to predict mortality.

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T217

EVALUATION OF THE CAPACITY TO IDENTIFY SARS COV-2 POSITIVE SAMPLES BY SECOND AND THIRD-GENERATION ANTIGENIC TESTS

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BACKGROUND-AIM

Comparison of the SARS Cov-2 detection sensitivity between the GOLD STANDARD molecular test, second-generation antigenic tests and third-generation antigenic tests.

METHODS

200 nasopharyngeal swab samples collected in UTM COPAN were examined from the Covid clinics of ULSS 8 Berica in Vicenza.

The samples were processed with the SARS Cov-2 Cobas 6800 Roche test, which identifies two target genes in Real Time PCR by evaluating their Ct (Threshold cycle). Then the samples were tested with the 2nd generation SD Biosensor RELAB antigenic method (STANDARDTM F COVID-19 Ag FIA) and with the 3rd generation Lumira DxTM SARS Cov-2 Ag Test method.

RESULTS

Of the 200 samples tested, 106 (53%) were negative with all 3 tests considered. 94 (47%) samples were positive in the molecular test. Of these, 47 (50%) agreed with all 3 methods (with the Ct between 14 - 29); while 44 (47%) were positive only with the molecular test and negative with both antigenic methods. Samples positive only with the molecular method have a Ct greater than 32.

Of the 93 positive samples, 4 (4%) gave positive results with the 3rd generation antigenic method and negative with the 2nd generation antigenic method. These samples have a Ct between 25 and 32.

CONCLUSIONS

In conclusion, laboratory tests showed a 75% concordance between second-generation antigenic tests and molecular tests, while the concordance for third-generation antigenic tests was 77%. The difference of concordant results with molecular tests between second and third-generation stems from the fact that second-generation tests are able to identify the positivity only when the Ct of the molecular test is included in the range between 14 and 29. On the other hand, for the third-generation antigenic tests, the positivity of samples can be identified up to 32 Ct.

Even if, contrary to molecular tests, samples demonstrating a positivity characterized by a Ct over 32 cannot yet be detected by third-generation antigenic tests, these have nonetheless proven to be useful as instruments for mass screening thanks to their lower cost and execution time.

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T218

CYTOKINE PROFILE AND TUMOR BIOMARKERS IN COVID-19 PATIENTS: PROGNOSIS OF DISEASE SEVERITY?

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BACKGROUND-AIM

Coronavirus disease 2019 (COVID19) is a rapidly evolving infectious/inflammatory disorder with uncertain progression and outcome. When deciding on aggressive treatment such as supportive mechanical ventilation, it is important to assess the severity of the disease. Several studies have adressed pro-inflammatory cytokine serum levels as biomarkers; recently tumor marker serum levels were assessed as well since they may increase due to the covid-induced lung fibrosis.

METHODS

We conducted a retrospective non-randomised study that included three groups patients diagnosed with COVID19: 1/ 30 patients with mild to moderate disease, 2/ 30 patients on mechanical ventilation who recovered, 3/ 30 deceased patients. Cytokine profile of pro-inflammatory (IL-1beta, IL-12p70, IL-2, IL-4, IFN gamma, TNF alpha, IL-6) and regulatory (IL-10) cytokines were measures in patients´sera using the ELLA microfluidic platform. Tumor markers (CEA, CA 15-3, CYFRA 21.1, HE4) were measured using automated immunoassays (CLIA). We compared cytokine levels and tumor marker levels among groups.

RESULTS

Levels of IL-10 were significantly higher in group 3 compared to group 2 and 1 ($p = 0.014$). IFN gamma levels in group 1 were higher than in group 2 and 3, although non-significantly ($p = 0.062$). IL-6 levels were higher in group 3 than in group 1 and 2 (mean 97.4 pg/mL vs. 63.8 and 63.0, respectively). CEA and CYFRA 1.1 levels were significantly higher in group 3 than in group 1 and 2.

CONCLUSIONS

According our data, levels of cytokines IL-10, IFN gamma and IL-6 and tumor biomarkers CEA and CYFRA 21.1 might predict the life-threatening outcome of covid-19 disease and help in treatment decisions. Studies with larger patient groups are needed in order to support these findings.

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T219

LABORATORY FINDINGS IN PATIENTS WITH HEMORRHAGIC FEVER WITH RENAL SYNDROME IN KOSOVO

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BACKGROUND-AIM

Hemorrhagic fever with renal syndrome (HFRS) are a group of acute diseases and zoonosis which belong to the Bunyaviridae order of viruses. It is an illness that usually is found in Europe and Asia, and is endemic in the Balkan region.
This work is an analysis of retrospective and descriptive cases with Hemorrhagic fever with renal syndrome in Kosovo. The aim is to describe HFRS laboratory abnormalities and their course during the time of hospitalization.

METHODS

The used method for this research is the descriptive-retrospective method. Data collected here are taken from the history of patients with HFRS hospitalized at Infectious Diseases Clinical at the Clinical and University Center of Kosovo. There were in total 6 clinical cases. A link has been made between the blood parameters and three weeks of hospitalization (decursus). The preferred serological test used was ELISA test (enzyme-linked immunoassay) for measuring the levels of antibodies (anti-HFRS IgM and anti-HFRS IgG) against hantavirus at the time of admission.Laboratory parameters: Urea, Creatinine, ALT,AST, LDH and Albumine were measured using I-LAB650 and compared with reference values given by manufacturer. Full Blood Count were measured and analyzed by Micros ABX 60

RESULTS

Levels of immunoglobulins at the time of admission at hospital: #1 (IgG 2.075, IgM 3.012), #2 (IgG 1.226, IgM 1.036), #3 (IgG 2.446, IgM 3.045); #4 (IgG 2.41, IgM 2.802); #5 (IgG 0.079, IgM 0.747), #6 (IgG 1.715, IgM 3.079). Mean levels of LDH at first week of admission: 525 u/L, second week 705 u/L, third week 726 u/L. AST mean serum levels: 69 u/L (first week), 36 u/L (second week), 39u/L (third week). Mean blood urea first week: 19.77 mmol/l, second week 12.44 mmol/l, third week 6.79 mmol/l. Creatinine first week 242.7umol/l, second week 179.7 umol/l, third week 123.6 umol/l. Thrombocytopenia first week <33000/uL; second week 164000/uL; third week 230000 u/L

CONCLUSIONS

HFRS is manifested by a wide range of laboratory abnormalities including low platelet count, high hematocrit, low blood albumins, high white cell count, elevated transaminases and high levels of creatinine and urea in blood. Comparing blood parameters in relation with the weeks of hospitalization, helps monitor the severity of disease and promises a good outcome

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T220

STUDY OF THE NEUTROPHIL/LYMPHOCYTE INDEX IN HOSPITALIZED PATIENTS WITH COVID19

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BACKGROUND-AIM

The neutrophil/lymphocyte ratio (INL) is the ratio of the absolute number of neutrophils to the absolute number of lymphocytes. The knowledge of new predictors of poor evolution is crucial to correctly and effectively approach the patient with COVID-19. INL appears as a potential marker of systemic endothelial dysfunction and has the advantages of being inexpensive, rapid, non-invasive, and independent of other known factors, in asymptomatic subjects.
The objective of this study is to elucidate the usefulness of the INL parameter as a prognostic biomarker of mortality in patients with COVID-19.

METHODS

Patient samples were obtained the same day as hospital admission. The inclusion criteria were the following: be older than 18 years and give the PCR test for COVID-19 positive. Mortality was followed for 28 days. Blood counts were performed on an Abbott Diagnostics® "Sysmex" autoanalyzer on whole blood samples. The data were processed in the SPSS v21. To elucidate the association of the parameters with mortality, the Mann-Whitney test was performed.

RESULTS

A sample of 39 patients was obtained who were admitted to the ward and met the inclusion criteria. Of which 29 (74.4%) were men and 10 (25.6%) were women. Mortality was 20.5% (8 patients). Regarding the origin, 17 (43.6%) came from Infectious Diseases, 5 (12.8%) came from Internal Medicine, 3 (7.7%) came from Pulmonology, and 14 (35.9%) from Intensive Care.
Significant differences ($p < 0.040$) of the INL were observed between deceased and survivors, the INL being higher in the deceased. No differences were observed in the number of leukocytes, neutrophils, and lymphocytes.

CONCLUSIONS

We verified that the neutrophil/lymphocyte index can be useful to make a prognosis of mortality in patients with COVID-19 and thus be able to optimize the therapeutic strategy in these patients. It could be used together with other predictors, as an early prognostic marker in COVID-19 due to its low cost and accessibility.

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T221

GLYCEMIC MARKERS IN COVID-19, WHAT ABOUT THEM?

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BACKGROUND-AIM

Epidemiologic studies indicate that diabetes is a central contributor to severe COVID-19 morbidity and mortality (1). Recent scientific literature suggest a causal association between COVID-19 and the onset of diabetes (2), assuming the hypothesis of a potential diabetogenic effect of COVID-19 beyond well-recognized stress response associated with severe illness. However, there is a certain degree of argument, since other studies suggest that the association between COVID-19 and the onset of diabetes is unlikely.
Our aim is to study if COVID-19 disease can cause permanent changes on glycemic markers.

METHODS

We studied 70 hospitalized patients in our hospital for COVID-19 disease. We selected those who were not diabetic, among which; at the moment of their admission into the hospital, we had a prior result of HbA1c within 6 months. The first day of admission, we performed an analytical profile that included glucose and HbA1c among other parameters. The same analytical profile was repeated again between 3 and 6 months after discharge. We performed a Wilcoxon test to see if there are differences among glycemic markers over time.

RESULTS

Participants had a median (interquartile range (IQR)) age 68 (58-79), glucose 97 mg/dL (87-108) and HbA1c 5,7% (5,4-5,9) at baseline (prior to being hospitalized). According to their HabA1c level, 54% were considered pre-diabetic (American Diabetes Association (ADA) classification of diabetes) and 46% were normo-glycemic.
At the time of admission, glucose levels were 99 mg/dL (90-110) ($p=0.251$ in relation to baseline level) and HbA1c 6% (5,8-6,2) ($p<0,01$). In this case, 71% of the patients were pre-diabetic.
At the end of the study, glucose levels were 93 mg/dL (87-109) ($p=0.845$ regarding baseline level) and HbA1c 5,7% (5,4-6,0) ($p=0.564$). Pre-diabetic patients were 46% in this instance.

CONCLUSIONS

During COVID-19 disease, HbA1c levels are significantly increased, but this alteration is not permanent, considering that HbA1c return to baseline levels after 3-6 months.

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T222

PERFORMANCE AND COST BENEFIT IMPROVEMENT OF MULTIPLEX REAL-TIME PCR PLATFORM FOR THE ROUTINE DIAGNOSIS OF INTESTINAL PROTOZOONOSIS

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BACKGROUND-AIM

Infections caused by intestinal protists are widespread, constituting a worldwide public health problem. The introduction of molecular diagnostic platforms have added an unquestionable advantage over microscopic diagnosis. The objective of our study was to evaluate the diagnostic performance of multiplex real-time PCR, as well as their economic impact for routine use in microbiological laboratories.

METHODS

Descriptive analysis of the main clinical and epidemiological manifestations of patients with a positive result for the molecular panel AllplexTM GI-Parasite Assay (Seegene, Seoul, South Korea) was carried out at a secondary level hospital in Spain.
Sample collection was longitudinal and prospective from September 2020 to August 2021.
The multiplex real-time PCR utilized detects: Blastocystis hominis, Cryptosporidium spp., Cyclospora cayetanensis, Dientamoeba fragilis, Entamoeba histolytica and Giardia lamblia.
Sensitivity data and costs associated with the replacement of microscopy by PCR as first line diagnostic methods in the laboratory were compared with those obtained in the same period of 2018-2019.

RESULTS

A total of 1728 stool samples were analysed, of which 627 (n=36.3%) were positive for at least one protist species. The diagnosed microorganisms were: B. hominis (378; 60.3%), D. fragilis (330; 52.6%), G. lamblia (30; 4.8%), Cryptosporidium spp. (8; 1.3%) and E. histolytica (1; 0.16%). There were 117 (18.7%) co-infections mainly caused by Blastocystis spp. and D. fragilis (109; 93.2%).
We investigated the epidemiology of 181 positive patients, from which (158; 87.2%) were nationals and only two of them had a travel history. Up to 20 patients (11.05%) had some type of immunosuppression, of which 13 (76.4%) were infected by Blastocystis spp., (P=0.482). The most common reported symptoms were diarrhoea (64; 35.4%) and abdominal pain (58, 32.0%).
Compared to microscopy, the use of the PCR increased the diagnostic sensitivity from 10.4% with a processing cost per sample of 4.6€ in 2018-19, to 36.3% and a cost of 13.8€ per sample in 2020-21.

CONCLUSIONS

- The use of molecular techniques increases the diagnostic sensitivity of gastrointestinal parasitosis, compared to microscopy.
- The most common causes of infections were Blastocystis spp. and D. fragilis.
- Future work will focus on detecting the relation of certain genotypes of Blastocystis spp. and a worse outcome.

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T223

SARS-COV-2 ANTIBODIES DETECTION IN SALIVARY SAMPLES FROM COVID-19 PATIENTS AND VACCINATED INDIVIDUALS.

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BACKGROUND-AIM

SARS-CoV-2 Ab determination in saliva could be suitable for monitoring the viral spread and vaccination efficacy, especially in pediatric patients. We investigated N/S1-RBD IgG antibody levels in salivary samples of infectious-naïve vaccinated subjects and COVID-19 patients, further comparing levels with serum anti-SARS-CoV-2 S-RBD IgG.

METHODS

A total of 72 subjects were enrolled at the Padova University Hospital: 36 COVID-19 patients, including 25 pediatric patients, and 36 health care workers (HCW), who underwent a complete vaccination campaign with BNT162b2 (BioNTech/Pfizer). All collected a salivary sample, using Salivette (Sarstedt, Nümbrecht Germany), and 9 HCW collected salivary samples at three different times within the same day (before breakfast, at 10 am and at 2 pm). In addition, a serum sample and medical history's (time post symptoms onset or time from the first vaccine) were collected for all individuals. Salivary COVID-19 N/S1 RBD (sal-IgG) ELISA (RayBiotech, GA, USA) and anti-SARS-CoV-2 S-RBD IgG Ab (ser-IgG) (Snibe Diagnostics, Shenzhen, China) were used for determining IgG Ab.

RESULTS

Overall subjects' mean age (±sd) was 35.8±18.2 yrs. Age significantly differed ($p<0.001$) from COVID-19 patients [29.7±17.3 yrs] and HCW [47.1±12.9yrs]. Positive sal-IgG were found in 70/72 (97.2%) samples; in sera, 71/72 (98.6%) samples were positive to ser-IgG. The sal-IgG median levels differed from COVID-19 to vaccinated HCW, being 0.21kAU/L and 0.8 kAU/L ($p = 0.030$), respectively; median levels for ser-IgG in COVID-19 and vaccinated HCW were 135 kBAU/L and 940 kBAU/L, respectively ($p < 0.001$). Sal-IgG levels were not influenced by time post-symptom onset or time post-vaccination, both on vaccinated HCW (Spearman's r = -0.147, $p = 0.402$) and COVID-19 subjects (Spearman's r=0.027, $p = 0.986$).

Differently, Ser-IgG levels was not influenced by the time post-symptom onset for COVID-19 subjects (Spearman's r=0.102, $p = 0.419$), while a strong significant correlation was found with time post-vaccination in HCW (Spearman's r=-0.6292, $p < 0.001$). Sal-IgG levels were not influenced by the daytime of collection (Spearman's r = 0.148, $p=0.373$). Considering overall results, Passing-Bablok regressions showed that sal-IgG and ser-IgG comparability was assessable only when ser-IgG values were divided by 1000, being slope and intercept 0.068 (95%CI: 0.069-0.341) and 0.221 (95%CI: -0.097 to 0.786), respectively.

CONCLUSIONS

Salivary and Serum S-RBD IgG are efficiently detectable both in COVID-19 and in vaccinated individuals, and results appeared to be not influenced by the daytime of collection. The analyses performed showed that, overall, sal-IgG were lower than ser-IgG, and thus comparability with serum levels need to be better explored.

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T224

MICROBIOLOGIC DIAGNOSTIC OF GASTROINTESTINAL INFECTIONS: PCR VS STOOL CULTURE.

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BACKGROUND-AIM

Routinely, diarrheal pathogens are mainly identified by stool culture. Although a major disadvantage is the time-to-result, there are some pathogens such as diarrheagenic strains of Escherichia coli (EC) which are underdiagnosed and virulence gene detection techniques are necessary for their identification.
Our aim was to evaluate the impact of multiplex gastrointestinal polymerase chain reaction (GI PCR) pathogen panels on clinical practice compared to stool culture.

METHODS

This prospective cohort study includes patients with gastrointestinal symptoms attended in a secondary hospital in Spain. The bacteria isolated by stool culture were identified by Matrix assisted laser desorption ionization-time of flight (Maldi-TOF). The results were compared with those obtained by PCR (multiplex Seegene Allplex™ GI-Bacteria (I y II)).
GI-Bacteria (I) Assay: Aeromonas spp. (Aer), Campylobacter spp. (Cam), Clostridium difficile toxin B (CdB), Salmonella spp. (Sal), Enteroinvasive E. coli (EIEC) / Shigella spp. (Sh), Vibrio spp. (Vib), Yersinia enterocolitica (Yer).
GI-Bacteria(II) Assay: Enteroaggregative E. coli (EAEC), Enteropathogenic E. coli (EPEC), E. coli O157 (EC O157), Enterotoxigenic E. coli (ETEC), Enterotoxigenic E. coli (ETEC), Hypervirulent Clostridium difficile (CD hyper), Enterohemorrhagic E. coli (EHEC).

RESULTS

1.014 patients were included, median age 66 years (± 37), 503 women (49,5%). There were 82 positive stool cultures (8,3%): 65 Cam spp., 15 Sal spp. and 2 Aer spp.
A total of 285 pathogen were detected from 251 gastrointestinal infections diagnosed by PCR:
- 101 Cam (35,4%)
- 68 CdB (23,9%)
- 48 EPEC (16,8%)
- 15 Aer (5,3%)
- 14 Sal (4,9%)
- 13 Vib (4,6%)
- 10 EAEC (3,5%)
- 6 EIEC / Sh (2,1%)
- 5 EHEC (1,8%)
- 2 ETEC (0,7%)
- 2 EC O157 (0,7%)
- 1 Yer (0,4%)

C. difficile toxin was studied by rapid test (DIFF QUIK CHEK COMPLETE® TECHLAB) in 34 of the 43 positive PCR; 29 were positive (85,3%), 3 indeterminate and 2 negative.
It must be considered that none of the 73 (25,6%) diarrheagenic strains of E. coli were identified in stool culture and only 45 Cam spp. (25%) were isolated.

CONCLUSIONS

Multiplex GI PCR panels have improved and accelerated microbiological diagnosis, detecting up to 3.5 times more microorganisms than stool culture. Therefore this PCR detected 285 pathogens compared to the 74 isolated in stool culture (29,5%).
PCR has also led to an improvement in the detection of toxin-producing C. difficile infections compared to rapid tests, consequently, 14.7% of these infections would have gone unnoticed using only the rapid test.

Microbiology - Infectious diseases including COVID 19

T225

THE TITERS OF ANTISPIKE ANTIBODIES , AN INSTRUMENT TO MONITOR THE LEVEL OF IMMUNE PROTECTION IN MEDICAL STAFF

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BACKGROUND-AIM

Medical staff have been very much exposed to SARS-CoV-2 infection during the pandemic year 2020, compared to the regular population. The titers of protective antibodies, induced either through natural infection or vaccination, were measured and were found to be variable overtime. Monitoring the level of anti spike antibodies prospectively will help us generate data on the length of protective effect and allow defining the duration of protection against new reinfections with COVID 19.

METHODS

The titers of antiSARS COV 2 antibodies were tested in 414 members of the medical staff, using the testing kit for SARS COV 2 Trimeric IgG, on Liaison XL analyzer. We devided the cohort in 3 groups , as follows : first category were staff members who passed through the COVID infection in 2020 (64 persons, 15%) and did not get vaccinated, second category was represented by vaccinated persons (272 persons, 66%) and third category were staff members who passed the infection and were also vaccinated (78 persons, 19%) All our staff have been vaccinated with Pfizer/ BioNTech vaccine during january and february 2021. We performed measurements of the anti spike antibodies at 2 time points, in april 2021, 2 months after vaccination and in august 2021, 6 months after vaccination.

RESULTS

In the first category group the antibody mean titers were, in april 87AU/ml and in august 61 AU/ml, for the second category were 445 AU/ml in april and 176 AU/ml in august and for the third category 795 AU/ml in april and 279 AU/ml in august.

CONCLUSIONS

There is an individual variability in anti spike antibodies level. 10 -12 months after infection there is a low, but constant level of antibodies in personnel who was infected with COVID 19 during 2020.The personnel who received the vaccine has high levels of antibodies 6 month after vaccination. The highest level of antibodies can be found in personnel who passed the infection and also received the vaccine. Medical staff should get vaccinated in order to preserve their work capabilities

Microbiology - Infectious diseases including COVID 19

T226

EVALUATION OF SARS-COV 2 VIRAL LOAD ESTIMATION BY THE COMPARATIVE METHOD IN DIFFERENT AGE GROUPS

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BACKGROUND-AIM

Reverse transcription-polymerase chain reaction (RT-PCR) is an essential diagnosis method of SARS-CoV-2 infection as it is sensitive and specific enough to detect carriers in early stages of the disease. Data of previous studies suggest that older and/or co-morbid adults were at increased risk of worse progression of the infection. The cycle threshold (Ct) value has been employed to estimate quantitative viral load and guiding public health interventions. However, to employ RT-PCR as a quantitative method should necessarily include a human housekeeping gene (HG) in the analysis. The present study pretends to analyse the relationship between age and viral load employing the comparative method to estimate viral load.

METHODS

275 nasopharyngeal swabs specimens were obtained. Those had been previously analysed and tested positive with the RT-PCR kit TaqPathTM COVID-19-CE-IVD RT-PCR from ThermoFisher. The amplification targets were N, S and ORF1ab genes. Specimens from patients of different age ranges (<40, 41-60 and >60 years old) were selected.

Specimens were analysed by RT-qPCR employing the TaqPathTM 1-Step Multiplex Master Mix kit from Applied Biosystems and the 2019-nCoV CDC Probe and Primer kit for SARS-CoV-2 from Biosearch Technologies. The amplification targets were N1 and N2 regions from the viral N gene and the human HG RNAse P.

The Ct values from both methods were obtained and the normalisation was performed using the comparative method obtaining the value of 2-∆Ct.

The hypothesis contrasting was performed by the Mann-Whitney test, considering that it was significative when p-value<0.05.

RESULTS

The Ct value showed a trend to decrease with age but not always statistical differences were found. When the comparative method was employed, the relative viral load tended to increase with age and more statistical differences were found.

CONCLUSIONS

- The inclusion of a HG in the SARS-CoV-2 detection improve the viral load estimation compared to the Ct value alone.
- The viral load tends to increase with age, what could be related with the increased risk of adverse outcomes and death in older adults.

Microbiology - Infectious diseases including COVID 19

T227

APPLIANCE OF PARAMETERS OF RT-PCR IN THE PREDICTION OF COVID-19 SEVERITY

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BACKGROUND-AIM

Reverse transcription-polymerase chain reaction (RT-PCR) is an essential diagnosis method of SARS-CoV-2 infection as it is sensitive and specific enough to detect carriers in early stages of the disease. The cycle threshold (Ct) value has been employed to estimate quantitative viral load and guiding public health interventions. However, to employ RT-PCR as a quantitative method should necessarily include a human housekeeping gene (HG) in the analysis. The present study pretends to analyse the relationship between viral load and severity degree employing the comparative method to estimate viral load.

METHODS

238 nasopharyngeal swabs specimens were obtained. Those had been previously analysed and tested positive with the RT-PCR kit TaqPathTM COVID-19-CE-IVD RT-PCR from ThermoFisher. The amplification targets were N, S and ORF1ab genes. Specimens from patients of different severity degree (mild if patient did not need hospital admission and moderate if patient need assistance from doctors in internal medicine) were selected.

Specimens were analysed by RT-qPCR employing the TaqPathTM 1-Step Multiplex Master Mix kit from Applied Biosystems and the 2019-nCoV CDC Probe and Primer kit for SARS-CoV-2 from Biosearch Technologies. The amplification targets were N1 and N2 regions from the viral N gene and the human HG RNAse P.

The Ct values from both methods were obtained and the normalisation was performed using the comparative method obtaining the value of 2-∆Ct.

The hypothesis contrasting was performed by the Mann-Whitney test, considering that it was significative wh en p-value<0.05.

RESULTS

The Ct value showed a trend to decrease in the group with moderate severity compared with the group with mild severity and statistical differences were found for all the regions except in N1. When the comparative method was employed, the relative viral load was higher in moderate severity than in mild severity groups and statistical differences were found for all the regions including N1.

CONCLUSIONS

- The inclusion of a HG in the SARS-CoV-2 detection improve the viral load estimation compared to the Ct value alone.
- The viral load calculated by the comparative method is higher in patients with moderate severity compared patients with mild severity

Microbiology - Infectious diseases including COVID 19

T228

BANNWARTH SYNDROME. A CASE REPORT.

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BACKGROUND-AIM

Bannwarth syndrome (BWS) is a manifestation of neuroinvasive Lyme disease (LD) caused by infection with members of Borrelia burgdoferi sensu lato (Bbsl) complex. It is characterized by painful radiculoneuritis and lymphocytic pleocytosis in the cerebrospinal fluid (CSF), often associated with cranial nerve involvement and occasionally with peripheral paresis.

METHODS

36-years old men presented with low back pain radiating to both lower extremities of 14 days of evolution, and bilateral facial paralysis of 48h of evolution. During hospitalization, he suffers from sixth nerve palsy and binocular diplopia.

RESULTS

Brain and spine MRI imaging were negative. CSF analysis was remarkable for lymphocytic pleocytosis (437 leukocytes, 100% mononuclear cells) and elevated proteins (275.9mg/dL, normal values: 15-45mg/dL).
Thorough anamnesis revealed a history of circular cutaneous erythema 3-4 weeks ago, orientated as tinea in primary care; and a trip to a Borrelia endemic area 2 months before the beginning of the symptoms.
LD serologic testing was performed in serum and CSF, with detection of positive levels of anti-Bbsl IgG in both serum and CSF, and IgM in CSF. LD antibody index (AI), comparing anti-Bbsl IgG and IgM levels between CSF and serum for determination of pathogen-specific immunoglobulin intrathecal synthesis, was also performed. Results showed increased intrathecal synthesis of both anti-Bbsl IgM and IgG (AI IgM of 11.36 and AI IgG of 6.96, normal values: 1.3-1.5).
Based on clinical presentation, antecedents and serological results, the patient was diagnosed with Lyme Neuroborreliosis (LNB).

CONCLUSIONS

We report a case of LD with BWS in a non-endemic area. LNB accurate diagnosis requires a suggestive clinical profile in the right epidemiological context, in addition to clear microbiological criteria. The preferred test (but not available in all laboratories) to detect LNB is the determination of intrathecal production of anti-Bbsl antibodies by the AI assay - sensitivity of about 80% in LNB of short duration (<6 weeks) and nearly 100% in LNB of longer duration. Moreover, AI may often be positive even preceding serum antibody positivity. However, it should be taken into account that positive results do not prove active infection since positivity remains for months to years following resolution.

Microbiology - Infectious diseases including COVID 19

T229

COMPARISON OF QUANTITATIVE SARS-COV-2 SEROLOGICAL ASSAYS IN ALIGNMENT WITH THE WHO INTERNATIONAL STANDARD (NIBSC 20/136)

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BACKGROUND-AIM

SARS-CoV-2 antibody tests are widely used to monitor the serologic response after SARS-CoV-2 infection or vaccination. The WHO has developed a standard reference material (NIBSC 20/136) for the purpose of calibration of anti-SARS-CoV-2 antibody assays and harmonization of test results. The present study assessed the comparability of three commercial assays aligned to NIBSC 20/136 and their titers expressed as binding antibody units per mL (BAU/mL).

METHODS

Anti-SARS-CoV-2 antibody titers were measured with the Elecsys® Anti-SARS-CoV-2 S (Roche Diagnostics, total anti-spike), the LIAISON® SARS-CoV-2 TrimericS IgG (DiaSorin S.p.A., IgG anti-spike) and the SARS-CoV-2 IgG II Quant (Abbott Laboratories, IgG anti-spike) in serum samples from 131 COVID-19 convalescent individuals. Blood collections were performed at least 150 days after the first positive PCR test. All results were converted from their arbitrary units to those defined by the WHO using the following conversion factors as provided by the manufacturers: $y_{Roche}=x*0.972$, $y_{DiaSorin}=x*2.6$, $y_{Abbott}=x*0.142$.

RESULTS

Passing-Bablok regression of the converted results from Roche and DiaSorin showed neither proportional nor constant bias. In contrast, both assays showed substantial constant and proportional bias when compared to the Abbott assay (Diasorin: 95% CI_{slope} from 2.9 to 4.1, 95% $CI_{intercept}$ from -44.9 to -8.9; Roche: 95% CI_{slope} from 0.2 to 0.3, 95% $CI_{intercept}$ from 2.1 to 9.4). The converted results by Abbott and DiaSorin also exhibited the worst degree of linearity ($p < 0.01$, CUSUM test) compared to each other. Bland Altman analysis verified a predominantly proportional bias (mean of differences = 297 BAU/mL) and wide limits of agreement (95% CI from -1008 to 1602 BAU/mL). The IgG-specific assays from DiaSorin and Abbott exceeded the corresponding results from the total anti-SARS-CoV-2 assay from Roche by more than 30% in 34 and 7 cases, respectively.

CONCLUSIONS

For some, but not all anti-SARS-CoV-2 antibody assays, conversion of results according to the WHO standard leads to comparable values. Furthermore, in a significant number of subjects IgG-specific anti-SARS-CoV-2 assays yield substantially higher results, raising doubts about the validity of this conversion procedure.

Microbiology - Infectious diseases including COVID 19

T230

INTERLEUKIN-6 AS A PREDICTOR OF COMPLICATIONS IN COVID-19 PATIENTS

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BACKGROUND-AIM

The clinical symptoms of COVID-19 range from asymptomatic and mild to severe respiratory failure. Interleukin-6 (IL-6) is considered to be an important cytokine in the cytokine storm occurrence and COVID-19 complications development. In this study we aimed to investigate the predictive value of IL-6 for the severity of COVID-19.

METHODS

We analyzed 149 patients admitted to the Department of infective disease in a two-month period in 2020. Patients were divided into two groups: I) without complications; N=70 and II) patients who needed mechanical ventilation; N=79. Blood sampling was performed into anticoagulant free tubes. IL-6 was measured in serum samples by electrochemiluminescent method (ECLIA) using the COBAS e411 immunochemistry analyzer (Roche Diagnostics, IN, USA). COVID-19 diagnosis was confirmed by the PCR test in all patients. Statistical analysis was performed using MedCalc for Windows, version 12.4.0.0 (MedCalc Sofware, Mariakerke, Belgium). The Mann-Whitney test was used for group comparisons. Receiver operating characteristic (ROC) analysis was performed for calculating the optimal cut-o# values for IL-6.

RESULTS

Patients who developed complications were in average older (median age 60 years vs 48 years for patients with the mild form of the disease). The group of patients who subsequently developed severe complications consisted predominantly of males (59 vs 32 in the mild group). IL-6 differed significantly between the groups and was higher in patients in the severe group (127.0 µg/L 95% CI 113.1-154.9 vs 28.5 CI 16.6-41.4). The calculated cut-off value for the prediction of severe COVID-19 was 41.6 µg/L (sensitivity=91.1%; specificity=64.3 %; AUC 0.870).

CONCLUSIONS

IL-6 could be a useful marker for the prediction of a severe form of COVID-19 disease.

Microbiology - Infectious diseases including COVID 19

T231

HEPATITIS B VIRUS REACTIVATION IN PATIENTS WITH SEVERE COVID-19 TREATED WITH TOCILIZUMAB

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BACKGROUND-AIM

Hepatitis B virus (HBV) infection is an important global public health problem. Approximately 95% of infections are self-limited, nevertheless the virus may persist after serologic resolution, with potential to reactivate.

The treatment with immunosuppressive agents has been associated with HBV reactivation, thus patients should be screened for Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody (anti-HBs) and Total Hepatitis B core antibody (anti-HBc) prior to immunosuppression treatment.

The risk of reactivation is higher in HBsAg positive patients and HBV DNA > 2000 IU/mL, medium in HBsAg negative, anti-HBc positive and anti-HBs negative and it is low in HBsAg negative, anti-HBc positive and anti-HBs positive patients.

The aim of the study was to assess the risk of HBV reactivation after administration of different doses of Tocilizumab in patients with severe COVID-19.

METHODS

Retrospective study of patients with severe COVID-19 who were treated with Tocilizumab between March 2020 and July 2021 in our tertiary care hospital.

RESULTS

A total of 308 patients were included (70.45% men, median age 63.5 years).

113/308 (36.70%) patients had a baseline serology and none of them were HBsAg positive. 5/113 (4.42%) patients were HBsAg negative, anti-HBc positive and anti-Hbs negative. 10/113 (8.85%) patients were HBsAg negative and positive for both anti-HBc and anti-Hbs.

In 236/308 (77%) patients a single dose of Tocilizumab was administered. 88/236 (37.30%) patients were followed with serology. Among those, after treatment, 19/88 (21.60%) were anti-HBc positive and 86/88 (97.73%) were HBsAg negative. None were HBsAg positive.

Of the 70/308 (22%) patients who received a second dose of Tocilizumab, 31/70 (44.29%) have follow-up serology results. After complete treatment, all of them were HBsAg negative and 3/31 (9.68%) were anti-HBc positive.

Only 2/308 (1%) patients received three doses of the immunosuppressant and none was anti-HBc positive nor HBsAg positive after the last dose.

CONCLUSIONS

According to our results, the risk of HBV reactivation in patients with severe COVID-19 and treated with Tocilizumab is low, regardless of the dose of immunosuppressant.

Microbiology - Infectious diseases including COVID 19

T232

EVALUATION OF THE LIAISON XL AUTOMATED SARS-COV-2 ANTIGEN TEST

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BACKGROUND-AIM

Despite having less sensitivity than RT-PCR, SARS-CoV-2 rapid antigen (Ag) tests are relatively inexpensive and have been reported to display a good specificity. This makes them very useful in places at high risk for virus transmission where turnaround time is critical. Recently, high-throughput automated antigen tests have been developed, minimizing reader bias and claiming to provide more consistent results than lateral flow techniques. We aimed to evaluate the automated Liaison SARS-CoV-2 Ag assay in comparison with a reference technique in asymptomatic individuals.

METHODS

861 asymptomatic individuals were enrolled during January 2021. 291 (34%) had tested positive for SARS-CoV-2: 111 with <25 Ct, 51 with 25-30 Ct, and 129 with 30-40 Ct. The remaining 570 selected samples (66%) were RT-PCR negative for SARS-CoV-2. All samples were collected in nasal swabs and deposited in 3 mL universal transport medium (UTM/VTM). RT-PCR (Allplex SARS-CoV-2, Seegene) results were compared to antigen (SARS-CoV-2 Ag, Liaison XL) with a positive cutoff of 200 $TCID_{50}$/mL and an undetermined cutoff of 100 $TCID_{50}$/mL.

RESULTS

A strong correlation was observed between SARS-CoV-2 Ag and RT-PCR (r=0.649; p<0.0001) with significant differences between Ct in PCR positive/Ag positive and PCR positive/Ag negative samples, with a median of 21 [IQR: 18-24] and 34 [IQR: 32-36] respectively. Both thresholds detected all samples with Ct<25 and were not able to detect any sample with Ct>35. In the range between 25 and 30 Ct, the undetermined cutoff displayed a sensitivity of 72% and the positive cutoff of 41%. Overall sensitivity for samples <30 Ct was 91% for the undetermined cutoff and 81% for the positive cutoff. 4 PCR negative samples were classified as Ag undetermined; no false positives were observed with the 200 $TCID_{50}$/mL cutoff.

CONCLUSIONS

The Liaison XL automated SARS-CoV-2 antigen test is able to detect highly infectious individuals (Ct<25) with very high sensitivity and specificity, minimizing human bias normally associated to rapid antigen tests. Conversely, it is not useful in individuals with Ct>30. In a context of new variants with reported high viral loads and increasing vaccination, rapid and inexpensive identification of highly infectious asymptomatic individuals can be useful for outbreak management.

Microbiology - Infectious diseases including COVID 19

T233

LONG TERM HUMORAL IMMUNITY RESPONSE TO SARS-COV-2 MRNA VACCINE IN A LARGE COHORT OF HEALTH CARE WORKERS

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BACKGROUND-AIM

SARS-CoV-2 vaccination of healthcare professionals in a Tertiary Care University Hospital started December 2020. We assessed 6-months humoral immune response kinetics following PfizerBioNTech vaccine.

METHODS

Anti-spike antibody titer was determined before the first dose (T0) and 15, 90 and 180 days (T1, T2 and T3, respectively) following vaccination. The SARS-CoV-2 IgG II Quant assay (Abbott) was used to assay serum IgG antibodies against subunit S1, of SARS-CoV-2.

RESULTS

Participants (n=2897; 22.3% were male; mean age 45 years) were mostly naive before vaccination (median=0.0, IQR= 0.0-0.0 AU/mL), although 2.1% of them had IgG anti-SARS-CoV-2 reactivity above cutoff (>50 AU/mL).
Post vaccination, test reactivity was maintained throughout the study in 99.9, 99.8 and 99.7%, at T1, T2 and T3 timepoints, respectively. At T1 (median IgG=21x10^3, IQR= 13.5-33.1 x10^3 AU/mL), 97.6% presented a robust humoral response (>4160 AU/mL), whereas at three months (median=3.2x10^3, IQR= 2.0-5.1 x10^3 AU/mL) it decreased by 6.5-fold to 35.1% and then by 3.0-fold to 3.3% at 6 months (median=1.0x10^3, IQR= 0.65-1.7 x10^3 AU/mL). The Friedman's test revealed that there was a statistically significant difference in SARS-CoV-2 IgG throughout the follow up [χ^2(3)=8652.4, P<0.0001], further confirmed by Willcoxon between each and every timepoint (P<0.0001). A multiple linear regression was run to predict IgG titer at T1, T2 and T3 from gender, age and previous IgG measurements. These variables significantly predicted the titer at T1 (P<0.0001, R^2=0.049), T2 (P<0.0001, R^2=0.573) and T3 (P<0.0001, R^2=0.518). The pre-vaccination IgG titer, gender and age added significantly to the prediction of IgG at T1 (P<0.0001), even though in subsequent time points only previous IgG measurements accrued to estimate (P>0.0001).

CONCLUSIONS

The SARS-CoV-2 IgG II Quant assay, showed good performance in assessing humoral immunity after vaccination. Although these results looked promising, we report continuous waning of humoral response following the peak, 15 days after vaccination completion, still at 6 months follow up. Establishing a serological monitoring scheme may effectively provide information on humoral response to the vaccine, adding to reasoning for a third dose of vaccine.

Microbiology - Infectious diseases including COVID 19

T234

MONITORING SARS-COV-2 ANTIBODY RESPONSE PRE- AND POST-VACCINATION IN CHILDREN AND ADULTS: POTENTIAL UTILITY AND APPLICATIONS

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BACKGROUND-AIM

Serological testing for antibodies against SARS-CoV-2 has potential utility in monitoring immune response to viral infection and vaccination in children and adults. Several covariates have been suggested to impact humoral response to SARS-CoV-2, including history of chronic disease and age. The current study aims to validate the utility of available SARS-CoV-2 antibody assays in the longitudinal monitoring of humoral response pre- and post-vaccination in Canadian children and adults.

METHODS

The DiaSorin Liaison SARS-CoV-2 Trimeric Spike assay for the quantitative detection of SARS-CoV-2 IgG antibodies was validated at the Hospital for Sick Children, including precision, specificity (n=30 historic specimens) and sensitivity (n=10 specimens from PCR positive patients). Asymptomatic children, adolescents, and adults of varying vaccination status were recruited from the community. Participation required completion of a health questionnaire, informed consent, and blood donation. Serum specimens were then evaluated on the DiaSorin Liaison SARS-CoV-2 Trimeric Spike quantitative assay. Results were compared to clinical reference information, including vaccination status and past SARS-CoV-2 infection.

RESULTS

Analytical validation demonstrated excellent precision (CV=<2%), sensitivity (100%), and specificity (100%). Of clinical study cohort (n=201), 58 had received two doses of an mRNA SARS-CoV-2 vaccine (93% Pfizer, 5% Moderna, 2% Combination) and 143 were not vaccinated, mostly due to age ineligibility. All individuals post-vaccination had detectable antibody tests results on the DiaSorin SARS-CoV-2 Trimeric assay. Antibody titres decreased significantly post-vaccination (range: 10-200 days, concentration range: 400-20,000 BAU/mL). All non-vaccinated individuals with no history of past SARS-CoV-2 infection had negative antibody results.

CONCLUSIONS

This study demonstrates high clinical specificity and sensitivity of the DiaSorin Liaison SARS-CoV-2 Trimeric Spike quantitative assay for the detection of SARS-CoV-2 IgG antibodies pre- and post-vaccination. Further immunological testing is needed to better define correlation to immune protection against SARS-CoV-2 in adolescents and adults post-vaccination, including neutralization assays and T cell phenotyping.

Microbiology - Infectious diseases including COVID 19

T235

AN EVALUATION OF PERFORMANCE OF THE VITROS ® IMMUNODIAGNOSTIC PRODUCTS ANTI-SARS-COV-2 IGG QUANTITATIVE ASSAY

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BACKGROUND-AIM

This study was designed to assess the clinical and analytical performance of the VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Quantitative Reagent Pack (VITROS IgG Quant) on the VITROS family of analyzers. The assay is for the quantitative detection of SARS-CoV-2 IgG antibodies with calibration traceable to the First WHO International Standard for Anti-SARS-CoV-2 Immunoglobulin, NIBSC (20/136). Results are reported in both qualitative (reactive/non-reactive) and quantitative values (Binding Antibody Units/mL (BAU/mL)).

METHODS

The assay reportable range was determined by calculation of the assay limit of detection/quantitation and demonstration of linearity across the range. Clinical specificity was assessed using 533 samples from healthy blood donors collected prior to the start of the COVID-19 pandemic. Sensitivity (Percent Positive Agreement (PPA)) was evaluated using samples from 232 RT-PCR positive individuals collected ≥14 days since reported symptom onset. PPA to PCR positivity was calculated. Reproducibility was evaluated consistent with CLSI EP05. Two replicates of 6 fluids, were tested on 2 occasions/day with 2 reagent lots across 2 instruments over 3 days. Quantitative results of 60 vaccinated individuals were assessed prior to vaccine, after the first dose and after the second dose.

RESULTS

The assay reportable range is 2-200 BAU/mL and, with dilution, up to 4000 BAU/mL.The clinical specificity of the VITROS Anti-SARS-CoV-2 IgG Quantitative assay for the 533 pre-pandemic healthy blood donors was 100.0% (533/533) [95% exact CI (99.3-100.0%)]. The PPA with RT-PCR for 232 PCR positive individual's samples collected 14 days or more from onset of symptoms was 94.0% (218/232) [exact 95% CI (90.1-96.7%)]. The observed total reproducibility for the 6 panel members ranged from 0.4 to 14.9 %CV. The assay demonstrated a quantitative increase in antibody titer throughout the course of vaccination and all but one vaccinated individual demonstrated a result >600 BAU/mL after the second vaccine dose.

CONCLUSIONS

The VITROS IgG Quant assay demonstrates excellent clinical and analytical performance and can detect quantitative antibody response to COVID-19 infection or vaccination.

Microbiology - Infectious diseases including COVID 19

T236

SARS-COV-2 VARIANTS OF CONCERN DETECTED BY THE SIEMENS HEALTHINEERS CLINITEST RAPID COVID-19 ANTIGEN TEST

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BACKGROUND-AIM

The COVID-19 pandemic has resulted in several variants of the virus. These arise from mutations within the genome that occur during replication and are characterized relative to the initial isolate from Wuhan, China, 2019. Variants with demonstrated increased transmissibility or virulence or that have an impact on vaccines, therapeutics, and diagnostics are defined as variants of concern (VOCs). As of August 12, 2021, the World Health Organization lists four SARS-CoV-2 strains as VOCs: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2). VOCs have mutations in the N gene that encodes the nucleocapsid protein target of antigen tests. Lateral flow rapid antigen assays provide quick, convenient, and inexpensive testing that has been critical for the pandemic response. This study examines the impact of the four VOCs on the Siemens Healthineers CLINITEST® Rapid COVID-19 Antigen Test*.

METHODS

To determine that the Siemens Healthineers CLINITEST® Rapid COVID-19 Antigen Test recognizes each VOC, multiple approaches were taken. Evaluation of nucleocapsid amino acid sequence data was performed in silico using the GISAID database and Clustal Omega sequence alignment tool. Multiple samples of each VOC with varying RT-PCR Ct values were also tested. A minimum of six replicates were run on each sample using multiple reagent lots and visual readers. Variant type was established through molecular methods, and the presence of antigen was also confirmed using the high-throughput Siemens Healthineers Atellica IM SARS-CoV-2 Antigen Assay.

RESULTS

Sequence analysis shows no more than four residues of the 419 amino acid nucleocapsid are mutated, with ~ 99% similarity compared to the original sequence. For each VOC clinical sample, the number of positive results out of the number tested is listed: Alpha (3/4), Beta (3/3), Gamma (8/8) and Delta (3/3).

CONCLUSIONS

Sequence analysis shows only minor mutations occur to nucleocapsid protein suggesting current VOCs will have negligible impact on assay performance. Additionally, data from clinical samples confirms the ability of the CLINITEST® Rapid COVID-19 Antigen test to detect these VOCs and supports its continued use in the ongoing pandemic.
* CLINITEST Rapid COVID-19 Antigen Test and CLINITEST Rapid COVID-19 Antigen Self-Test are distributed by Siemens Healthcare Diagnostics Inc.
Not available for sale in the U.S. Product availability may vary from country to country and is subject to varying regulatory requirements.

Microbiology - Infectious diseases including COVID 19

T237

EVALUATE THE EVOLUTION RATE OF THE NEW SARS-COV-2 DELTA VARIANT

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BACKGROUND-AIM

The global monitoring of the SARS-CoV-2 genetic sequence has enabled the surveillance of variants. Among all the SARS-CoV-2 variants, those with specific genetic markers that are predicted to affect transmission, diagnostics, therapeutics, or immune escape.

Recently, the WHO has named 4 variants: Alpha, Beta, Gamma and Delta. The Delta variant (PANGO lineage B.1.617.2) was first detected in India in October 2020. This new variant caused more transmissible than the previously-dominant Alpha variant due to mutations located at the S-gene sequence L452R and P681R.

The aim of this study was to follow up the incidence of the SARS-CoV-2 Delta variant in the geographical area covered by our health area.

METHODS

Nasopharyngeal samples with a positive result at the SARS-CoV-2 RT-PCR (TaqPathTM COVID-19, ThermoFisher) were retested with the intention of looking for the specific genetic markers of the different variants. For this purpose, we used the multiplex RT-PCR ALLPLEX™ SARS-CoV-2 Variants I and Variants II (Seegene) and the TaqMan SARS-CoV-2 Mutation L452R (ThermoFisher).

RESULTS

From 1st May until 31st August 2021, we tested 52134 nasopharyngeal swabs with 3687 positive results (7.07%). During this period, we detected these variants: 1 Eta (B.1.525), 1 Lambda (C.37) 5 Gamma (P.1), 23 unknown, 58 inconclusive, 1280 Alpha (B.1.1.7) and 2319 Delta (B.1.617.2).

CONCLUSIONS

In May 2021, when the Delta variant was first detected in our laboratory, the prevalence of the Alpha Variant was more than 95%. Since July, the prevalence of the Delta variant was more than 90% reaching the 97.5% prevalence in August. The Results show how in less than 2 months, Delta variant has replaced the Alpha variant as the most prevalent variant of the SARS-CoV-2 in our health area.

Microbiology - Infectious diseases including COVID 19

T238

MENINGOENCEPHALITIS CAUSED BY WEST NILE VIRUS IN SOUTHERN SPAIN

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BACKGROUND-AIM

Central nervous system infections can appear with nonspecific symptoms (fever, disorientation), so the correct anamnesis together with the study of cerebrospinal fluid (CSF), serological and imaging tests are essential to establish the diagnosis in these patients.

West Nile Virus (WNV) is a zoonotic arbovirus, belonging to the Flaviviridae family, transmitted to humans through arthropods (mosquitoes Culex) from its usual reservoir (usually birds). Mammals, mainly humans and horses, are accidental hosts and lack the ability to transmit the virus to mosquitoes.

Under certain environmental conditions, the number of infected vectors can increase, and therefore the risk of transmission to humans. Most human WNV infections are asymptomatic or produce moderate to high fever, weakness, and myalgia. In less than 1% of cases, the infection manifests as a neuroinvasive disease.

During the years 2017 to 2019, the activity of the WNV was in decline in the Spanish state, with very few notifications of equine foci and no human cases. On the other hand, during the past year, up to October 2020, 71 cases of meningoencephalitis were reported in the Andalusia region, in southern Spain; 57 in Seville and 14 in Cádiz.

The predominant clinical symptoms are fever, headache, myalgia, and meningitis or flaccid paralysis. The diagnosis is made by detecting antibodies (IgM or IgG) or nucleic acid amplification tests (NAAT) in cerebrospinal fluid (CSF), blood, or serum.

The aim of the present study was to describe the West Nile meningoencephalitis occured in our hospital area and to evaluate the main clinical symptoms and analitical parameters found in the patients.

METHODS

We studied patients in our hospital area with symptoms of meningoencephalitis compatible with West Nile virus infection in the season of onset of symptoms.

Analytical and imaging tests are performed. Among the analytical tests carried out, the determination of IgG and IgM antibodies in serum and cerebrospinal fluid (CSF), and the determination of nucleic acids (NAAT) are also carried out in both samples.

We carried out a descriptive study analyzing the main clinical symptoms and altered analytical parameters found in the patients.

RESULTS

The 11 patients analyzed (45.5% women and 54.5% men), between the end of August and September 2020, had ages between 44 and 87 years (with a median age of 72 years).

In 45% of the cases, the days until admission from the onset of symptoms was 1 day. The days of hospital stay were between 5 and 18, with a median of 11 days.

Among the previous predominant pathologies in these patients, we found that 54% had hypertension or type 2 diabetes mellitus, and 45% had dyslipidemia or some type of immunosuppression.

In 72% of the cases the presenting symptom was fever, 100% of the patients studied had fever during admission. Other predominant symptoms found were disorientation (90.9% of cases) and digestive symptoms such as vomiting or nausea (81.8%).

Imaging tests found 63.6% of patients with signs of chronic ischemia on Magnetic resonance imaging (MRI) or Positron emission tomography (PET).

Among the most notable analytical parameters, we found hyponatremia, increased c-reactive protein, and increased cerebrospinal fluid proteins:

The median of the sodium values was 133 mEq/L, with values between 126 and 140 mEq/L, the reference values being 136 to 145 mEq/L.

The median C-reactive protein was 21mg/L, with values between 3 and 580 mg/L, with reference values from 0 to 5 mg/L.

The median protein in cerebrospinal fluid was 63.25 mg/dL, with values between 29.1 and 94.2 mg/dL (reference values 15-50 mg/dL).

The etiological diagnosis was established by the detection of antibodies or by detection of NAAT.

Of the 11 patients analyzed, IgM antibodies were detected in serum in 100% of cases, and IgG antibodies in serum in 54.5%. The detection of IgM antibodies in cerebrospinal fluid occurred in 72.7% of the cases.

Virus nucleic acids were only detected in 1 patient in CSF and in 2 patients in urine.

CONCLUSIONS

Most patients with meningoencephalitis with suspicion of West Nile Virus infection were diagnosed by detection of serum IgM antibodies.

All patients presented fever during admission, being in 72% of cases the symptom of the onset of the disease. There was a significative number of patients with disorientation and digestive symptoms.

The decreased medians of sodium, and increased C-reactive protein and protein in cerebrospinal fluid in those patients were common indications in these patients. Chronic ischemia in imaging tests was also found frequently.

It is possible that the decrease in measures aimed at reducing the reproduction of mosquitoes, due to the COVID-19 pandemic, has caused an increase in the reproduction of vector mosquitoes and in transmission to humans in southern Spain. The time of greatest risk for the transmission of the disease is the end of summer, since the birds have already begun their migrations and the mosquitoes therefore turn to humans for their food.

Symptoms of WNV meningoencephalitis are common to other causes of meningoencephalitis, so the patient's medical history can guide the diagnosis, and infection with this virus should be considered in endemic areas, especially during the summer months. This would have repercussions in a shorter hospital stay and a lower consumption of resources and less comorbidity associated with the infection.

Microbiology - Infectious diseases including COVID 19

T239

SARS-COV-2 IGG TARGETTING RBD OF S1 PROTEIN LEVELS AFTER COMIRNATY VACCINATION MEASURED ON UNICEL DXI800

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BACKGROUND-AIM

COVID-19 vaccination is an important tool to help stop the pandemic situation. SARS-CoV-2 vaccination in Czech Republic started on December 27th, 2020. First vaccination was provided by mRNA Comirnaty vaccine. This study is evaluating level of IgG antibodies after vaccination in during the time.

METHODS

Samples were measured in Beckman Coulter Unicel 800 DxI analyzer using Access SARS-CoV-2 IgGII assay. That assay is semiquantitative targeting RBD of the S1 protein. Many studies indicated that an antibody response from this region may be neutralizing to SARS-CoV-2. The initial cohort was 37 people, 25 of them provided all data. Comirnaty vaccine was administered according to guideline in two doses, 3-4 weeks apart. Blood samples were taken on day 14 (ie 14 days after the 1st dose), then on day 7 after the 2nd dose and then 3 weeks after the 2nd dose of the vaccine. The obtained data were processed by statistical software SigmaPlot (Systat, USA) and MS Excel. Results were presented as box plot graph and as a MS excel (each proband in separate line). Some probands were monitored for long time IgG levels. The hypothesis of the dependence of antibody production on age was verified by Spearman correlation for 37 probands 14 days after 1st dose. Site effects of vaccine was evaluated after 1st and 2nd dose.

RESULTS

The median of IgG level 14 days after the first dose of vaccine was 32.3 AU/ml. This value is above the cut off value specified by the manufacturer (10 AU/ml). The initial response to the vaccine corresponds to the primary response after the first exposure to the virus. Another dose of vaccine causes a fold increase in this component of the immune response. This increase was found in all probands in the test group 1 week after the 2nd dose of vaccine. Three weeks after the 2nd dose, a decrease in antibody production was found in 84% of probands, 16% of people had an increase in production even after this time. This increase was verified in these individuals by repeated sampling in the next week, when a decrease in antibodies was found.

CONCLUSIONS

In our study group an effect on B cells in terms of antibody production was found after mRNA vaccination. The Beckman Coulter SARS-CoV-2 IgGII semiquantitative assay is suitable for monitoring antibody levels. The study is still on going to monitor more long term IgG antibodies levels.

Microbiology - Infectious diseases including COVID 19

T240

EVALUATION OF SENTINEL DIAGNOSTICS COVID-19 ANTI-S1 TOTAL AB TEST, A NEW IMMUNOTURBIDIMETRIC ASSAY FOR THE DETECTION OF TOTAL ANTI-S1 ANTIBODIES IN HUMAN SERUM AND PLASMA.

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BACKGROUND-AIM

Serology methods are extremely helpful in understand the SARS-CoV-2 spread in the population and to monitor the immunity coverage given by vaccination programs rolled out, especially with methodology able to discriminate neutralizing from non neutralizing antibodies.

METHODS

Sentinel Diagnostics has developed the COVID-19 Anti-S1 Total Ab test, a new immunoturbidimetric assay for the semiquantitative determination of human total antibodies specific for Spike protein S1 of SARS-CoV-2 in human serum and plasma. The test is based on PETIA technology. The product has the advantage to be applicable on most of the clinical chemistry open platforms.
Aim of the study was to evaluate the analytical and clinical performances of the COVID-19 Anti-S1 Total Ab in a clinical lab on clinical chemistry platform SENTiFIT® 270. Study design and acceptance criteria were from "IFCC Interim Guidelines on Serological testing of Antibodies against SARS-CoV-2" document.

RESULTS

The COVID-19 Anti-S1 Total Ab showed a limit of detection of 11.39 BAU/mL. A clinical sensitivity of 98.5 % and a clinical specificity of 93.2 % were observed on 264 PCR tested serum samples (205 negative samples). No cross reactivity on a panel of 90 serum samples IgG positive for major infective viruses or on a panel of 60 IgG positive samples to Cytomegalovirus was found. 479 (105 positive and 374 negative) serum samples have been, under routine conditions, tested with the COVID-19 anti-S1 Total Ab and with the routine CLIA method. The clinical concordance resulted 98.1% (95% CI: 96.8%-99.3%) as Area Under ROC curve (AUC).

CONCLUSIONS

Overall, the COVID-19 Anti-S1 Total Ab test met the requirements of robustness in terms of analytical endogenous interferences, of absence of cross-reactivity versus other respiratory virus diseases and of clinical specificity and sensitivity versus PCR. Clinical Performance of the PETIA reagent was comparable with the routine CLIA method, thus offering a valid alternative testing method on an open clinical chemistry platform. Additionally , the use of COVID-19 Anti-S1 Total Ab test in combination with COVID-19 Anti-NC Total Ab test can support a differential analysis of the immunological status of the COVID-19 seropositivity (vaccination vs infection).

Microbiology - Infectious diseases including COVID 19

T241

EVALUATION OF SENTINEL DIAGNOSTICS COVID-19 ANTI-NC TOTAL AB TEST, A NEW IMMUNOTURBIDIMETRIC ASSAY FOR THE DETECTION OF TOTAL ANTI-NUCLEOCAPSIDE (NC) ANTIBODIES IN HUMAN SERUM AND PLASMA.

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BACKGROUND-AIM

Different studies bring evidence that the amount and persistence of Abs generated by an exposure to SARS-CoV-2 and by vaccination is different. This may have several implications on immunity, from the efficiency of infection coverage to the length of protection. The use of a combined system to efficiently quantify and discriminate anti-SARS-CoV-2 antibodies generated by different antigenic determinants can efficiently help to monitor the virus spread, the vaccination program follow-up and population immunity coverage.

METHODS

Sentinel Diagnostics has developed the COVID-19 Anti-NC Total Ab test, a new immunoturbidimetric assay for the semiquantitative determination of human total antibodies specific for Nucleocapsid protein (NC) of SARS-CoV-2 in human serum and plasma. The test is based on PETIA technology.
The aim of the study was to evaluate the analytical and clinical performances of the COVID-19 Anti-NC Total Ab in a clinical lab on clinical chemistry platform SENTiFIT® 270. Study design and acceptance criteria were from "IFCC Interim Guidelines on Serological testing of Antibodies against SARS-CoV-2" document.

RESULTS

The COVID-19 Anti-NC Total Ab showed a limit of detection of 5.4 BAU/mL. A clinical sensitivity of 93.7 % and a clinical specificity of 78.0 % were observed. No significant cross reactivity on a panel of 90 serum samples IgG positive for other major infective viruses and no cross reactivity on a panel of 60 IgG positive samples to Cytomegalovirus was found. 479 serum samples have been tested with the COVID-19 anti-NC Total Ab and with a routine CLIA method. The clinical concordance resulted 95.8 % as Area Under ROC curve (AUC).

CONCLUSIONS

Overall, the COVID-19 Anti-NC Total Ab test met the requirements of robustness in terms of analytical endogenous interferences, of absence of cross-reactivity versus other viral diseases and of clinical specificity and sensitivity versus PCR. Clinical Performance of the PETIA reagent was comparable with the routine CLIA method, thus offering a valid alternative testing method on an open clinical chemistry platform. Additionally, the use of COVID-19 Anti-NC Total Ab test in combination with COVID-19 Anti-S1 Total Ab test, can support a differential analysis of the immunological status of the COVID-19 seropositivity (vaccination vs infection).

Microbiology - Infectious diseases including COVID 19

T242

PERFORMANCE OF ROCHE ELECSYS SARS-COV-2 ANTIGEN ASSAY IN DIFFERENT SETTINGS

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[1]*Elblab, Elblandkliniken, Meißen*

BACKGROUND-AIM

SARS-CoV-2 antigen rapid diagnostic tests (Ag-RDT) are an important tool in patient management on hospital admission. While a plethora of publications are comparing different Ag-RDT versus RT-qPCR, this study will examine the performance of Roche Elecsys SARS-CoV-2 Antigen assay in different settings.

METHODS

For antigen testing Roche Elecsys SARS-CoV-2 Antigen assay (Mannheim, Germany) and for RNA testing Seegene Allplex SARS-CoV-2/Flu/RSV RT-qPCR (Seoul, Republic of Korea) were used according to the manufacturer's instructions. We retrospectively analyzed 3434 Ag-RDT results with corresponding RT-qPCR results. Ag-RDTs were performed on four different sites (Hospital Radebeul and Ambulatory Test Center Meißen with Roche Cobas e411, Hospital Riesa and Hospital Meißen with Roche Cobas 6000/e601) between 10.03.21 and 24.05.2021. At hospital admissions all patients were tested in parallel for antigen and RNA, whereas at the Ambulatory Test Center only antigen positive persons received an RT-qPCR test.

RESULTS

From all 3434 records 255 were tested positive and 3179 were tested negative using RT-qPCR. In the beginning we observed a high rate of false positive Ag-RDT results on both e411 averaging 5,0% (5/101) at Ambulatory Test Center Meißen and 6.5% (38/582) at Hospital Radebeul). After implementation of specimen centrifugation the false positive rate of e411 in Radebeul decreased to 0.4% (1/269), which is comparable to the performance of both Cobas 6000 with uncentrifuged samples (0.5% (6/1186) in Hospital Meißen and 0.8% (9/1157) in Hospital Riesa). Another issue was the concomitant performance of Elecsys SARS-CoV-2 Antigen assay and Elecsys Anti-SARS-CoV-2 assay on the same Cobas 6000, which led to a false positive rate of 12% (14/113).

CONCLUSIONS

The e411 test performance might be impaired by suspended matter like mucus or cellular debris and can be recovered by centrifugation. Whereas the e601 module showed good performance with native specimens. Due to reagent carryover, Ag-RDT should not be combined with the Elecsys Anti-SARS-CoV-2 assay on the same device using standard protocols.

Microbiology - Infectious diseases including COVID 19

T243

DIRECT COMPARISON OF FOUR DIFFERENT SARS-COV-2 ANTIGEN RAPID DIAGNOSTIC ASSAYS

J. Gebauer [1], C. Kutschker [1], A. Prantz [1], D. Krnac [1], S. Hofmann [1], B. Rolinski [1]
[1]*Elblab, Elblandkliniken, Meißen*

BACKGROUND-AIM

SARS-CoV-2 antigen rapid diagnostic tests (Ag-RDT) are a key tool in controlling Covid-19 pandemic. While there are many publications describing Ag-RDT in parallel with RT-qPCR results, the direct comparison of different Ag-RDT is limited due to preanalytical issues. Here we compare for different Ag-RDT avoiding preanalytical bias.

METHODS

We collected UTM or VTM-N specimen of combined oropharyngeal and deep nasal swabs from patients either symptomatic or with preceding positive Ag-RDT. UTM (Copan, Mast Group, Reinfeld, Germany) specimens were stored at -20°C and VTM-N (Citoswab, Nal von Minden, Moers, Germany) specimens at +4°C until analysis. We used Standard Q SARS-CoV-2 Rapid Antigen Test(SQ), Standard F COVID-19 Ag FIA (SF) (both SD Biosensor Inc., Gyeonggi-do, Republic of Korea), Lumipulse G SARS-CoV-2 Ag(FR)(Fujirebio, Tokyo, Japan) and Roche Elecsys SARS-CoV-2 Antigen assays(RE) (Mannheim, Germany). For SQ and SF the specimen was mixed 1:1 with the corresponding extraction buffer. For FR 20% of sample extraction solution and for RE 10% of extraction solution C were added to the specimen. The assays were performed according to the manufacturer Instructions.RNA testing was done with Seegene Allplex SARS-CoV-2/Flu/RSV RT-qPCR (Seoul, republic of Korea).

RESULTS

For Copan UTM we analyzed 26 pcr-negative specimens and 30 pcr-positive specimens with a mean ct-value of 19.73, whereof 4 specimens had ct-values > 30. For VTM-N we analyzed 46 negative and 66 positive specimens with a mean ct-value of 22.31, whereof 8 specimens had ct-values > 30. Using UTM accuracies were 87,5% for SQ and SF and 92,9% for FR and RE. Using VTM-N accuracies were 56,4% for SQ, 64,6% for SF, 86,4% for FR and 84,6% for RE.

CONCLUSIONS

With all four tests UTM showed a better performance than VTM-N, most likely caused by the protein degrading guanidine salt in VTM-N. FR and RE showed a significantly higher accuracy than SQ and SF. Interestingly we found no difference between SQ and SF, although SF is advertised as more sensitive as SQ, justifying the much higher price. While FR has the convenience of quantitative results, RE has the advantage of shorter measurement time (RE 18 min vs FR 35 min). In summary, higher accuracy and automation are clear benefits of Ag-RDT by laboratory tests.

Microbiology - Infectious diseases including COVID 19

T244

INTERNAL EVALUATION STUDY OF ACTIVITY DETERMINATION IN SARS-COV-2 REACTIVE T CELL

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BACKGROUND-AIM

Both, humoral and celullar inmune response, play a decisive role in the development of protective immunity to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).
The aim of this study was to evaluate Wantai SARS-CoV-2 IGRA test in vaccinated and not vaccinated population, according to the anti-spike protein receptor-binding domain (S-RBD) IgG levels.
This study involved 53 participants using two-stage random sampling stratified by:
a)Covid-19 fully vaccinated
b)Covid-19 not vaccinated
c)Covid-19 not vaccinated with previous coronavirus infection

METHODS

The study was scheduled to be completed within 2 weeks. So that, blood serum and whole-blood in heparin specimens were collected in all participants 1 week apart.
Wantai Interferon-gamma Releasing Assay (IGRA) is based on the incubation of whole blood samples in specific antigen derived from SARS-CoV-2. T lymphocytes from most persons that have benn infected with SARS-CoV-2 will release interferon gamma (IFN-γ) in response to the antigen stimulation.
Whole-blood was incubated in each SARS-CoV-2 stimulation tube (3 tubes each subject). IFN-γ levels were evaluated by ELISA method, according to manufacturer's instructions.
S-RBD SARS-CoV-2 IgG levels were measured by chemiluminescent microparticle immunoassay in Abbott Alinity C automate in serum specimen.
Data were analyzed using Origin Software.

RESULTS

Results have been interpretated according to Wantai’s IFU instructions

- Of the total 32 of the SARS-CoV-2 vaccinated patients involved in the study, 100% of them were positives for S-RBD SARS-CoV-2 IgG antibodies and showed T cell response.
- 100 % of the subjects with previous coronavirus infection who were unvaccinated (3 participants) were positives for S-RBD SARS-CoV-2 IgG antibodies and showed T cell response.
- Of the total of 18 participants not vaccinated and with no reported symptoms of previous coronavirus infection 17 were negative for both S-RBD IgG antibodies and celullar inmune response, except for one patient how showed a weak positive result for S-RBD IgG antibodies and T cell response

CONCLUSIONS

In summary, Wantai SARS-CoV-2 IGRA test can be easily integrated in the laboratory as an aid in the diagnosis of specific T cellular immune response of SARS-CoV-2 spike protein after vaccination or infection.

Microbiology - Infectious diseases including COVID 19

T245

GOOD CONSENSUS IN SARS-COV-2 EQA SCHEMES

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BACKGROUND-AIM

External quality assessment (EQA) provider Labquality responded to the urgent need for proficiency testing (PT) for methods detecting SARS-CoV-2 by arranging international EQA pilot schemes for nucleic acid testing (NAT), antigen (Ag) detection, and antibody (Ab) detection in 2020. This year, the three individual schemes are conducted four times each. Participation in EQA schemes should be part of the quality routine of clinical laboratories and point-of-care testing sites to produce reliable patient results.

METHODS

A SARS-CoV-2 NC_045521 (wild-type, WT) whole genome cDNA swab sample was used on every NAT round and variants B1.1.7 and B1.351 positive swab samples were used on different rounds. For the Ag rounds, a synthetic SARS-CoV-2 nucleocapsid protein swab sample was used as positive sample. Proprietary excipient matrix formulations were used as negative samples. For the Ab rounds, one-donor SARS-CoV-2 ab positive sera/plasmas were used as positive samples and negative sera/plasmas, donated before the outbreak of the pandemic, were used as negative samples. Samples were shipped at ambient temperature conditions as they are stable at +2 - +30 °C. Results and methods were reported through Labquality's electronic LabScala portal for processing.

RESULTS

For the NAT rounds (3 swabs/round), the number of total reported results using tests by 77 different manufacturers were 1379 for the WT positive samples (success rate 99.6%), 420 for B.1.1.7 positive sample (SR 100%), 351 for B1.351 positive sample (SR 99,1%), and 1058 for the negative samples (SR 98.4%). For the Ag rounds (2 swabs/round), reporting included 698 results for the positive (SR 99.0%) and 699 results for the negative (SR 98,7%) samples using test by 37 manufacturers. For the Ab rounds (3 samples/round), reporting included in total 1919 results for 6 different SARS-CoV-2 antibody positive samples (average SR 89,4%) and 955 results for the 3 different negative samples using tests by 45 manufacturers.

CONCLUSIONS

The excellent SRs for NAT (WT and variants) and Ag rounds showed that there was a very good consensus between the many different test platforms represented in the EQA schemes. The SRs for the Ab rounds were also very good, although there were some challenges to be seen in tests detecting specific Ig classes.

Microbiology - Infectious diseases including COVID 19

T246

THE TITER CONCENTRATION OF IGG ANTIBODIES OF COVID-19 IN VACCINATED INDIVIDUALS AND INFECTED PATIENTS

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BACKGROUND-AIM

SARS-CoV-2, the causative agent of Coronavirus Disease 2019 (COVID-19), is an enveloped, single-stranded RNA Betacoronavirus. Upon infection with SARS-CoV-2, the host mounts an immune response against the virus, typically including production of specific antibodies IgM and IgG against viral antigens and both arise in blood. There is significant inter-individual difference in the levels and chronological appearance of antibodies in COVID-19 patients, but median seroconversion has been observed at approximately 2 weeks. Antibodies against SARS-CoV-2 with strong neutralizing capacity, especially potent if directed against the receptor-binding domain (RBD) on the S1 subunit of this virus, have been identified. Numerous vaccines for COVID-19 have been developed many of which focus on eliciting an immune response to the RBD.

METHODS

A total of 124 samples were collected from three groups. 23 from non-infected; non-vaccinated asymptomatic individuals; 12 samples from previously infected patients in which 5 of them had AstraZeneca vaccine later. The last third group, 89 samples collected from non-infected vaccinated individuals in which 25 had received Pfizer and 64 AstraZeneca vaccines. The Elecsys anti-SARS-CoV-2 S assay from Roche was used to detect the antibodies. It uses a recombinant protein representing the RBD of the S antigen in a double-antigen sandwich assay format. Any value above (>0.8 U/mL) was considered positive.

RESULTS

All the 23 non-infected; non-vaccinated individuals showed a detection levels below the cut-off value of the assay (<0.4 U/mL). The average titer for those who have previously infected patients with no vaccine (n=7) were 616±875 U/mL compared to infected but received extra vaccine (n=5) 1424±1223 ($p=0.1048$). In the third group with artificial immunity, the average of the titer was 1368±927 U/mL in individuals with Pfizer vaccine while it was 256±618 U/mL in those with AstraZeneca vaccine($p<0.00001$). The average titer of both vaccines was 569±872 U/mL.

CONCLUSIONS

The natural immunity of previously infected patients has demonstrated a good antibodies production against SARS-CoV-2. The immunity may be enhanced and improved by the administration of the artificial vaccine. Further study is required to establish sold conclusion.

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T247

PERFORMANCE EVALUATION OF THE ATELLICA IM SARS-COV-2 ANTIGEN ASSAY*

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BACKGROUND-AIM

A fully automated Atellica® IM SARS-CoV-2 Antigen (CoV2Ag) Assay has been developed for qualitative detection of nucleocapsid antigen in nasopharyngeal (NP) and anterior nasal (AN) swab samples using the Atellica IM Analyzer. The CoV2Ag assay is a one-step sandwich immunoassay using anti-SARS-CoV-2 antibodies to capture and detect antigen in a chemiluminescent format.

METHODS

The limit of detection (LoD) of the CoV2Ag assay was determined using gamma-irradiated SARS-CoV-2. The performance of the CoV2Ag assay was evaluated with NP and AN swab samples in the following populations: a) symptomatic; b) asymptomatic; c) SARS-CoV-2 RT-PCR negative; and d) SARS-CoV-2 variants. The NP and AN samples collected in Siemens Healthineers sample inactivation media were also evaluated. CoV2Ag assay precision, cross-reactivity, and interference were assessed.

RESULTS

The CoV2Ag assay's LoD ranged from 18.2 to 114.2 TCID50/mL in different transport media (TM). In the symptomatic population, the CoV2Ag assay's positive agreement with RT-PCR was 86.83% in NP and 80.65% in AN samples. In symptomatic NP and AN samples with <30 Cycle thresholds (Ct), the CoV2Ag assay's positive agreement was 96.49% and 98.59% respectively. The CoV2Ag assay demonstrated negative agreement of 99.64% in NP and 100.00% in AN samples. In the asymptomatic population, the CoV2Ag assay's positive and negative percent agreement was 80.56% and 100.00% in NP and 98.00% and 98.00% in AN samples respectively. In asymptomatic NP and AN samples with <30 Ct, the positive agreement was 93.10% and 98.00% respectively. The CoV2Ag assay detected SARS-CoV-2 Alpha, Beta, Gamma, and Delta variant samples. The NP and AN samples collected in Siemens Healthineers inactivation media showed 97.50% positive agreement and 95.77% negative agreement when compared to samples collected in viral TM and tested using the CoV2Ag assay. The CoV2Ag assay demonstrated no cross-reactivity with the tested microorganisms. The Atellica IM CoV2Ag Assay demonstrated good precision, with <10% CV for repeatability and <20% CV for within-laboratory precision.

CONCLUSIONS

These results demonstrate good performance of the Atellica IM CoV2Ag Assay.

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SEROLOGICAL INVESTIGATION OF COVID 19 ANTIBODIES IN ARMENIA

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BACKGROUND-AIM

In December 2019 in Wuhan China the new coronavirus outbreak emerged and quickly spread in all parts of the world resulting to more than 200 000 000 infection cases and around 4 500 000 deaths. The global incidence of the infection is still rapidly growing as well as number of deaths. Due to the fact that COVID19 is a new virus, not much is known about the immune response of infected organism, which is crucial not only for vaccination policy development, but also for identification of public health strategies.

Current research aims to describe COVID 19 IgG levels depending on symptoms, antibiotic and antiviral medications intake history, existing chronic condition and smoking status during March-December of 2020 in Armenia. Furthermore, the study aims to help elucidate the fraction of asymptomatic or pre-symptomatic/ subclinical infections in the population and understand the main risk factors for infection complication.

METHODS

The cross-sectional study with convenience sampling of individuals who turned to “EcoSense” laboratories to be tested for COVID 19 IgG were examined. The NovaTec SARS-CoC-2 (COVID-19) IgG COVG940 96 Determinations ELISA test kits were used. The questionnaire was filled regarding the COVID 19 status, symptoms, exposure history, disease history, pre-existing chronic conditions, medication and vaccination history. The descriptive as well as multivariate analysis was performed.

RESULTS

Overall 1573 testing was performed 837 of subjects agreed to participate in the interview. 57.5% were females. 24.1% of participants had laboratory confirmed COVID 19 but by the time of interview were already recovered. 212 (25.3%) participants had positive IgG levels, among 126 (15.1%) participants IgG levels were in the grey zone. Out of PCR confirmed cases only 58.7% had positive IgG levels and 3.9% IgG level was in the grey zone.

4.9% of participants was previously symptomatic, however were not tested to confirm COVID 19 infection. Out of all previously symptomatic but non-confirmed cases 31.5 % had positive IgG levels. Headache was the most common symptom among participants (37.2% among all participants and 53.1% among participants who previously had positive COVID 19 PCR test). Second most common symptom was anosmia (23.7% among all participants and 48.9% among participants who previously had positive COVID 19 PCR test).

5.4% of participants mentioned previous hospitalization due to COVID 19, 71(8.5%) mentioned being diagnosed with pneumonia and 24 (2.9%) participants mentioned being admitted to ICU 20 mentioned receiving oxygen therapy and 4 (0.5%) of the participants mentioned receiving an artificial ventilation of lungs. 26 of the participants mentioned being pregnant gestation age varied from 6-35 weeks. There was a weak correlation between symptom sum score and IgG titers. The Correlation coefficient was 0,273, $p<0.05$, $R2=0.075$. The linear regression analysis was also performed. The obtained results indicate that the number of symptoms patients have is a significant predictor for IgG level $F(1, 711) = 57.45$, $P<0.01$, $R2=0.075$.

CONCLUSIONS

Our study reviled that around half of PCR confirmed COVID 19 patients do not have positive titer for IgG, most importantly the number of symptoms is a weak predictor for IgG levels, which contradicts the existing misassumption regarding severity of clinical manifestation of COVID 19 and post-infection immunity.

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INSUFFICIENT STANDARDIZATION IN SARS-COV-2 SEROLOGY: QUALITATIVE AND QUANTITATIVE DIFFERENCES IN COMMONLY USED SARS-COV-2 ANTIBODY ASSAYS

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BACKGROUND-AIM

Antibodies against the SARS-CoV-2 spike protein (S) are quantified for both research and routine purposes in convalescent patients and in vaccinated individuals. Meanwhile, test systems from several manufacturers have been CE-IVD labeled and are therefore widely used in medical laboratories. Because these test systems provide numerically different values, the manufacturers attempted to reference their systems to a common standard, the WHO standard for SARS-CoV-2 immunoglobulin. Our group aimed to evaluate whether this effort sufficiently improved the comparability of the tests.

METHODS

N=69 individuals receiving BNT162b2 (Pfizer/BioNTech) and N=50 individuals receiving AZD1222 (Astra-Zeneca) were recruited. Previous infection with SARS-CoV-2 was ruled out by anamnesis and detection of anti-nucleocapsid antibodies. Antibodies against S were quantified after the first dose (weel 3 for BNT162b2; week 3 and 11 for AZD1222) and after the booster shot (week 3) using tests from Roche, Abbott and DiaSorin. As a reference, surrogate virus neutralization tests were performed.

RESULTS

Despite standardization, results from different test systems were not interchangeable and BAU/mL (binding antibody units per milliliter) deviated relevantly when measured with two different assays. This was observed after both vaccination with BNT162b2 and AZD1222. Moreover, the differences between the test systems were time-dependend, with changing correction factors over time from vaccination; e.g., 3 weeks after the first dose, Abbott provided values three times higher than Roche, 11 weeks after the first dose (which was right before the booster shot), Roche quantified twice as high as for Abbott, and 3 weeks after the second dose even 5-6 times higher.

CONCLUSIONS

Our results clearly demonstrate that the standardization efforts undertaken to date in SARS-CoV-2 serology are unsatisfactory. Clinicians must always consider the test system used when interpreting SARS-CoV-2 antibody levels, even when results are reported in an apparently standardized format in BAU/mL. Sequential testing with different assays should be avoided because the effect of time on agreement between test systems makes direct comparison nearly impossible.

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PLEURAL AND PERITONEAL CAVITIES INFECTION, DRUG-RESISTANCE PROFILE AND ITS ASSOCIATED FACTORS IN SOUTHERN ETHIOPIA

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BACKGROUND-AIM

Background: Body fluids play a critical role in the diagnosis of the organ it surrounds. Pleural and peritoneal fluids are the most common body fluid. Drug resistant pathogenic bacteria isolated from the body fluids are becoming a major public health concern. The study aimed to assess drug-resistance profile and associated factors of bacterial infection of pleural and peritoneal cavities in Southern Ethiopia.

METHODS

Methods: Institutional based cross-sectional study was conducted. Semi-structured questionnaire was used to collect the data and appropriate culture media were used to isolated and identified the pathogens. Kirby-Bauer disk diffusion technique was used for antimicrobial susceptibility test. Binary logistic regression was used to determine the associated factors. Adjusted odds ratio (AOR) with 95% confidence interval (CI) at ≤0.05 level of significance was used to determine the presence and strength of associated factors.

RESULTS

Results: A total of 252 study participants were involved in the study to provide survey response and body fluid specimen. Of these the overall bacterial culture positive samples were 16.7% with a total 43 bacteria isolates. E. coli (31%) and Klebsiella spp (19%) were the predominant isolates. The overall prevalence of MDR was 53.3%. Patients with surgery [AOR= 3.17, 95%CI: (1.16-8.67)], hospitalization [AOR= 2.86, 95%CI: (1.28-6.33)], Cirrhosis [AOR= 2.70, 95%CI: (1.21-6.02)] and alcoholism [AOR= 2.78, 95%CI: (1.20-6.42)] were independently associated with the case.

CONCLUSIONS

Conclusion: Gram negative pathogens were the predominant isolates. High MDR bacteria were observed in our study. History of surgery, prolonged hospitalization, Cirrhosis and alcoholism were important factor. Working on the identified problem is important to reduce the problem. Strengthening antibiotic stewardship program is important to reduce the high MDR.

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NASAL MICROBIOME IN COVID-19 PATIENTS: A POSSIBLE RELATION BETWEEN CORYNEBACTERIUM AND HYPOSMIA.

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BACKGROUND-AIM

The microbiome seems to play a relevant role in the development of SARS-CoV-2 infection and the relative severity COVID-19 symptoms. In literature the possible relationships between the gut, lung, nasopharyngeal, or oral microbiome and COVID-19 are reported. Recently, we analyzed the nasopharyngeal swab and found a different microbic composition in patients positive to SARS-CoV-2 respect to healthy subjects. Here, we analyzed the microbiota obtained from the nasal swabs of both positive and negative SARS-CoV-2 individuals, in order to define possible differences in terms of bacterial composition in this niche of respiratory tract in relationship to patient’s symptoms.

METHODS

The hypervariable V1-V2-V3 regions of the bacterial 16S rRNA gene were analyzed using the Microbiota solution A (Arrow Diagnostics).

RESULTS

We found a significant reduction of Actinobacteria in COVID-19 positive patients respect to controls ($p=0.001$, FDR= 0.001). The significant reduction of Actinobacteria among groups was evaluated from the class up to the genus ($p<0.001$) by using the ANOVA test. At the genus level, a significantly reduced relative abundance of Corynebacterium in patients with mild or severe symptoms, as compared to controls ($p=0.001$, FDR=0.013), was verified.

CONCLUSIONS

Interestingly, the reduced abundance of Corynebacterium is reported in association with anosmia, a common symptom of COVID-19: overall our patients suffered from anosmia (very or moderately severe), while the Corynebacterium genus is highly represented in the nasal mucosa of healthy subjects. Further studies on larger cohorts are needed to establish functional relationships between nasal microbiota content and clinical features of COVID-19.

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EVALUATION OF THE ROLE OF ROUTINE LABORATORY TESTS IN COVID-19 PATIENTS

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BACKGROUND-AIM

Our aim is to evaluate the role of routine laboratory biomarkers like C Reactive Protein (CRP), Lactate Dehydrogenase (LDH), Interleukin 6 (IL6), Ferritin, Creatinine, Procalcitonin (PCT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Serum Albumin, Total Bilirubin (T Bil), High Sensitive Troponin I (hs troponin I), N Terminal-pro B-type Natriuretic Peptide (NT proBNP), Blood Urea Nitrogen (BUN) and Blood Gases in COVID 19 patients who are admitted with SARS CoV-2 positive test results by real time reverse transcriptase polymerase chain reaction (rRT PCR) in Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute, Mumbai, India.

METHODS

80 individuals detected with COVID-19 belonging to the age group 12-83 years (median age 62 years) were studied. The case group consisted of 62 males and 18 females. 40 healthy adults without any history and clinical evidence suggestive of COVID-19 and without any comorbidities, like diabetes, hypertension chronic lung disease, cardiac disease, cancer and immune compromised individuals were considered as a control group for the study.

Routine laboratory findings of these 80 patients were used to evaluate the abnormalities found in COVID-19 patients. Statistical analysis was carried out on the data after determining whether the data had a normal / log-normal distribution and their significance was determined by calculating the p-value. The percentage of patients showing a decrease or increase from the normal value was calculated.

Trend analysis was carried out for the 80 patients considered in the case group.

Among them, 5 patients were used as representatives to show the trend in these biomarkers during the course of hospital stay. These 5 patients include 2 adult males, 2 adult females and 1 adolescent girl.

Case studies of 5 patients during first wave and second wave of COVID 19 and paediatric multi system inflammatory syndrome were described with laboratory investigations.

RESULTS

We found significant increase in mean values of values of AST, ALT, Total Billirubin, Creatinine, CRP, PCT, LDH, IL6, Ferritin, Lactate, hsTroponiI, NT Pro BNP and decrease in mean values of Albumin, SO2 and PO2 in COVID 19 cases than control.We applied Receiver Operating Curve (ROC) curve to discriminate case population more precisely than control population.

To explain the role of routine laboratory parameters in disease monitoring, two adult males, two adult female and one adolescent girl as representative examples of different manifestations of COVID 19 with Adult Respiratory Distress Syndrome (ARDS), Cardiac Injury, Neurological manifestations and Paediatric Multi system inflammatory syndrome (PIMS) admitted in the Intensive care unit (ICU) of the hospital were discussed.

CONCLUSIONS

The diagnostic and clinical care team at Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute, India, have evaluated the role of routine laboratory biomarkers and observed statistically significant abnormal values of biochemical and immunoassay parameters, for example, that of: C Reactive Protein (CRP), Procalcitonin (PCT), Lactate Dehydrogenase (LDH), D-Dimer, Interleukin 6 (IL6), Ferritin, Blood Gases, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Serum Albumin, Total Bilirubin(T Bil) and Creatinine, in COVID 19 patients. Various biomarkers that include inflammatory markers, coagulation parameters, tissue-specific tissue injury indicators (liver, kidney, cardiac), cytokines and derangement of the haematological markers. Routine laboratory monitoring of COVID-19 patients through testing is critical for assessing disease severity and progression, as well as monitoring therapeutic intervention.

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THE PROGNOSTIC IMPORANCE OF INTERLEUKIN 6 IN CORONA VIRUS DISEASE 2019

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BACKGROUND-AIM

Inflammation has a leading role in increasing the severity of COVID-19 due to inducing a cytokine storm. Inflammatory biomarkers such as Interleukin 6 (IL-6), C-reactive protein (CRP) and procalcitonin (PCT) could be used to predict the progression and mortality of COVID-19. We performed emergency IL-6, CRP and PCT measurements from COVID-19 patients and a prognostic value was evaluated based on these results.

METHODS

We analysed 215 patients retrospectively and devided them into three groups according to severity: 1.) outgoing patients with mild symptoms, 2.) moderate symptoms (administered to the infectology department) and 3.) severe symptoms (that required ICU care). IL-6 values were measured with chemiluminescence (Siemens Advia Centaur XPT) and was compared to CRP and procalcitonin values.

RESULTS

Results [median (min.-max)]: The median of IL-6 was 6.9 pg/ml (<2.7-23.2) in mild COVID (n=25), 27,2 pg/ml (2.7-355.4) in the moderate group (n=110) and 98.0 pg/ml (9,1->55000) in severe cases (n=80). All patients with IL-6 level over 15000 pg/ml died (76% of all deceased patients). We found no correlation between CRP and procalcitonin levels compared to baseline IL-6 levels, however in 90% of severe cases PCT was over 2 mg/ml, indicating bacterial or fungal infection. We confirmed bacterial and fungal infection with positive haemoculture.

CONCLUSIONS

According to our results, in covid-19 patients IL-6 can be used as a prognostic marker, forecasting the cytokine storm, which can also be caused by secondary sepsis, supported by high PCT levels and positive haemoculture in our study.

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POOLING TO SAVE SARS COV-2 PCR RESOURCES

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BACKGROUND-AIM

Since the first detection in Wuhan, China, in December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogen of coronavirus disease 2019 (COVID-19), has spread worldwide to now be considered a pandemic. Worldwide is experiencing an acute shortage of certain reagents important for performance of assays for the detection of SARSCoV-2, and some have stopped testing due to lack of test supplies. The spanish society of infectious diseases (SEIMC) justifies the use of pools in low prevalence situations when the capacity to perform individual PCRs is exceeded and/or if material resources are scarce.
The main objective of this study was to evaluate the efficiency of the use of pools of nasopharyngeal samples for the diagnosis of infection by SARS-COV-2.

METHODS

Nasopharyngeal swab samples from patients with a low probability of SARS-COV-2 infection were studied. The technique used was real time RT-PCR (Cobas SARS-COV and Influenza A/B - Cobas Liat). The samples were analyzed consecutively, grouped in pools of 5. Those pools with TC< 37 were considered positive. All samples included in positive pools were retested individually.

RESULTS

898 samples from different patients were analyzed, grouped in 227 pools, of which 24 were positive, which contained 30 positive samples. The prevalence was 3.34%.
The CT values of the pools and positive samples, and their differences can be seen in table 1 and graph 1. The median difference (CT-pool / CT-sample) was 2.3 (0.04 - 4.83). The CT of the pools and the positive samples showed an adequate correlation (r 0.9267, P <0.0001) (graph 2).
The economic saving when using pools (226) compared to the total of samples (898) was 75%.

CONCLUSIONS

This study demonstrates that the use of pools for the detection of SARS-COV-2 by real time RT-PCR in samples of nasopharyngeal exudate is useful saving resources guaranteeing the reliability of the result.

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CLINICAL AND ANALYTICAL PERFORMANCE EVALUATION OF A NEW EPSTEIN-BARR VIRUS IMMUNOASSAY PANEL

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BACKGROUND-AIM

Epstein-Barr virus (EBV) causes the majority of Infectious mononucleosis (IM), serological assay in combination with clinical evaluation are essential for diagnosis, especially for the management of antibiotic therapy. Panel of antibody against EBV, including IgM, IgG antibodies against the viral capsid antigen (VCA) and IgG antibody to EBV nuclear antigen (EBNA), are widely used. Accurate test results enables a correct EBV infection diagnosis. The evaluation of analytical performance and clinical reliability should be performed by laboratory medicine before implementation. We evaluated independently the new Elecsys EBV immunoassay panel (Roche Diagnostics International Ltd, Rotkreuz, Swisterland) under routine laboratory conditions.

METHODS

The precision, trueness, carry-over and linearity were assessed.
Forty-one sera with potential cross-reactivity on EBV IgM antibody were analyzed to assess the analytical specificity. Clinical reliability was performed by using 143 sera from patients who have been prescribed an EBV panel. We compared the results of the three test panels (EBV IgM, EBV VCA IgG and EBV EBNA IgG) to the final infection status according the clinical patient settings. Percentage (%) of samples classified by the individual assays panel was calculated using Wilson's score interval (95% confidence interval). Statistical analysis was performed with Medcalc® for Windows, version 14.8.1 (Ostend, Belgium).

RESULTS

This Elecsys EBV immunoassay panel showed excellent analytical performance. Two false positives were found among the samples with potential cross-reactivity.
Among the seronegative, acute infection and past infection samples: 85.29%(95%,CI: 69.87-93.55%), 93,75% (95%,CI: 71,67-98,89%) and 92,86% (95%,CI: 68,53-98,73%), respectively, have been correctly identified.

CONCLUSIONS

This study shows that Elecsys EBV immunoassay panel has satisfactory analytical and clinical performance for a routine used.

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COMPARISON OF RT-PCR REAL-TIME EUROREALTIME WITH RT-PCR PLATFORM OF THE LIGHTCYCLER 480 II (ROCHE) AND CEPHEID XPERT XPRESS ON THE GENEXPERT DX INSTRUMENT (CEPHEID) FOR DETECTION SARS-COV-2

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BACKGROUND-AIM

An outbreak of severe acute respiratory syndrome CoV-2 happened in Albania at the beginning of 2020. In this situation, a quick diagnosis in the early stages of the CoV-2 would help prevent the spread of the disease. During the outbreak phase, molecular and serologic tests for the SARS-CoV 2 became available, but the detection of viral RNA is the most sensitive method to aid in the diagnosis of SARS-CoV-2 infections. In this situation it was necessary to develop and adapt safe, accurate, fast, and simple technologies for the detection of SARS-CoV-2. Our laboratory in accordance with the equipment and supply possibilities made its own choices. In this study, we compare the detection performance of two molecular assays for SARS-CoV-2, respectively Xpert Xpress SARS-CoV-2 (Cepheid) and Euroimun EURORealTime SARS-CoV-2.

METHODS

A total of 100 nasopharyngeal swab samples were tested with both methods. The samples were collected from ambulatory patients at Intermedica Clinic, and were tested: (I) with EURORealTime SARS-CoV-2 assay with RT-PCR platform of the LightCycler 480 II (Roche); (II) by the Cepheid Xpert Xpress SARS-CoV-2 assay on theGeneXpert Dx instrument (Cepheid). RNA was extracted with The QIAamp Viral RNA Mini Kit for EURORealTime SARS-CoV-2 assay.

RESULTS

From the 100 specimens, 37 tested negative and 62 tested positive (LDT Ct<30) with both systems, for an agreement of 99%. Testing was repeated only for a single specimen with discrepant results. For this specimen, the Cepheid assay was repeatedly negative for SARS-CoV-2. The initial EURORealTime SARS-CoV-2 assay result was positive for SARS-CoV-2, though the cycle threshold (Ct) values for detection was 32.0 (low positivity). These results suggest that SARS-CoV-2 was present at a very low concentration, near the positivity limit of the EURORealTime assay. For an accurate result we decided to test all the samples with low level of viral load with two methods.

CONCLUSIONS

Sensitive and specific assays for SARS-CoV-2 detection are essential to the global response to the COVID-19 pandemic. During the pamdemic and in the middle of huge demand for testing, Intermedica clinic alongside Cepheid successfully adapted FDA-approved test kits from EURORealTime SARS-CoV-2 assay with the RT-PCR platform of the LightCycler 480II (Roche). The Cepheid assay is simple and very fast, while the EURORealTime assay with the RT-PCR platform of the LightCycler 480II (Roche) analyzes multiple samples simultaneously, but both methods, Cepheid assay and EURORealTime assay, show a very good detection performance for SARS-CoV-2.

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IMPACT OF HYPOCALCEMIA ON HOSPITAL ADMISSION FOR PROGNOSIS OF COVID-19 PATIENTS: WHERE THE WAVES THE SAME?

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BACKGROUND-AIM

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the etiological agent for the pneumonia cases of unknown origin in Wuhan (China), a disease named as coronavirus disease 2019 (COVID-19). Its clinical presentation varies from asymptomatic or mild upper respiratory tract symptoms to severe viral pneumonia with respiratory failure and death. Hence, there is a need to identify risk factors for early prediction of progression of COVID-19 patients.

The role of laboratory medicine in this viral outbreak includes prognostication of COVID-19 patients. Recent studies have reported that hypocalcemia is associated with severe COVID-19. It would be caused by an interaction of calcium with unsatured fatty acids released during the disease, which can bind calcium with a favourable enthalpy (-20KJ/mol) and trigger acute hypocalcemia. Mostly of studies have reported hypocalcemia, defined by a low total calcium, as a common biochemical feature in COVID-19 patients possibly associated to a worse prognosis. However, calcium interacts with fatty acids when in an ionized state and the role of ionized calcium levels have been scarcely studied. We aimed to evaluate the role of hypocalcemia, defined by low ionized calcium levels, for prognosis of severity of hospitalized COVID-19.

METHODS

We conducted a retrospective cohort study including adult patients (≥ 18 years) with laboratory confirmed COVID-19 admitted to the hospital from October to November 2021. Only patients with blood ionized calcium levels from blood gas analysis on admission to the Emergency Department were included. Ionized calcium levels were expressed as actually measured levels and hypocalcemia was defined as ionized calcium level, measured by direct potenciometry on a ABL 90 analyzer (Radiometer), below 4.60 mg/dL.

The primary endpoint was severe COVID-19 progression, defined as a composite of admission to Intensive Care Unit (ICU) and/or need for invasive mechanical ventilation and/or in-hospital mortality.

The association between hypocalcemia and the risk for the endpoint was assessed by bynary logistic analysis. Software package SPSS vs. 22 (SPSS Inc), was used for statistical analysis, with a $p < 0.05$ considered statistically significant.

RESULTS

During the study period, 305 patients were admitted to hospital. Study population included 261 patients (mean age: 59.1 years (SD: 16.3); 166 (63.6%) male), of whom measurement of ionized calcium was available. Upon arrival to the Emergency Department, mean ionized calcium was 4.49 mg/dL (SD: 0.21). Hypocalcemia was found in 185 patients (70.9%). Severe hypocalcemia (ionized calcim levels < 3.97 mg/mL) was detected in only 2 patients (0.8%). During hospitalization, 78 (29.9%) cases progressed to severe disease, of whom 74 (28.4%) required intensive care, 21 (8.0%) underwent invasive mechanical ventilation and 9 (3.4%) died of any cause.

No significant difference was observed in the rate of patients progressing to severe disease in patients with hypocalcemia vs. normo- or hypercalcemia (25 (32.9%) vs. 53 (28.6%); p=0.496). In univariate analysis, hypocalcemia was not a predictor of severity (OR: 1.221 (95%CI: 0.680-2.170; p=0.496).

When mean ionized calcium levels were compared, no difference was found between severe COVID-19 patients in comparison to non-severe patients (4.50 mg/dL (SD: 0.23) vs. 4.49 mg/dL (SD: 0.20); p=0.750). Concerning other thromboinflammatory biomarkers widely recommended for prediction of severity in COVID-patients, no differences were observed for median (interquartile range (IQR) ferritin (529 ng/mL (281-794) vs. 514 ng/mL (286-920); p=0.730), C-reactive protein (CRP) (6.5 mg/dL (2.8-9.9) vs. 6.3 mg/dL (3.3-10.8); p=0.717), interleukin-6 (IL-6) (32.8 pg/mL (16.5-54.0) vs. 28.6 mg/dL (15.1-56.3); p=0.483) and D-dimer (483 ng/mL FEU (341-944) vs. 590 ng/mL FEU (IQR: 375-971); p=0.167) levels between severe and non-severe patients.

CONCLUSIONS

Conversely to previous studies reporting the potential value of calcium for predicting worse outcomes in COVID-19 patients, our study did not confirm this first finding described in cohorts collected during the first wave and hypocalcemia was not associated with severity.

A possible explanation would be the differences previously reported on characteristics and severity of COVID-19 patients admitted to hospitals in the second outbreak in comparison to the first wave, with laboratory tests on admission showing a lower inflammatory component, also confirmed in our study, in which differences not were observed for some biomarkers such as CRP, ferritin, IL-6 and D-dimer. Because inflammation and thrombosis, two hallmarks in severe COVID-19, are mediated by signaling molecules derived from unsaturated fatty acids, which can bind calcium to trigger hypocalcemia, the fact that patients were admitted to the Emergency Department earlier in the disease course and with a less inflammatory condition during the second wave would explain our results, confirming that hypocalcemia is a frequent finding in COVID-19 patients but it is not associated with a worse prognosis.

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T258

ANTI-RBDS1 IGG RESPONSE TO COVID-19 VACCINES IN INDIVIDUALS WITH NO PRIOR INFECTION

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BACKGROUND-AIM

Knowledge on the kinetics and duration of immunity after COVID-19 vaccination is limited. Although cellular immune response plays a major role in SARS-CoV-2 immunity, serological tests show good agreement with virus neutralization tests and are routinely more accessible. Our aim was to compare the levels of anti-receptor binding domain spike subunit 1 (anti-RBDS1) IgG antibodies (Ab), in a cohort of individuals with no prior infection vaccinated with different COVID-19 vaccines.

METHODS

Venous blood was drawn in apparently healthy individuals with no prior COVID-19 infection at time intervals up to 4 months after the second dose of two mRNA vaccines (BNT162b2/Comirnaty by Pfizer-BioNTech (N=85, 43(21-63) years) and mRNA-1273 by Moderna (N=32, 45(27-78) years)) and one adenoviral vector-based vaccine (ChAdOx1-S by AstraZeneca-Oxford, N=26, 25(23-68) years). Anti-RBDS1 Ab were measured in serum using the SARS-Cov-2 IgG II assay on the Abbott Architect i2000SR analyzer. Statistical analysis was performed using the MedCalc software (version 11.5.1, Mariakerke, Belgium).

RESULTS

Two weeks after the second dose, the Pfizer and Moderna groups showed the highest levels of anti-RBDS1 Ab compared to the AstraZeneca group (15463(9075-32370) and 18737(14267-29499) vs. 1207(874-1879 AU/mL, $P<0.001$). The same differences were observed after a month (Pfizer 11212(7128-21187) and Moderna 16338(10270-28227) vs. AstraZeneca 1071(756-1545) AU/mL, $P<0.001$). After 1.4 months, the highest anti-RBDS1 Ab levels were observed in the Moderna group (11034(9006-17220) AU/mL), while in the Pfizer and AstraZeneca group the decline was more pronounced (6909(4983-11348) and 852(575-1236) AU/mL, respectively, $P<0.001$). After 2 months, the differences persisted with the lowest levels found in the AstraZeneca group (Moderna 8818(7032-13875), Pfizer 5247(3514-11037) and AstraZeneca 800(466-976) AU/mL, $P<0.001$). After 4 months, data for the AstraZeneca group was not available, however higher anti-RBDS1 Ab levels were observed in the Moderna compared to the Pfizer group (4499(3495-5599) vs 2021(1333-4826), $P=0.012$).

CONCLUSIONS

The results of our study confirm significant differences in the anti-RBDS1 IgG response to different COVID-19 vaccines in individuals with no prior COVID-19 infection.

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T259

COVID-19 VACCINES: COMPARISON OF IMMUNOGENICITY OF PFIZER/BIONTECH, MODERNA AND ASTRAZENECA VACCINES

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BACKGROUND-AIM

After 18 months of the declaration of COVID-19 pandemics, more than 220 million cases have been diagnosed and 4,5 millión deaths have been recorded. Vaccines are able to prevent new cases and decrease severity and lethality. Humoral response has been studied individually for the different available formulations and scarce data has been reported about cellular immunity. The aim of this project was to compare cellular and antibody responses after one and two doses of 3 different vaccines inoculated to healthcare workers in Navarra (Spain).

METHODS

This study was conducted among 707 healthcare workers of University of Navarra (Spain) who had received Moderna (n=273), Pfizer (n=165) or AstraZeneca (AZ) (n=269) COVID-19 Vaccine. Humoral response was evaluated by testing Anti-S-RBD (Roche) and Anti-N (Roche) in the cobas e601 platform. Antibody levels were determined twice in march and april/july 2021, 3 weeks after the first and the second dose administration. Cellular immunity was measured using SARS-CoV-2 IGRA Test (Euroimmun), from a subset of 158 individuals (51 Moderna, 51 Pfizer and 56 AZ) collected 3 weeks after the first dose. Descriptive statistics were obtained using Stata 12.0.

RESULTS

Sixteen per cent of participants had serologic evidence (Anti-N (+)) of previous SARS-CoV-2 infection at baseline. Among those without a previous infection, the first dose response (Anti-S-RBD Units/mL) showed a median (interquartile range) of 119 (50-193), 45 (16-96) and 31 (14-68) for Moderna, Pfizer and AZ vaccines, respectively ($p<0.05$). Among those vaccinated after infection, levels were 20145 (7896-26660), 14143 (9444-21695) and 7104 (2880-8868) for Moderna, Pfizer and AZ, respectively ($p<0.05$). Anti-S quantifications (Moderna, Pfizer and AZ, respectively) 3 weeks after the second dose were: 4508 (2841 - 6677), 2239 (1311-4314) and 3226 (1508-9474), without previous COVID-19, and 4879 (2783 - 9262), 15775 (3735 - 25397) for those previously infected receiving Moderna and Pfizer vaccines, respectively.
The cellular immunity after one dose was positive for 94.1%, 96.1% and 98.2% participants who had received Moderna, Pfizer or AZ vaccines, respectively.

CONCLUSIONS

Moderna, Pfizer and AZ vaccines showed very high humoral and cellular immunogenicity. Moderna vaccine generated higher antibody levels and lower cellular response, so vaccines response dynamics must be studied to establish the duration of the immune response.

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T260

T-CELL AND ANTIBODY RESPONSE TO SARS-COV-2

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BACKGROUND-AIM

SARS-CoV-2 vaccination is a game-changing tool and critical to ending the COVID-19 pandemic. Although antibody determination is widely available, both T-cell and antibody responses are crucial in understanding immune defense against SARS-CoV-2 infection. We aimed to evaluate T-cell response by Interferon Gamma (IFN-γ) Release Assay (IGRA) and its added value to antibody testing.

METHODS

Peripheral blood samples from 14 volunteers (10 females and 4 males) were analyzed. The study group consisted of 10 vaccinated (more than 6 weeks ago) individuals without history of SARS-CoV-2 infection and 3 individuals that had infection more than 6 months ago, but no vaccination and one individual was SARS-CoV-2 infection and vaccine naïve. SARS-CoV-2 IgG antibody determination in serum was done on Architect platform (Abbott, USA) with a cut-off value 50 AU/ml. Samples for the IGRA assay were collected in QuantiFERON Control Set Blood Collection Tubes (negative and positive control) and QuantiFERON SARS-CoV-2 Starter Set (peptides from the Spike antigen for specific $CD4^+$ and $CD4^+/CD8^+$ T-cell stimulation) and the plasma levels of IFN-γ were determined by ELISA (Qiagen, Germany). To exclude background IFN-γ levels, results from the negative control tube were subtracted from the results in stimulated tubes.

RESULTS

The mean antibody level in 10 vaccinated individuals (mean ± SEM) was 3124 AU/ml ± 1248 (range 126.5-11530.0) and the IFN-γ level response from $CD4^+$ T-cells 0.30 IU/ml ± 0.13 (0.01-1.42) and $CD4^+/CD8^+$ T-cells 0.31 IU/ml ± 0.09 (0.04-0.84). Three individuals with history of infection had antibody level 605 AU/ml ± 511.8 (68.0-1628.0) and IFN-γ response from $CD4^+$ T-cells 0.08 IU/ml ± 0.01 (0.06-0.10) and $CD4^+/CD8^+$ T cells 0.15 IU/ml ± 0.05 (0.08-0.25). Individual without history of infection and vaccination had antibody level 2.7 AU/mL and IFN-γ response from $CD4^+$ T cells 0 IU/ml and $CD4^+/CD8^+$ T-cells 0.01 IU/ml. Antibody levels showed a strong correlation with IFN-γ response from $CD4^+$ T-cells (P = .0009, Pearson r = 0.7830) and $CD4^+/CD8^+$ T-cells (P = .0282, r = 0.5844).

CONCLUSIONS

SARS-CoV-2 antibody response correlates with IGRA results. T-cell immunity assessment can give additional information on immune response. Vaccination induces a higher humoral and cellular immune response than infection.

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T261

MONITORING ANTI-SARS-COV-2 ANTIBODIES AFTER COVID-19 VACCINATION IN HEALTHCARE WORKERS AND THE UNIVERSITY STAFF, ASSOCIATED WITH THE VIRAL CIRCULATION CONTROL

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BACKGROUND-AIM

The professional world has been profoundly affected by the COVID-19 pandemic since working closely with co-workers and customers can lead to transmission of SARS-CoV-2 and compromise the business continuity. The pandemic has pushed the demand for workplace surveillance tools, through the administration of different types of vaccines to the population, that can decrease the viral circulation and protect against the disease by reducing hospitalization and deaths. The University of Urbino, in association with the Azienda Sanitaria Area Vasta 1 Marche, has launched a project involving volunteers recruited in a cohort of fully vaccinated among University and Healthcare workers to longitudinally monitoring i) anti-S antibodies post-vaccination and ii) anti-N antibodies as an indicator of virus circulation. Here we report the preliminary results.

METHODS

Antibody titer tests in serum samples were performed using IVD kit for IgG against spike protein (LIAISON) and IgM/IgG against the Nucleocapsid protein (Diatheva). Despite vaccination, a positive value for anti-N Ab indicated that the subject has been exposed to the SARS-CoV-2, therefore subsequently direct search for viral RNA was performed. Total RNA from nasopharyngeal swabs and saliva samples were subjected to a real-time RT-PCR multiplex for the detection of viral ORF1b and N genes, and the internal control (human RNase P) using Diatheva COVID-19 PCR kit.

RESULTS

300 serum samples have been processed. The analysis of the anti-S Ab titer showed that all except two (99.3%) University workers developed anti-S Ab, 2 months after the second dose of the vaccine. 19% had very high titer >2080 BAU/mL, in the remain the titer was 577±527 BAU/mL (mean±SD; range 37.9-2070). Twelve subjects resulted positive for IgM anti-N (4%). Nevertheless, the nasopharyngeal swabs and salivae were SARS-CoV-2 RNA negative.

CONCLUSIONS

The monitoring of anti-S Ab offers the advantage to evaluate the kinetic and duration of Ab post vaccination, meanwhile, the anti-N screening enables to trace possible new infections minimizing the risk of viral spread in the workplace to colleagues and students or patients, keeping the work environment safe and COVID-free and proving vaccine efficacy in disease prevention and transmission.

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T262

AN OPTIMIZED STEPWISE ALGORITHM COMBINING RAPID ANTIGEN AND RT-QPCR FOR SCREENING OF COVID-19 PATIENTS

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BACKGROUND-AIM

Diagnosing SARS CoV-2 infection with certainty is essential for appropriate case management. We investigated the combination of rapid antigen detection (RAD) and RT-qPCR assays in a stepwise procedure to optimize the detection of COVID-19.

METHODS

From August 2020 to November 2020, 43,399 patients were screened in our laboratory for COVID-19 diagnostic by RT-qPCR using nasopharyngeal swab. Overall, 4,691 of the 43,399 were found to be positive, and 200 were retrieved for RAD testing allowing comparison of diagnostic accuracy between RAD and RT-qPCR (Figures 1-2). Cycle threshold (Ct) and time from symptoms onset (TSO) were included as covariates.

RESULTS

The overall sensitivity, specificity, PPV, NPV, LR-, and LR+ of RAD compared with RT-qPCR were 72% (95%CI 62%–81%), 99% (95% CI95%–100%), 99% (95%CI 93%–100%), and 78% (95%CI 70%–85%), 0.28 (95%CI 0.21-0.39), and 72 (95%CI 10-208) respectively.
The interactions between Ct values and TSO showed a predictably higher sensitivity for Ct ≤ 25 regardless of TSO: TSO ≤ 4 days 92% (95% CI 75%–99%), TSO > 4 days 100% (95% CI 54%–100%), and asymptomatic 100% (95% CI 78-100%). For patients with Ct > 25, sensitivity was higher when TSO ≤ 4 days than when TSO > 4 days or even in asymptomatic patients but was still not significant: 56% (95% CI 21%–86%), 50% (95% CI 19%–81%), and 25% (95% CI 9%-49%) respectively (p = 0.2099) (Figure 2).
Figure 3 shows a stepwise algorithm that combines RAD and RT-qPCR for the screening of COVID-19 patients. As sensitivity of RAD is over 80% only in patients with TSO ≤4 days (i.e., 83%), RAD might be useful only in such patients. Overall, combining RAD and RT-qPCR would allow reducing from only 4% the number of RT-qPCR needed.

CONCLUSIONS

This study highlights the risk of misdiagnosing COVID-19 in 28% of patients if RAD is used alone. Thus, negative results from RAD needs to be confirmed by RT-qPCR prior to making treatment decisions. A stepwise analysis that combines RAD and RT-qPCR would be an efficient screening procedure for COVID-19 detection and may facilitate the control of the outbreak.

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T263

PLASMA MR-PROADM, A SIGNATURE OF ENDOTHELIAL DYSFUNCTION, FOR PROGNOSIS OF COVID-19 SEVERITY. COMPARISON WITH CANONICAL BIOMARKERS

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BACKGROUND-AIM

Severe COVID-19 is characterized by multiorgan syndrome in which the vascular endothelium is the main damaged organ. Because endothelial dysfunction is one of the major hallmarks of COVID-19, mid regional-pro-adrenomedullin (MR-proADM), measured as a surrogate of adrenomedulin secretion, may be of interest as indicator of COVID-19 induced endotheliitis. In this study, we aimed to evaluate the role of MR-proADM for prediction of severity in hospitalized COVID-19 patients, in comparison with other canonical biomarkers.

METHODS

Design and study population: Two-centre prospective observational study recruiting consecutive adult laboratory-confirmed COVID-19 during the second wave in Spain.
Laboratory methods: Blood samples for biochemical analysis (creatinine, albumin, bilirubin, C-reactive protein (CRP), procalcitonin and lactate dehydrogenase (LDH), hematological analysis, including cell blood counts, and D-dimer, were drawn on arrival to the Emergency Department and results reported to physicians. For ferritin, interleukin-6 (IL-6) and MR-proADM, leftover serum and plasma were immediately frozen and stored at -80 °C until testing. Plasma MR-proADM levels were measured by a homogeneous sandwich immunoassay with fluorescent detection using a time-resolved amplified cryptate emission (TRACE) technology assay (KRYPTOR® Gold, Brahms ThermoFisher).
Outcome: Ninety-day all-cause mortality following enrolment was the primary outcome.

RESULTS

Study population included 359 hospitalized patients by SARS-CoV-2 infection. Thirty two patients (8.9%) died.
Concerning laboratory tests, creatinine, procalcitonin, IL-6 and MR-proADM were significantly higher and albumin and lymphocyte count significantly lower, in deceased patients.
Admission MR-proADM levels were two-fold higher in non-survivors (1.50 nmol/L #IQR: 0.98-2.31# vs. 0.73 nmol/L #IQR: 0.56-1.06#). This biomarker showed the highest discrimination ability for the outcome (ROC AUC: 0.832), without a significant difference in comparison to procalcitonin (ROC AUC: 0.795). A cutoff of 0.80 nmol/L achieved a sensitivity of 96.9% and a specificity of 58.4%, with a very high negative predictive value (99.5%). In regression analysis, after adjusting by a propensity score including 11 potential confounders, inverse transformed MR-proADM levels showed an independent association with the endpoint (HR: 0.162 #95% CI: 0.043-0.480#; p=0.002).

CONCLUSIONS

MR-proADM, on arrival to the ED, has a potential role to establish the prognosis of COVID-19 patients. Low MR-proADM levels might assist to physicians in early identifying low-risk patients and clinical decision makings.

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T264

SARS-COV2 SEROLOGICAL MONITORING IN A TUNISIAN MILITARY POPULATION

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BACKGROUND-AIM

Determination of SARS-COV2 antibodies (AB) can help for indirect infection diagnose especially in asymptomatic patients. It’s useful for epidemiological surveillance by establishing and monitoring the extent of viral spread in the community. But how persistent and how protective these AB can be, remind till now unclear. Monitoring of AB evolution over time seems therefore to be essential to understand the virus serological response and it’s clinical implications hence the interest of our study.

METHODS

We conducted a prospective study over 10 months (October-July 2021) including 22 voluntary non vaccinated patients working in the Aeronautical Medicine Expertise Center of Tunisia having already contracted the virus (positivity of RT-PCR, antigen or AB for SARS-COV2). We evaluated their serological response at the first month then at 3 months after infection using a rapid serological test (BIOSYNEX COVID-19 BSS IgG/IgM) for the qualitative detection of AB (IgM and/or IgG) based on immune-chromatographic technique.
Sample was a finger veinupuncture whole blood or plasma obtained by immediate centrifugation and separation from venous whole blood drawn on EDTA and analyzed at most 2h from sampling.
Positivity was evaluated as high (≥ +++) medium (++) or low (≤+).
Patients epidemiological data were collected.

RESULTS

All samples were positive for SARS-COV2 AB (IgM+IgG) at the first month of infection except 3 patients having a weakly positive RT-PCR. At 3 monthes, 59% had a negative serology, 18.2% presented low IgG response and 22.8% had medium or high IgG response associated to a low persistent IgM AB, this last category had a mean age of 50 years (46-53) and involved diabetic type 2 or dystyroid patients.

CONCLUSIONS

Serological response to covid-19 seems to be more pronounced and more long lasting in elderly patients with history of chronic disease. Wider studies are needed to approve these results.

Microbiology - Infectious diseases including COVID 19

T265

KINETIC OF ANTIBODIES RESPONSE TO COVID-19 VACCINES IN A TUNISIAN MILITARY POPULATION

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BACKGROUND-AIM

Vaccination against SARS-COV2 reduces propagation of the virus and severe forms of the disease, but how long these vaccines can be protective is not yet well known. The aim of our study is to assess kinetic of antibodies response to different vaccines in a Tunisian military population.

METHODS

We conducted a prospective trans-sectional study involving 40 military volunteers working in the Aeronautical Medicine Expertise Center who were eligible to vaccination against SARS-COV2 virus. Non inclusion criteria were a positive serology of covid-19 before vaccination or prior documented covid-19 infection.
After informed consent, all subjects benefited from a serology of SARS-COV 2 just before vaccination, after 3 weeks of the first vaccine dose, 2 weeks after the second vaccine dose and finally 3 months after the second vaccine dose. A rapid serological qualitative screening test (Biosynex IgG/IgM antibodies) based on immune-chromatographic technology was used.
Positivity was evaluated as high (≥ +++) medium (++) or low (≤+).
Clinical and epidemiological data were collected.

RESULTS

Twenty eight subjects benefited from Pfizer BioNTech vaccine, seven from Sputnik and five from Sinovac Vaccine.
At 3 weeks from the first dose, all Sinovac vaccinated subject (SV) had negative serology, from Sputnik vaccinated persons (SP), 42,86% had low serology response (IgG and IgM) whereas the others were negative and for Pfizer vaccinated persons (PFZ), 36% were negative, 36% presented low IgG response, 20% medium IgG response and 8% high IgG response.
At 2 weeks from the second dose, SV presented low or negative serology, SP had medium or high IgG response whereas all screened PFZ had high IgG response With almost 20% associated low IgM antibodies response.
At 3 months from the second dose, SV presented negative or low serology with 2 covid-19 infected patients, SP had low or negative IgG response with no infection, PFZ presented 57.7% high IgG response, 23.1% medium IgG response 15.4% low IgG response and 3.8% negative serology with 2 infected patients.

CONCLUSIONS

Antibodies response to vaccinated persons was the highest after 2 weeks from the second Vaccine dose in PfZ and SP, it tended to disappear in SV and SP after three months from the second vaccine dose whereas it stayed high in more than half PFZ. Vaccination reduces the risk of infection but does not abolish it. Wider studies are needed to have more accurate results.

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T266

LOW LEVELS OF GRANULOCYTIC MYELOID DERIVED SUPPRESSOR CELLS MAY BE A GOOD MARKER OF SURVIVAL IN THE FOLLOW-UP OF PATIENTS WITH SEVERE COVID-19

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BACKGROUND-AIM

SARS-CoV2 infection cause a disease (COVID-19) that may develop a systemic disease with immunosuppression and death in severe forms of COVID-19. Myeloid derived suppressive cells (MDSCs) are inhibitory cells that contribute to the immunosuppression in patients with cancer and infection. Increased levels of MDSCs have been found in COVID-19 patients although their role in the pathogenesis of severe COVID-19 has not been clarified. That is why we raised the question whether MDSCs could be useful in the follow-up of patients with severe COVID-19 in the intensive care unit (ICU).

METHODS

We have monitored the immunological cells, including MDSCs, Treg, Exhausted T cells and activated T cells, in 80 patients at admission in the Intensive Care Unit (ICU), and after 1, 2 and 3 weeks to look for the possible association with mortality (40 patients). Samples were analized by flow cytometry. The endpoint was death or the discharge from ICU. Mann-Whitney U test was used to compare cell distributions between discharged and deceased COVID-19 patient. Wilcoxon test was used to compare cell distributions in each group of patients at ICU admission vs. last determination

RESULTS

Even though the basal level of circulating MDSC did not discriminate between both groups, the last measurement before the endpoint (death or ICU discharge) showed that patients discharged alive from the ICU had lower levels of granulocytic MDSCs (G-MDSCs), higher level of activated lymphocytes and lower level of exhausted lymphocytes, compared with patients with bad evolution (death).

CONCLUSIONS

In conclusion, a steady increase of G-MDSCs during the follow-up of patients with severe COVID-19 was found in those who finally die, whereas a low level of G-MDSC may be a good marker of survival in the follow-up of patients with severe COVID-19.

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T267

PROGNOSTIC VALUE OF LONG PENTRAXIN 3 IN COVID-19 PATIENTS

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BACKGROUND-AIM

After the December 2019 outbreak in China, the novel Coronavirus infection (COVID-19), has quickly overflowed worldwide. We observed that several laboratory tests have been shown as characteristically altered in COVID-19, but these biomarkers were not accurate to rapidly evaluate outcome of patient. Different than the short pentraxin prototype CRP, produced by the liver in response to interleukin (IL)-6, the long Pentraxin 3 (PTX3) is a key component of humoral innate immunity. We investigated the prognostic role of PTX3 serum levels.

METHODS

PTX3 serum levels was measured by ELISA commercial Kit in patients with COVID-19 (n=96) and in healthy controls (n=50). Statistical analysis were made and PTX3 serum levels were compared into groups and between other laboratory markers.

RESULTS

High PTX3 serum level was a strong independent indicator of ICU-transferred patients and it worked substantially better than other molecules, including CRP, IL-6, ferritin and LDH. In particular, a cut-off of PTX3 > 18 ng/mL yielded a sensitivity of 96% and a specificity of 99% in identifying patients requiring ICU. Univariate and multivariable logistic regression models demonstrated that, after controlling for comorbidities, the only significant biomarker was PTX3.

CONCLUSIONS

Although our results need to be confirmed in larger cohorts, we support the use of PTX3 as quick and practical biomarker to predict COVID-19 severity, also in Emergency Room

Microbiology - Infectious diseases including COVID 19

T268

A MACHINE LEARNING APPROACH TO PREDICT THE PROGNOSTIC OF PATIENTS ADMITTED FOR COVID-19 USING POCT EQUIPMENT

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BACKGROUND-AIM

Pulmonary involvement by COVID-19 is frequently associated with alterations in oxygenation and acid-base balance. The aim of our study is to generate a Machine Learning model (MLM) capable of predicting patient prognosis at 7 days using Point Of Care Testing (POCT) arterial blood gas parameters of admitted patients.

METHODS

Retrospective single-center study between March 2020 and June 2021. Data were obtained from 3345 arterial blood gases (ABG) of 1159 patients who were distributed into two groups: ABG of patients who died in <=7 days (n=2086) and ABG of patients who were discharged from the hospital in <= 7 days (n=1259). This database was randomly divided into a subgroup (n=2677) to train the model and another (n=668) with balanced groups to validate the model.

ABG were drawn into heparinized syringes and analyzed using POCT equipment (GEM Premier 4000, Werfen). The parameters of Sex, Age, pH, pCO2, pO2, HCO3, sO2c, Sodium, Potassium, Glucose, Lactate, Hemoglobin and Base excess were studied.

A classical inference analysis was performed by logistic regression, calculating the odds ratio (OR) of each variable versus prognosis and applying a threshold p-value of 0.004 (0.05/12 parameters) after Bonferroni correction.

Finally, a Support Vector Machines (SVM) MLM was developed using the Rstudio software and the Caret (Classification and Regression Training) library.

RESULTS

In the inference analysis, 9 of 12 parameters showed statistical significance ($p<0,001$) between discharged and deceased patients.

The performance of the MLM was analyzed using a confusion matrix, where the model predicts the prognosis of each patient in the validation subgroup, with data not included in the training subgroup. An area under the ROC curve of 0.945 was obtained, and a sensitivity and specificity of 94% and 76% respectively, with an overall model accuracy of 85%. Some of the most important parameters were pCO2, hemoglobin and sodium.

CONCLUSIONS

The creation of predictive algorithms based on POCT device parameters allows to determine quickly and efficiently the probability of death before 7 days. Machine Learning models could represent a great advance in the detection of patients with poor prognosis for the implementation of early support measures with the consequent clinical benefit.

Microbiology - Infectious diseases including COVID 19

T269

COAGULATION AND ROTATIONAL THROMBOELASTOMETRY PROFILING OF COVID-19 PATIENTS

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BACKGROUND-AIM

In addition to typical respiratory symptoms, COVID-19 is associated with coagulation abnormalities that lead to thromboembolic complications.

METHODS

Retrospective study of critically ill patients admitted to an intensive care unit (ICU) a cause of severe COVID-19 pneumonia (Group 1) and we evaluated coagulation function using coagulation standard parameters on day of admission (T0) and 10 (T10) days after admission to ICU and rotational thromboelastometry (ClotPro). In addition, we compared coagulation standard parameters to patients with severe non–COVID-19 pneumonia (Group 2).

RESULTS

Eighty-four patients participated in our study. Traditional coagulation parameters were similar between group 1 and group 2. Only D-dimer levels (2442.11 ng / ml vs 370 ng / ml, p = 0.03) were significantly higher in COVID-19 pneumonia than in non-COVID-19 pneumonia. In addition, we concluded an increase in D-dimer levels during the hospital stay (T0 = 2442.11 ng / ml vs T10 = 8564.39 ng / ml, p = 0.000). Finally, patients with SARS-CoV-2 pneumonia exhibited hypercoagulant thromboelatometry profiles, characterized by elevated maximum clot firmness (MCF) values.

CONCLUSIONS

The results observed in our study support hypercoagulability in a severe inflammatory state, rather than a disseminated intravascular coagulation (DIC). More studies are needed to allow a better understanding of the coagulopathy produced in patients with severe COVID-19 pneumonia.

Microbiology - Infectious diseases including COVID 19

T270

ASSOCIATION OF VITAMIN D WITH THE MODULATION OF THE DISEASE SEVERITY IN COVID-19

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BACKGROUND-AIM

COVID-19 disease is one of the most common respiratory diseases found in 2019 in Wuhan, China. In addition to common risk factors, other factors such as salts seem to be effective in the progression or clinical course of this disease. The aim of this study was to evaluate the serum levels of vitamin D, ACE concentrations, and neutrophil to lymphocyte ratio (NLR) were measured in patients with confirmed COVID-19 in comparison with the control group

METHODS

In this case-control study, blood samples from 30 patients (12M and 18F) with covid-19 and 30 healthy age- and sex-matched individuals (12M and 18F) were collected. Sera were separated and vitamin D and ACE levels were determined using colorimetric Biorexfars kits.

RESULTS

Results:
There was no difference in mean age between covid-19 patients and control subjects (42.9±3.23 vs 43.8±2.97 years, respectively). The mean age of men (42.2±0.28 years) did not significantly differ from that of women (44.3±0.41 years). The differences between COVID-19 positive and negative groups, in terms of the vitamin D and angiotensin-converting enzyme (ACE) concentrations, are depicted in The results show that vitamin D had an important change in the group of COVID-19 positive individuals. Four patients have unfortunately deceased during this study for whom, the mean vitamin D concentration was significantly decreased compared to the control group and other COVID-19 patients ($p < 0.0001$). Serum ACE concentration showed a significant increase in the patient's group, compared to the control group ($p < 0.0001$). The ACE concentration was significantly higher in deceased individuals even compared to the other COVID-19 patients ($p < 0.0001$). Looking at individuals with different vitamin D levels, the ACE concentration showed a higher quantity among individuals with insufficient vitamin D concentration ($p < 0.0039$). A significant decrease in lymphocyte count and lymphopenia has been observed in this study ($p < 0.0001$). There was also a significant increase in the patients' group in terms of the neutrophil count ($p < 0.0001$). In the case of the deceased patients, the change in lymphocyte and neutrophil count had an important difference not only with the control group but also with other COVID-19 patients ($p < 0.0001$). Consequently, the ratio between neutrophils and lymphocytes (NLR) was also considerably higher in the COVID-19 group, and beyond in the case of the deceased patients ($p < 0.0001$).

CONCLUSIONS

Results demonstrated significant alterations in vitamin D and ACE levels as well as NLR in the patients' group. The contribution of those factors to the prognosis and severity of the disease has been shown.

Microbiology - Infectious diseases including COVID 19

T271

SPECIFIC DIAGNOSTIC TESTS TO DETECT SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2.

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BACKGROUND-AIM

Specific diagnostic tests to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and resulting COVID-19 disease are not always available and take time to obtain results. Routine laboratory markers are used to assess the clinical status of a patient. The ain is to demonstrate that the routine laboratory test, such as the case of white blood cell count, platelet count, measures of anticoagulation, ferritin level, serum albumin, C-reactive protein (CRP) and procalcitonin , allows a more selective and efficient diagnosis to detects SARS-CoV-2.

METHODS

Retrospective cohort study including 80 patients who presents COVID symptoms. All patients underwent basic biochemistry test as part of the diagnosis and reverse transcriptase polymerase chain reaction (RT-PCR) to confirm the presence of SARS- CoV-2.

RESULTS

We included 80 patients (N=80) in our study, 51 men and 29 women. The RT-PCR was positive in 15 patients. We observed an increase in the levels of inflammation and infection markers in patients with a COVID-19 diagnosis confirmed by RT-PCR: ferritin (373,06 vs 341,23 ng/ml, p=0,384), C-reactive protein (81,73 vs 57,55 mg/l, p=0,02) and procalcitonin (0,7 vs 0,25 ng/ml ,p=0,125). In addition, this patients with SARS-CoV-2 diagnosis presents elevated levels of coagulation markers or d-dimers: 8923,7 vs 1573,4 , p=0,03). On the other hand, we conclude a decrease in the levels of white blood cell count (7,88 vs 9,27/µl p=0,335, platelet count (251.300 vs 260.400/µl , p=0,331) and albumin (2,9 vs 3,2 g/dl, p=0, 390).

CONCLUSIONS

These easy-to-measure, time-saving and very low-cost parameters have been shown to be effective in the early prediction of the COVID-19 severity. Their use at the early ad- mission stage can improve the risk stratification and management of medical care resources in order to reduce the mortality rate.

Microbiology - Infectious diseases including COVID 19

T272

PERFORMANCE OF MONOCYTE DISTRIBUTION WIDTH AS AN AID IN SEPSIS DETECTION IN ADULT PATIENTS WITH MONOCYTOPENIA AND LEUKOPENIA

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BACKGROUND-AIM

Early sepsis detection in the emergency department (ED) is critical but is often delayed due to time needed for clinical assessment and laboratory tests to be ordered and results become available. We recently evaluated new sepsis biomarker, Monocyte Distribution Width (MDW) as an aid in sepsis detection for general adult ED population. We were specifically interested to assess the performance of MDW in patients with leukopenia or monocytopenia.

METHODS

Study cohort included 73 patients with leukopenia (WBC<4000 cells/μl) and 97 patients with monocytopenia (monocyte count<300 cells/μl). Blood samples were collected in K2-EDTA tubes and tested on the Beckman Coulter DxH 900 hematology analyzer to obtain MDW results as part of a CBC-Diff. Sepsis diagnosis was established with sepsis-3 criteria. The diagnostic performance of MDW was evaluated by ROC analysis, calculating area under the curve (AUC), sensitivity, specificity, Positive Predictive Value and Negative Predictive Value. Statistical analysis was performed with Medcalc software, version 19.6.4 (MedCalc Software bvba, Ostend, Belgium).

RESULTS

97 patients with monocytopenia were classified into 4 groups: no infection no sepsis (44), infection (15), sepsis (35), septic shock (3). 73 patients with leukopenia were classified as no infection no sepsis (26), infection (16), sepsis (30), septic shock (1). In the group with monocytopenia there were 57 patients with cancer (58.8%), 15 of them with hematological malignancies. Among leukopenic patients, there were 58 patients with cancer (79.5%), including 12 with hematological malignancies.
Performance characteristics of MDW as an aid in sepsis detection in sub-groups of ED patients were the following: for monocytopenia - AUC 0.736, sensitivity 87% and specificity 61% at a cutoff >20.35*; for leukopenia - AUC 0.705, sensitivity 74% and specificity 69% at a cutoff >24.13*; for the whole cohort, MDW demonstrated AUC 0.71, sensitivity 81% and specificity 51% at a cutoff of 20.

CONCLUSIONS

Our results demonstrated that MDW performance in the ED is not affected in patients with monocytopenia and leukopenia, even in the cohort with high prevalence of patients with cancer.
*Cutoff is applicable to the specific patient population in the study.

Microbiology - Infectious diseases including COVID 19

T273

CORRELATION OF ROUTINE COAGULATION TESTS AND CRP WITH THE DAYS OF HOSPITALIZATION OF COVID-19 PATIENTS

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BACKGROUND-AIM

COVID-19-related systemic cytokine response induces the production of procoagulant factors, which predisposes patients to a prothrombotic state. CRP, the main acute inflammatory protein, has been related to the disease outcome. The aim of our study was to investigate the ability of routine coagulation tests and CRP values for the prognosis of the length of hospital stay of CoVID-19 patients.

METHODS

Coagulation tests (PTratio, aPTTratio, Fibrinogen (FIB), DDimers) and CRP were performed in 244 CoVID-19 patients upon hospital admission and prior to the initiation of anticoagulation therapy. Coagulation tests were performed on BCS XP System (SIEMENS). Pearson's correlation coefficient was used for comparison between the above parameters of 244 patients and their days of hospitalization. P values<0.05 were considered statistically significant. All statistical analyses were performed using SPSS27.

RESULTS

The Median, minimum and maximum values were: PTratio: 0.97 (0.85-1.22), aPTTratio: 1 (0.72-1.19), FIB: 508mg/dl (60-1068 mg/dl), DDimers: 694μg/dl (170-30,938 μg/dl), CRP: 38.4mg/L (0.4-383 mg/L). The comparison between Coagulation tests, CRP and days of hospitalization revealed statistical significant correlation in aPTTratio (p=0.045), FIB (p=0.035) and CRP (p<0.001).

CONCLUSIONS

aPTT, FIB and CRP values upon admission in COVID-19 patients, may be utilized for the prognosis of the duration of hospitalization.

Microbiology - Infectious diseases including COVID 19

T274

EVALUATION OF COAGULATION STATUS USING CLOT WAVEFORM ANALYSIS IN PATIENTS WITH COVID-19

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BACKGROUND-AIM

The activation of coagulation is one of the most severe complications of CoVID-19. The automatic optical end-point coagulation analyzers have the ability to present the clot reaction curve of the PT, APTT and Fibrinogen(FIB) which is referred as a clot waveform analysis (CWA). CWA has not been adequately described as a tool for the evaluation of coagulation.

The aim of our study was to comparatively assess the changes in clot waveform analysis (CWA) parameters between COVID-19 patients upon hospital admission and healthy individuals.

METHODS

In this retrospective observational study, we evaluated 227 CoVID-19 patients upon their hospital admission, prior to the initiation of anticoagulation therapy relative to 84 healthy individuals. Coagulation tests and CWA were performed on BCS XP System (SIEMENS). The CWA parameters of PT, aPTT and Fifrinogen (FIB) were 1. the change in Absorbance (dmA) which is considered to reflect to the “thrombin burst” as real hemostatic ability and 2. the time difference between the starting time of the reaction (mixing of reagent and sample) until a specific absorbance change (absorbance threshold). All statistical analyses were performed using SPSS27. Variables were tested with Student’s t tests or Mann–Whitney U tests for differences in distributions of dmA and dsec of PT, aPTT and FIB between two groups. P values <0.05 were considered statistically significant.

RESULTS

A statistically significant increase (p<0.001) was detected in the dmA values of PT, aPTT and FIB in CoVID-19 patients compared with the healthy individuals: PT: 2201mA vs 1071mA, aPTT: 2217mA vs 1139mA, FIB: 1238mA vs 523mA. The dsec values of CoVID-19 patients were: PT: 3.51sec, aPTT: 8.28sec, FIB: 5.6sec. The dsec values of healthy individuals were: PT: 3.55sec, aPTT: 10.73cec, FIB: 12.9sec. A statistically significant decrease was found in CoVID-19 patients only for the dsec values of FIB (p<0.001).

CONCLUSIONS

CWA variables upon admission in COVID-19 patients may be used for the evaluation of their hypercoagulopathy. Our results may help to identify patients at a high risk of thromboembolism.

Microbiology - Infectious diseases including COVID 19

T275

EVOLUTION OF DIAGNOSTIC TESTS FOR ACUTE COVID-19 INFECTION IN OUR AREA IN DECEMBER 2021 VS. DECEMBER 2020 AS A CONSEQUENCE OF THE UPDATE OF THE PROTOCOL FOR EARLY DETECTION, SURVEILLANCE AND CONTROL OF COVID-19 IN SPAIN

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BACKGROUND-AIM

As a result of the accelerated increase in community transmission of SARS-CoV-2 infection in Spain, the Ministry of Health published an update of the "Protocol for Early Detection, Surveillance and Control of COVID-19" on December 22 in order to review the management of cases and contacts. Thus, the quarantine time for positive cases is reduced to 7 days instead of 10, an Acute Infection Diagnostic Test (AIDT) is not necessary for discharging a patient unless it is a healthcare worker, the vaccinated contacts do not require isolation measures and antigen testing such as AIDT is prioritized 3-5 days after contact with the confirmed case, as well as in symptomatic patients.

METHODS

We have analyzed the number of AIDT and the percentage of positives in the month of December 2021 coinciding with the sixth wave of COVID-19 and we have compared it with the same dates of the previous year when the second wave was taking place and the control and surveillance measures were stricter.

RESULTS

A total of 12406 AIDT (1818 Ag, 15.51% positive and 10588 PCR, 25.53% positive) were performed in December 2021, compared to 7397 PDIA (533 Ag, 9.94% positive, and 6864 PCR, 19.61% positive) in December 2020. An irregular distribution of AIDT by weeks is observed in 2021 (1120, 1564, 2524, 4553 and 2645, respectively), but not in 2020 (1845, 1605, 1703, 1418 and 826). There is an increase of 154% in PCR and 341% in Ag in 2021 compared to 2020, which only justifies a positive difference of 3.44% in the positive detection rate (3.92% PCR; 5.57% Ag). The Ag/PCR ratio was 0.17 in December 2021 and 0.07 in December 2021.

CONCLUSIONS

The performance of AIDT during the month of December 2021 in our hospital increased markedly compared to the same dates of the previous year, particularly in the fourth week of the month, coinciding with Christmas. Antigen testing was prioritized over PCR, so that for every 100 PCR, 17 antigen tests were performed (compared to 7 in December 2020). This change in the protocol meant a saving of almost 17000 euros only in the month of December. Despite the change in protocol and the relaxation of prevention measures imposed by the Government, hospital pressure was minimal probably thanks to the high percentage of vaccination in our population.

Microbiology - Infectious diseases including COVID 19

T276

ANALYSIS OF MDW AS A BIOMARKER OF SEVERITY AND OUTCOME IN COVID-19 PATIENTS

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BACKGROUND-AIM

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has infected almost 356 million people and caused over 5.6 million deaths worldwide as of January 2022. We studied a novel biomarker, Monocyte Distribution Width (MDW), in COVID-19 patients, evaluating potential utility of MDW as a biomarker of severity and prognosis in SARS-CoV-2 infections. Monocytes are critical immune cells; we hypothesized that morphological changes in monocytes detected by MDW may correlate with disease severity and assist in predicting patient outcomes. We also analyzed changes in LY count (LY#), NE count (NE#), and Neutrophil-to-Lymphocyte Ratio (NLR) in COVID-19 patients.

METHODS

The study included 99 uninfected adult controls and 715 adult COVID-19 patients admitted to the ED in a tertiary care facility in Dubai, UAE, during the initial wave of the pandemic in 2020. The SARS-CoV-2 RNA was detected by a nasal swab reverse transcription-polymerase chain reaction. Blood collected in K2-EDTA was analyzed on a Beckman Coulter DxH 900 analyzer, within two hours from blood draw. Patients were classified according to disease severity in the following groups: asymptomatic, mild, moderate, and severe. Medcalc v.19.6.4 (MedCalc Software bvba, Ostend, Belgium) was used for statistical analysis.

RESULTS

The COVID-19 patient groups studied included 100 asymptomatic, 107 mild, 189 moderate and 319 severe cases (37 deceased). We observed increasing trends for MDW, NE# and NLR, and decreasing trends for LY#, moving from the controls to asymptomatic, mild, moderate, severe survived and severe deceased patients. The corresponding values for MDW by groups were: 17.1, 18.5, 21.8, 23.1, 24.5, 26.4; for LY# (x10^3 cells/μl): 2, 1.2, 1.3, 1.1, 1, 0.9; for NE# (x10^3 cells/μl): 4.3, 4.1, 3.9, 4.4, 5.7, 7.4; for NLR: 2.1, 3.4, 2.7, 4.0, 5.6, 6.8. ROC analysis for MDW to predict patient outcomes (survived vs. deceased) demonstrated AUC 0.735, sensitivity 75.7%, specificity 65.7%, positive predictive value 12.4% and negative predictive value 97.7% at cutoff 24.7. With MDW cutoff at 30.6, the specificity for a negative outcome was 95.2%.

CONCLUSIONS

MDW may be considered as a potentially useful and inexpensive tool to aid the management of COVID-19 patients in the ED.

Microbiology - Infectious diseases including COVID 19

T277

VITAMIN D STATUS IN COVID-19 PATIENTS AND IT'S INFLUENCE ON DISEASE SEVERITY

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BACKGROUND-AIM

Vitamin D is involve in the modulation of the innate and acquired immune system and also in the production of antimicrobial peptides, as well as in the expression of genes involved in the intracellular destruction of pathogens. Low serum 25OHD levels are frequently found in elderly individuals or in those with chronic conditions, which have also been reported as poor prognostic factors for COVID-19. The downregulation of ACE2 by SARS-CoV-2 leads to a dysregulation of the renin–angiotensin system, which contributes to the "cytokine storm" that precedes the acute respiratory distress syndrome characteristic of the severe form of COVID19. In this sense, vitamin D can inhibit proinflammatory cytokine production in human monocytes/macrophages , and chronic vitamin D deficiency may induce RAS activation, leading to the production of fibrotic factors and, therefore, lung damage .

Considering the differences in the severity and fatality of COVID-19 in the globe, it is important to understand the reasons behind it. Improvement of immunity through better nutrition might be a considerable factor. This study evaluated the correlation of vitamin D concentrations with COVID-19 cases and its impact on the severity and mortality of COVID- 19.

METHODS

Serum levels of total 25(OH)D were quantified using a commercially available immunoassay (ADVIA Centaur Vitamin D Total Assay®, Siemens Healthcare GmbH, Erlangen, Germany).

RESULTS

Included in the study were 83 patients (55.2% men, mean age was 57 years and 45.8% women mean age 56 years) with confirmed COVID-19 pneumonia, diagnosed and treated , between 1 June and 12 August 2020 in City General Hospital "8th of September"-Skopje.

In the entire study, very low vitamin D levels are observed in both genders. Median Vitamin D level was significantly lower in the female (28.01 nmol/L) versus the male subgroup (43.96 nmol/L). It has been noticed that in women, although the percentage of hospitalized from COVID- 19 is lower, they have a higher mortality rate which is 18.42% , compared to men where although we have a higher percentage of hospitalized, mortality is lower and is 8.9% . Also the length of hospitalization among women is longer, 19 days , compared to men which is 15.5 days.

CONCLUSIONS

In summary, this observational study among patients with COVID-19 who have experienced a definite outcome, shows an association between Vitamin D status and severity of and mortality from COVID-19.

Microbiology - Infectious diseases including COVID 19

T278

LUNG CELL DESTRUCTION IN COVID-19 IS PRIMARILY CAUSED BY CELL LYSIS DURING THE INTRACELLULAR REPLICATION OF THE VIRUS.

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BACKGROUND-AIM

Covid-19 has caused high morbidity and mortality in society. Mechanisms behind the lung destruction and need for artificial ventilation has been debated. From cytokine storm to inflammation. We have investigated the importance of viral load for the outcome

METHODS

We have developed a quantitative method for measuring the number of SARS-CoV-2 virus in the blood, with a CFU/mL from 10 to 108 CFU/mL and CV below 15%, on the Simoa from Quanterix.com

RESULTS

Daily monitoring of virus load from admittance, together with markers of inflammation and cell damage, to discharge, artificial ventilation or death, showed that
1. disease severity and death were predicted by virus concentration (AUC 0.86)
2. inflammatory markers were depending on virus concentration (p<10-6)
3. virus blood load preceded IL-6, antibody response and coincided with clinical severity

CONCLUSIONS

The time dependent events in markers points to a pathophysiological process of cell destruction by the uncontrolled production of virus in the cells.
Our finding is in agreement with the lack of good clinical effect of antibody treatment or dexamethasone.
Drugs for treatment should be focused on inhibition of RNA/DNA replication.
At the moment we follow the effect of Remdesivir and Paxlovid using this method, stopping medication, when virus in undetectable below 0.1 ng/L of N-Antigen (~<10 CFU/mL)

Microbiology - Infectious diseases including COVID 19

T279

DEXAMETHASONE AND TOCILIZUMAB TREATMENT NULLIFIES THE VALUE OF C-REACTIVE PROTEIN AND PROCALCITONIN TO DETECT SECONDARY BACTERIAL INFECTIONS IN COVID-19 PATIENTS

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BACKGROUND-AIM

Procalcitonin (PCT) and C-reactive protein (CRP) were previously shown to have value for the early detection of secondary infections in critically ill COVID-19 patients(1). However, since the introduction of immunomodulatory therapy, the value of these biomarkers is unclear. We investigated PCT and CRP kinetics in critically ill COVID-19 patients treated with dexamethasone with or without tocilizumab, and assessed the value of these biomarkers to detect secondary bacterial infections.

METHODS

Critically ill COVID-19 patients were divided into three treatment groups: no dexamethasone and no tocilizumab (D-T-, n=66), dexamethasone but no tocilizumab (D+T-, n=47), and dexamethasone and tocilizumab (D+T+, n=73). Serial data of PCT and CRP was analyzed between 6 days prior to cessation of dexamethasone treatment and 10 days afterwards. Furthermore, the D+T- and D+T+ groups were subdivided based on the occurrance of secondary infection to analyze detection accuracy of these biomarkers for the occurrence of a secondary infection.

RESULTS

Following cessation of immunomodulatory treatment, there was a rebound in PCT and CRP levels, most pronounced in the D+T- group. Upon occurrence of a secondary infection, no significant increase in PCT and CRP levels was observed in the D+T- group ($p=0.052$ and $p=0.08$, respectively). CRP levels remained suppressed in the D+T+ group. ROC analysis of PCT and CRP levels yielded AUC of 0.52 and 0.55, respectively, which are both markedly lower than we found in COVID-19 patients not treated with immunomodulatory drugs (0.80 and 0.76, respectively)

CONCLUSIONS

Cessation of dexamethasone treatment in critically ill COVID-19 patients results in a rebound increase in PCT and CRP levels unrelated to the occurrence of secondary bacterial infections.

Microbiology - Infectious diseases including COVID 19

T280

ARE ADENOSINE DEAMINASE AND MONONUCLEAR CELLS GOOD BIOMARKERS FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS?

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BACKGROUND-AIM

Adenosine deaminase (ADA) is an enzyme that takes part in purine metabolism and is related to the maturation of T-lymphocytes. In pleural fluid (PF), high values of ADA together with a high percentage of mononuclear cells (%MN), are related to the presence of Mycobacterium tuberculosis (MT) and the diagnosis of Pulmonary Tuberculosis (PT).
The objective of the study was to determine the sensitivity (S), specificity (E), positive predictive value (PPV) and negative predictive value (NPV) of the determination of the value of isolated ADA and, on the other hand, ADA together with %MN in the diagnosis of PT.

METHODS

From the Laboratory Information Systems of our Hospital (Modulab and Gestlab), the results of all the PFs that had been requested to determine ADA, %MN and microbiological study during the years 2017 to 2019 were collected.
The variables studied were the determination of ADA (Biosystems), %MN (Sysmex XN-3100), and the bacteriological study (PCR).
Pathological results in PF of ADA ≥ 35IU/L and %MN ≥ 50% were considered.

RESULTS

Taking into account only the ADA value, a total of 655 PFs were studied. Of these, 194 samples (29.61%) had a pathological ADA result, of which 17 (8.76%) had microbiological confirmation of MT. Of the samples with a non-pathological ADA result, 461 (70.38%), none were positive for MT. The S was 100%, the E was 72.26%, the PPV was 8.76%, and the NPV was 100%.
Studying together ADA and %MN, 599 PF were assessed, presenting 84 (14.02%) samples of pathological ADA and %MN results. Of these, in 11 (13.09%) the presence of MT is guaranteed. Of the PFs with a non-pathological %MN result, 515 (85.97%), none had PT. The S was 100%, the E was 87.58%, the PPV was 13.09%, and the NPV was 100%.

CONCLUSIONS

This study confirms the excellent S and NPV of ADA determination, as well as the improvement of E and PPV when ADA and %MN are combined. These results allow the start of treatment to be accelerated until microbiological confirmation, which can take up to several weeks.

Microbiology - Infectious diseases including COVID 19

T281

ADENOSINE DEAMINASE AND MONONUCLEAR CELLS: BIOLOGICAL PARAMETERS FOR RAPID AND AUTOMATED DIAGNOSIS OF PULMONARY TUBERCULOSIS.

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BACKGROUND-AIM

The diagnosis and prompt treatment of Pulmonary Tuberculosis (PT) are essential for the best quality of life for the patient. Adenosine deaminase (ADA) is related to the maturation of T-lymphocytes, so the high catalytic concentration of ADA in pleural fluid can help diagnose PT. In pleural fluid (PF), high values of ADA together with a high percentage of mononuclear cells (%MN), are related to the presence of Mycobacterium tuberculosis (MT).
The objective of the study was the statistical comparison of ADA and %MN values in patients with PT versus negative for PT.

METHODS

The PFs that had been requested to determine ADA, % MN, and microbiological study from 2017 to 2019 were collected. The results were obtained from the Laboratory Information Systems of our Hospital (Modulab and Gestlab).
The determination of ADA (Biosystems), %MN (Sysmex XN-3100), and the bacteriological study (PCR) were the variables that were studied.
Results of ADA ≥ 35IU/L and %MN ≥ 50% in PF were considered pathological.
To conduct the statistical study, the SPSS v.27 program was used. We compared the results of the samples that had tested positive for MT with those that had tested negative for MT. The Mann-Whitney test was applied when dealing with non-parametric variables (Kolmogorof-Smirnov test).

RESULTS

Considering only the ADA value, a total of 655 PFs were studied. Of these, 17 samples (2,59%) had a microbiological confirmation of MT. The median of ADA in patients with PT was 91,06 (63 - 140) IU/L, in patients without PT was 35,97 (0 - 480) IU/L. Statistically significant differences were found comparing both groups.
Studying only the %MN, 599 PF were evaluated, presenting 11 (1,84%) the microbiological confirmation of MT. The median in patients with PT was 99 (76-100) % and, in patients without PT was 78 (0-100) %. Significant differences were also found when comparing the %MN values.

CONCLUSIONS

In this study, it has been shown that ADA and %MN are good markers for the diagnosis of PT. In patients with high suspicion of PT, the rapid determination of ADA in PF together with the %MN allows the diagnosis and initiation of treatment to be advanced until diagnostic confirmation by microbiology.

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TWO- YEARS FEED-BACK ON BIOCHEMISTRY AND HEMATOLOGY PRESCRIPTIONS IN OVERSEAS FRANCE AND IN FRENCH-SPEAKING COUNTRIES DURING COVID-19 PANDEMIC

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BACKGROUND-AIM

The French Society of Clinical Biology (SFBC) "Biochemical markers of COVID-19" has set up a working group whose primary purpose is to review, analyze and monitor biological prescriptions according to patient's care path.

METHODS

This study covers all public and private sectors of medical biology in metropole and overseas France (OF) and extends to the French-speaking world (FSW). OF and FSW feed-back is reported herein.

RESULTS

After 2 years of pandemic, the time has come for reasoned prescription in Biochemistry and Hematology analyses. Clinicians have mostly prescribed tests according to their habits or recommendations of their scientific societies, dealing with non-specific Acute Respiratory Distress Syndrome (ARDS), rather than following recommendations of IFCC. In all regions questioned (New Orleans, French Polynesia, Mayotte, Guadeloupe, New Caledonia), necessary/sufficient parameters for COVID-19 Emergency Room patients are: CBC, serum electrolytes, creatinine, urea, CRP, SGOT-SGPT, APTT-TCA-fibrinogen and according to signs of severity: troponin, D-dimers, blood gas (BG) with calculation of ratio PaO_2/FiO_2 (P/F) and possibly lactate. For intensive care unit patients, Procalcitonin is added. A large increase in BG prescriptions has been noted. Point-of-care testing (POCT) devices have demonstrated effectiveness in orientation and/or for clinical follow-up of critical cases. Diffuse thickening of pulmonary inter-alveolar septa resulted in hypoxemia illustrated by a collapsed P/F ratio being a part of Berlin criterion for ARDS: $P/F < 300$ mmHg is correlated with mortality in “usual” ARDS. COVID-19 would be special in causing a presentation of extremely severe hypoxemia, but correlation with mortality has not yet been fully established.

CONCLUSIONS

At the early stage of COVID-19, with regard to OF and FSW surveyed, common symptomatology with local zoonosis (dengue, zika, malaria, leptospirosis...) complicates diagnosis of COVID-19. At a more advanced stage, it is a question of managing an influx of patients with ARDS; biology is then simpler and POCT devices have proven their effectiveness. As a result, resuscitators can better manage frequent comorbidities encountered in these regions: i.e. obesity, diabetes, chronic renal failure, cardiovascular diseases.

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POTENTIAL OF NEUTRALIZING ANTIBODIES AGAINST SARS-COV-2 INFECTION: IMPACT OF COVISHIELD VACCINATION

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BACKGROUND-AIM

The vaccination efficacy can indirectly be assessed through the quantification of neutralizing antibodies. Very few data are available on Covishield efficacy in terms of neutralizing antibody expression upon vaccination. This study is focused on profiling of neutralizing antibody expression during and after the Covishield two shot vaccination and observing COVID-19 infection in vaccinated participants during the period.

METHODS

SARS CoV-2 neutralizing antibody concentrations in samples were estimated using electrochemiluminescence immunoassay kit for Lifotronics eCL8000. The sampling had been done sequentially at 45th, 85th day after 1st dose and 15th day after 2nd dose Covishield vaccination. Parallelly, in order to confirm the total SARS CoV-2 IgG response in COVID-19 infection, measured the IgG using SARS CoV-2 IgG lateral flow immunoassay test kit.

RESULTS

The subjects previously infected with COVID-19 before 1st dose vaccination demonstrated high neutralizing antibody (>10AU/ml). In COVID-19 uninfected subjects, there was a sudden incline in neutralizing antibody after the 2nd dose. Infection with SARS CoV-2 between 1st and 2nd dose of Covishield vaccination implicate that the level of neutralizing antibody in serum after 1st dose was not adequate to combat the virus and prevent infection. We observed COVID-19 infection in participants even after 2nd dose of vaccination.

CONCLUSIONS

Interestingly, there was no protection against SARS CoV-2 even with a high neutralizing antibody expression of 188.5 AU/mL after the 2nd dose. Findings of Covishield efficacy in different cohort samples before and after 2 doses of Covishield vaccination provide impetus for improvement or development of next generation vaccines.

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PATTERN OF SARS-COV-2 GENES DETECTED IN OUR LABORATORY

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BACKGROUND-AIM

SARS-CoV-2 viral genome serves as mRNA, while shorter subgenomic RNAs (sgRNAs) are produced and thought to encode the virus proteins among them Spike (S), Envelop (N) and Nucleocapside (N). The aim of our study is to highlight the pattern of SARS-CoV-2 RNA genes detected in our Laboratory, the corresponding Cycle threshold (Ct) values and to investigate any correlation between the genes' Ct values.

METHODS

We retrospectively recorded for six months period all samples presented with genes generating a positive signal with Ct value below the upper limit provided by the manufacturer, the corresponding Ct values, the patient's medical data and the given interpretation.The applied methodology was real-time Reverse Transcription Polymerase Chain Reaction using GeneFinder™ COVID-19 Plus RealAmp Kit, on ELITeInGenius® instrument by amplification of RNA-dependent RNA polymerase (RdRp) gene, E gene and N gene. Statistical analysis: Multilinear regressions, Pearson's correlation, one-way ANOVA (Tukey), were applied. Values of P <0.05 were considered to indicate statistical significance.

RESULTS

According to the pattern of simultaneous targeted genes the samples were divided into 4 groups; Group A: RdRp, E, N (N=193), Group B: RdRp, N (N=16), Group C: E, N (N=36) and Group D: single N (N=85). In Groups A, B and C there were no statistically significant differences in Ct(s) between the detected genes; all P <0.05. The four groups differ in statistical significant degree in Ct(s) of N gene (P=0.000) and according to the Post Hoc Tukey the Ct(s) of N gene in group D (median 39.2 / minimum 34.3 / maximum 43.8) were higher (P=0.000) except from group B (P=0.202). According to the medical data all samples with single detection of N gene were originated from patients during convalescence period in order to be discharged either from COVID-19 ward or from the quarantine and the interpretation given was “retest” or “negative”.

CONCLUSIONS

Among the targeted genes, only N gene was detected in a single pattern. This is probably due to the fact that N protein is abundantly produced within infected cells.The persistence of N gene in high Ct should be interpreted with caution and in a close communication with clinician in order to a better patient management.

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T285

SURVEY ON BIOLOGICAL PRESCRIPTIONS OF 327 HEALTHCARE SERVICES IN SUSPECT OR POSITIVE PATIENTS FOR COVID-19 IN FRANCE AND FRENCH SPEAKING TERRITORIES

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BACKGROUND-AIM

In April 2020, the French Clinical Biology Society (SFBC) set up a working group of medical biologists «Biochemical Markers and COVID-19» including: metropolitan University Hospitals (mUH), metropolitan General Hospitals (mGH), metropolitan Army Training Hospitals (ATH), overseas and french speaking territories (OS-FST), itself divided into french overseas (FOS), french speaking Africa (FSA), french speaking America-Europ (FSAE). One of the goals was the analysis of the biological prescriptions (March-May 2020) in suspect or positive patients for COVID-19, in adult emergency, critical care, post-intensive care, pediatrics, geriatrics, according to the IFCC guidelines.

METHODS

This survey included the prescriptions of 327 healthcare services: mUH=203, mGH=55, ATH=11, OS-FST=58 (FOS 27, FSA 15, FSAE 16). The methodology focused on systematic prescriptions and retained a rate of prescriptions >10%. Herein results summarized only biomarkers of guidelines. Statistics: Stata software (V15 Stata Corp) and R. Comparisons of the prescription rate: Chi2 or Fisher tests. Multiple match analysis: ADE4.

RESULTS

ATH and mUH were bigger prescriptors than mGH and OS-FST. Geriatry and pediatry prescripted less than medicine, emergency or critical care. The only biomarkers prescripted >75% by both mUH, mHG, ATH and OS-FST were full blood count (FBC), urea (BUN), creatinine, CRP. mATH were alone to have a 100% prescription of FBC, prothrombin time (PT), BUN, creatinin, CRP. Among OS-FST, FSAE were the highest prescriptors of D-dimers, blood gases (BG), LDH, Troponin (Tn), bilirubin, ferritin. FOS were the global lowest prescriptors (D-dimers, ASAT, ALAT, PCT, ferritin, Tn, BG). In all groups, IL6 was ≤25% prescripted. mUH and mHG highlighted a comparative distribution of health units with a similar prescription profile except for PT, hepatic parameters, IL6, Tn and albumin, more analyzed in mUH.

CONCLUSIONS

This multicentric study gives for the 1st time an original point of view on the prescription of biomarkers in France and french speaking areas during the 1st wave of COVID-19. The IFCC guidelines have been immediately inconstantly applied, depending on the type of health structures and units, the availability of biomarkers; all pointing out a controlled prescription.

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INCREASING DIAGNOSTIC PRODUCTIVITY OF A RAPID REAL TIME-PCR FOR SARS-COV-2 BY APPLYING THE POOLING TECHNIQUE TO NASOPHARYNGEAL SPECIMENS

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BACKGROUND-AIM

Real time PCR represents the gold standard in SARS-CoV-2 research. The use of Real Time platforms that allow to reduce the turn around time (TAT) is very important for urgent cases. We used a M10 SARS-CoV2 SD Biosensor rapid real time instrument to test pooled samples in order to reduce TAT and increase productivity at the same time.

METHODS

1530 nasopharyngeal specimens, collected in UTM (Universal Transport Medium) and E-swab (Copan), were used to create 306 pools. Each pools were composed by 5 samples.Each pool was created in two aliquots, one to be analyzed with SARS-CoV-2 Roche Cobas 6800 and the other with M10 SARS-CoV-2 SD Biosensor. Every pool was assembled with 5 different nasopharyngeal specimens picking up 120 μl of each sample (for a total of 600 μl), loaded on STANDARDTM M SARS-CoV-2 System cartridge and processed on a STANDARDTM M10 platforms (SD Biosensor Inc, Korea), following Manufacturer instructions. Each platform is equipped with 4 modules. ORF1ab and Gene E was amplified, together with an internal control (IC) included in cartridge extraction reagents, in 59 minutes runs.

RESULTS

The results obtained with M10 Sars-Cov2 SD Biosensor are described in the list:
- Negative: 277
- Positive: 14
- Presumptive positive (only E gene positive)
- Invalid: 14

The sample of positive pools were retested individually.In all 14 pools with invalid results there was a sample collected in E-swab, a medium not validated by the manufacturer. Results with the M10 SarS-Cov2 SD Biosensor instrument showed 100% agreement with those obtained with Cobas 6800 Roche. Invalid samples with M10 were negative with Cobas 6800.

CONCLUSIONS

The collected data demonstrate an excellent repeatability of the test and reliability of the employed processing method.The concordance with the Roche Cobas 6800 system was 100%. The concordance of the results also concerns the values of the Ct which are in turn overlap.Therefore, pooling from nasopharyngeal samples can also be used with Real time random access PCR platforms to decrease TAT.The pooling technique also allows to contain the consumption of analytical kits, a great advantage considering production problems and the consequent supply delay by manufacturing companies.

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USE OF A NEW RAPID REAL TIME-PCR SYSTEM FOR URGENT DIAGNOSIS OF SARS-COV-2 IN A HOSPITAL

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BACKGROUND-AIM

Molecular test is the gold standard for diagnosis of SARS-CoV-2 infection. Traditional RT-PCR Systems requiring nucleic acid extraction and amplification has been recently fully automated for 96 plate formats, but the entire process requires normally more than 3 hours to complete. For some departments or conditions like emergency room or surgery admission, time factor in reporting is crucial. We tested a new Rapid Molecular PCR platform, M10 Sars-Cov2 SD Biosensor, in a Microbiology Laboratory dedicated to emergency analysis to shorten turn around time (TAT) for suspected COVID Patients.

METHODS

Nasopharyngeal swabs were eluted in UTM medium (Copan 3 ml). A few samples were collected in 1 ml E-swab (Copan) medium. 600 µl of sample was loaded on STANDARDTM M SARS-CoV-2 System cartridge and processed on StandardTM M10 platforms (SD Biosensor Inc, Korea) equipped with 4 modules each, following Manufacturer instructions. ORF1ab and Gene E was amplified, together with an internal control (IC) included in cartridge extraction reagents, in 59 minutes runs.

RESULTS

After validating new system versus traditional RT-PCR with 100% of concordant results, from 20 September to 11 November 2021 we performed on StandardTM M10 totally 324 amplification runs on random access emergency samples arrived at Vicenza Hospital Microbiology Laboratory from Emergency and Surgical Rooms, needing a fast report for patient’s admission or treatment. Results are summarized in this list:

- Negative: 273
- Positive: 35 (gene ORF: 11,49 <Ct< 35,12)
- Presumptive positive (only E gene): 4 (gene E: 30,13 <Ct< 33,74)
- Invalid: 12

Invalid samples were all collected in E-swab medium, not validated by Manufacturer. M10 runs last 59 minutes, but early calls are highlighted on instrument display before that time when E and/or ORF1ab signals overcome the thresholds in high positive samples.

CONCLUSIONS

New StandardTM M10 System resulted a very reliable platform, suitable for low/middle routines, with optimal performance in emergency situations due to short TAT, safety of entire process in sealed cartridges easy to handle even for not specialized operators, and high detection capacity demonstrated by 100% of valid results in UTM medium, suggested by Manufacturer. E-swab medium is confirmed unsuitable for amplification on M10. More samples will be collected to complete our evaluation.

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PLATELET-TO-LYMPHOCYTE RATIO (PLR), A BIOMARKER TO PREDICT THE SEVERITY OF COVID-19 DISEASE. CORRELATION WITH C-REACTIVE PROTEIN IN COVID-19 PATIENTS.

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BACKGROUND-AIM

The rapid spread and potential lethality of the virus SARS-CoV-2, generated an urgent need to identify laboratory parameters of prognostic significance that can predict the severity and risk associated with infection. Platelets play a role in immune response and immunomodulation by activating P-Selectin Glycoprotein in the site of inflammation. Lymphocytes play a role through CD4 T-cells, B-cells producing specific viral antibodies, and CD8 cytotoxic T-cells by directly killing the virus in infected cells. The aim of this study was to determine the usefulness of PLR and C-Reactive Protein as prognostic biomarkers in COVID-19 patients.

METHODS

This study was a retrospective observational study with cross-sectional approach, involving ambulatory COVID-19 patients confirmed positive with SARS-CoV-2 PCR test, between November 2021 and January 2022. We calculated PLR for all patients and divided patients into two groups, first with $PLR<150$ and second group with $PLR>150$. CRP was measured in two groups, to evaluate the severity of disease. Spearman test was performed for analyzing data.

RESULTS

A total of 145 patients to 18-75 years old, with COVID-19 were enrolled in this study: 89 (61%) have $PLR<150$ (mean 108), and 56(39%) have $PLR>150$ (mean 319). In the second group CRP was higher than the first one (52.3 versus 26). There is a statistically significant difference between two groups in PLR and CRP values ($p<0.012$). There was a strong correlation between PLR and CRP ($r= 0.398$) in all patients in two groups. Our results show that PLR is a significant predictor parameter in COVID-19 patients.

CONCLUSIONS

The increase of PLR and CRP in COVID-19 patients was caused by an inflammatory process mediated by the immune response with positive correlation. These parameters are cost-effective and readily available and can help to identify patients at high risk of developing severe disease.

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COMPARISON OF VACCINE IMMUNOGENICITY 2ND AND 3RD DOSE IN HEALTHCARE WORKERS IN G.GENNIMATAS GENERAL HOSPITAL OF ATHENS, GREECE

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BACKGROUND-AIM

Worldwide vaccination campaigns against COVID-19 were started by the end of December, 2020. Despite the initially promising results of vaccination, many countries, including Greece, are currently experiencing their 4th pandemic wave, dominated by the omicron and delta variants of SARS-CoV-2. After studies indicated that protection against COVID-19 was waning 6 months after vaccination, a 3rd dose was suggested for all two-dose schemes.
Aim: To compare vaccine immunogenicity after 2nd and 3rd dose of healthcare workers in Greece.

METHODS

SARS-CoV-2 IgG II Quant assay on the ALINITY System was used for measuring the titers of IgG antibodies against SARS-CoV-2. In our study, 127 (86 females, 41 males) not previously infected (Covid-) and 11 (9 females, 2 males) previously infected (Covid+) participants were included and tested 1 month after the 2nd and 3rd vaccine dose. Positivity threshold was set at 50 AU/ml. For the analysis, the participants were divided based on age in five groups (20-70 years) and on sex. Data analysis for this study was generated by “Analyse-it” software.

RESULTS

All vaccinated individuals developed antibodies against SARS-CoV-2 after the 2nd dose while in all Covid- cases IgG titers were increased after the 3rd dose (mean value 11585 AU/ml compared to 23747 AU/ml, respectively). In Covid + cohort, statistically significant increased variance in IgG titers was observed between the 2nd and 3rd dose in age groups over 50. Also, a significant increased variance was found between the two doses in males versus females (120% vs 97% increase in IgG mean value, respectively). In Covid+ group, a statistically significant decreased variance was found between the two doses in males and females. Additionally, no significant increased variance was observed in age groups over 50. However, the sample studied was limited and the conclusions should not to be considered representative.

CONCLUSIONS

The 3rd dose seems to boost IgG titers in all ages in Covid- individuals and in almost all ages in Covid+ individuals. Sex may play a role in immunization after the 3rd dose. However, further work is needed for understanding and evaluating this difference. Similarly, Covid+ cohort should be expanded for exporting reliable and representative results.

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SERUM IL-6, CRP, PROCALCITONIN AND FERRITIN AS INDICATORS OF SEVERITY OF COVID-19

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BACKGROUND-AIM

The coronavirus 2019 disease (COVID-19) is characterized by a heterogeneous clinical presentation and viral mutation, a complex pathophysiology and a wide range of laboratory findings, depending on disease severity. It is still not well understood why some patients are asymptomatic or have mild influenza-like manifestation while others reveal a hyperinflammatory state ("cytokine storm") followed by an acute respiratory distress syndrome and even death. Scientists face challenges related to the management, prognosis and treatment of the infection and try to identify laboratory predictors for progression towards severe and fatal forms of this disease. We aimed to study some laboratory parameters, including serum IL-6, C-reactive protein (CRP), procalcitonin (PCT) and ferritin representing the inflammatory state and hospital mortality in COVID-19 patients.

METHODS

We analysed a total of 122 hospitalized patients with PCR proven symptomatic COVID-19 infection divided into two groups (50 with lethal outcome – 36 male and 14 female and 72 recovered patients – 56 male and 16 female). Serum IL-6, ferritin, PCT and CRP (Access 2, Olympus AU 480, Beckman Coulter) were measured. Collected data was analyzed using SPSS software, version 19.0. Continuous variables were expressed as means and standard deviations were carried out (mean ± SD). Statistical differences were considered significant at $P < 0.05$.

RESULTS

The mean age of the patients with lethal outcome was significantly higher than the recovered group (63.04 ± 2.23 yrs vs 55.22 ± 7.66 yrs, $P < 0.05$).
On admission patients with lethal outcome exhibited significantly higher serum IL-6 (425.39 ± 215.48 pg/ml vs 32.89 ± 29.28 pg/ml, $P < 0.05$), CRP (204.53 ± 110.56 mg/l vs 91.73 ± 68.80 mg/l, $P < 0.0001$), PCT (7.14 ± 2.10 ng/ml vs 0.28 ± 0.15 ng/ml, $P < 0.05$) and ferritin (1417.75 ± 1288.33 ng/ml vs 811.76 ± 768.08 ng/ml, $P < 0.05$) than those in the recovered group.

CONCLUSIONS

Our results show that elevated serum IL-6, CRP, PCT and ferritin can predict the severity of the disease. They can be useful markers of risk scarification in COVID-19 and can be considered in combination with clinical details and other laboratory tests while designing the patient treatment.

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SARS-COV-2 REINFECTIONS IN A HOSPITAL IN THE VALENCIAN COMMUNITY

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BACKGROUND-AIM

SARS-CoV-2 was detected in the city of Wuhan in December 2019, and since then nearly 400 million cases and almost 6 million deaths have been reported worldwide.

The emergence of new variants and their fast growth have increased cases of reinfection throughout the world.

According to the Health Ministry of Spain, in the "Strategy for early detection, surveillance and control of Covid-19" document, a suspected case of reinfection is one with or without compatible symptoms, who had a confirmed SARS-CoV-2 infection 90 days ago or more and a new positive result currently. The suspected case will be classified as possible, probable or confirmed depending on whether the results of the second infection have been obtained (antigen test, PCR or if the sample has been sequenced, respectively).

According to the latest report published on reinfection by Carlos III Institute, in our country, 125811 reinfections have been reported to date, of which 56.6% are possible, 42.3% are probable and only 1.1% are confirmed.

The aim of this study is to establish the number and proportion of reinfections in our Health Area and classify them as possible, probable or confirmed.

METHODS

The number of PCRs carry out in our Health Area as well as their results have been obtained from the start of the pandemic to present. They have been sorted according to date and have been grouped by patient.

Health Ministry criteria have been followed to classify suspected cases as possible, probable or confirmed.

RESULTS

PCRs carry out in the 100-week study period (March 1, 2020 to January 30, 2022) were 249695, and it has been found that 128932 correspond to different patients. Of these, 61243 (47.5%) had 2 or more determinations; and 13078 (21.35%) had more than one positive PCR, this being the population of interest for the study of reinfections.

According to the Health Ministry criteria, and with the genetic analysis data available in our laboratory, it is found that 432 (3.3%) cases are suspicious. Of these, 387 are classified as probable due to the 2 positive PCRs results with at least 90 days between them and no available data of the variant. On the other hand, 45 are classified as confirmed reinfections because they have the variant's result for the second infection, and it is not possible that said lineage circulated when the first infection occurred.

CONCLUSIONS

Obtained results show a similar percentage of reinfections in our Health Area (0.17%) with those obtained by Carlos III Institute (0.2%).

It stands out in this study that 10% are classified as confirmed reinfections. This is due to the genomic typing of the variant that has been carried out in our laboratory on many of the suspected cases of reinfection, adding to the healthcare work carried out during the pandemic.

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POST- VACCINE PRODUCTION OF IGM ANTIBODIES IN A COHORT OF COVID-19 POSITIVE AND COVID-19 NEGATIVE ITALIAN HEALTHCARE WORKERS.

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BACKGROUND-AIM

Pfizer/BioNTech Comirnaty was one of the first vaccines developed against SARS-CoV-2. This study wanted to evaluate anti-SARS-CoV-2 IgM production to Comirnaty.

METHODS

Approved by the local ethics committee, it enrolled 248 healthcare workers(HCW) from Desio Hospital, ASST Brianza, Desio, Italy. Those not previously infected with the virus before vaccination were categorized as COV NEG, while those with a positive PCR test due to prior infection were classed as COV POS. Antibody testing was done at: t0, before the first dose; t1, 21 days after the first dose; t2, 14 days after the second dose; and t3, 120 days after the first dose. Measurements were by ECLIA as reference technique (Roche c602, Roche Diagnostics GmbH, Mannheim, Germany), and by microarray-CLIA (Sunlant SLXP-002, Sunlant Bioengineering Co., Ltd., Wuxi City, China). Total anti-S RBD immunoglobulins(Ig) were assessed by ECLIA at a 0,8 U/mL cut-off for reactivity. For total anti-NCP, total anti-S1 RBD, anti-NCP IgM, and anti-S1 IgM, microarray-CLIA determined reactivity at 1 UA/mL.

RESULTS

Result interpretation focused on IgM response. The COV NEG group had a more prominent one. At t2, production equaled that of the COV POS, showing that both groups generate similar amounts of IgM after two doses of vaccine. Even if infection occurred 2-3 months before vaccination, 22% of COV POS HCWs had IgM at t0, suggesting viral restimulation. IgM decreased at t3 and were found in 3 and 10% of COV NEG and COV POS people, respectively. Production in the COV POS HCWs remained stable throughout the various time points. In 40% of the COV POS group, this was consistent at t0, t1, t2, and t3, while in 20% IgM was generated exclusively at t3, maybe due to new viral contact or to a false positive result. After two vaccine doses, #60% of the COV NEG group produced IgM at t2 and 33% at t3, as expected from secondary immune response. A subgroup of 21 HCWs in the COV NEG group showed a minor and slowly growing trend in total Ig and IgM from t0 to t2 (late response, LR). At all time points, the IgM recorded in this subgroup was lower than in the COV POS and COV NEG groups.

CONCLUSIONS

In conclusion, IgM production and kinetics in response to Comirnaty are dependent on the subject's initial condition, that is, whether or not they were previously infected.

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ANALYSIS OF INITIAL VALUES OF D-DIMER IN HOSPITALIZED PATIENTS WITH COVID-19 AND ITS CORRELATIONS WITH MORTALITY

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BACKGROUND-AIM

D-dimer is the smallest fibrin-degradation product. A significant increase of plasma D-dimer concentrations is a consequence of the activation of the coagulation and fibrinolytic system. COVID-19 infection is characterized by a hypercoagulable state which is caused by inflammatory thrombosis, initiated by the entry of SARS-CoV-2 into the human cell.The aim of this study was to analyze the initial values of D-dimer in hospitalized patients with COVID-19 and the possibility of its correlation with the hospital mortality.

METHODS

The retrospective study included 624 SARS-CoV-2 positive patients, 297 women (47.6%) and 327 men (52.4%), who were consecutive hospitalized in the COVID Hospital of the Health Center Vranje (Serbia), in January 2021. Twenty-five patients died. The D-dimer analysis was performed within 24 hours from admission at hospital, on a Sysmex CA-1500 System Analyzer, using immunoturbidimetric method (normal range <0.5 mg/L).

RESULTS

Independent samples t-Test showed that there was no statistically significant difference in D-dimer levels in relation to the gender of patients ($p > 0.05$). The mean D-dimer value on admission in patients who died (2.55 mg/L) was higher than the mean D-dimer value in patients who survived (1.72 mg/L). Our research using t-tests of independent samples did not confirm statistical significance in d-dimer values in relation to patient mortality ($p > 0.05$), although we know that elevated d-dimer values significantly correlate with poor prognosis. The intrahospital mortality was 4%. Pearson's correlation analysis confirms the existence of a statistically significant positive correlation between D-dimer values and patients age ($p < 0.01$). This study was based on the measurement of initial values of D - dimers in patients of different sex, age and possible comorbidities, which contribute to the heterogeneity of results.

CONCLUSIONS

The degree of coagulopathy correlates with a disease severity in COVID-19 patients and significantly contributes to the morbidity and mortality of these patients. Elevated value of D-dimer is the most sensitive coagulation laboratory parameter in COVID-19 infection and that make it a clinically useful biomarker of thrombotic disease. However, the low specificity of D-dimer limits its diagnostic application.

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LYMPHOPENIA AS A BAD PROGNOSTIC FACTOR IN COVID-19 PATIENTS

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BACKGROUND-AIM

Changes in complete blood count in COVID-19 patients may be useful in monitoring of disease progression and and could be a predictive factor in group of patients who could develop sever form of disease. These changes could help clinicians in a recognizing of patients which will have a fatal outcome. The severe form of COVID- 19 is characterized by severe lymphopenia and these patients are in the risk of developing of a cytokine storm, which is one of the most important reasons for patient mortality. The aim of this study was to determine the role of lymphopenia in the prognosis of COVID-19 patients..

METHODS

A total of 100 SARS-CoV-2 PCR-positive patients were analyzed. All patients were hospitalized in Covid-Hospital in Hospital Vranje (Serbia) in the period from 01.1.2021 to 01.12.2021. Complete blood count tests were performed on ADVIA ® 120 Hematology System from Siemens Healthineers. Patients were divided into 3 groups. In the first group were patients with mild clinical symptoms, in the second patients with pneumonia but without hypoxemia and in the third one the patients with pneumonia and hypoxemia who therefore required the oxygen therapy. Independent samples t-Test was performed to analyze the results, using statistical analysis software.

RESULTS

Patients with mild clinical had a complete blood count within the reference ranges. Neutrophilia with or without leukocytosis was observed in second and third group of patients, also among patients who had developed pneumonia. The main abnormality in these both groups of patients was lymphopenia. It was found a statistically significant difference in lymphocyte count between the second and third group of patients ($p=0,001$). Patients who required admission to the intensive care unit showed a statistically significant more severe lymphopenia than patients who did not require therapy in the intensive care unit ($p<0,0005$). Severe lymphopenia was associated with more severe clinical manifestations of COVID-19. Patients who required intensive care unit admission were definitely at risk to develop cytokine storm, caused by hyperesecretion of proinflammatory cytokines (IL-6, IL 2, IL 8, IL 10, TNF alpha), which was clinically manifested in the development of the life-threatening conditions.

CONCLUSIONS

Our results indicate the importance of lymphopenia in COVID-19 patients and a positive correlation of lymphopenia with a poor clinical outcome. Lymphopenia could be an early hematological indicator for severe clinical manifestations, so monitoring of this parameter could be a good predictor for poor outcome.

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T295

IMMUNE RESPONSE AND TIME COURSE AFTER DIFFERENT FORMS OF VACCINATION

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BACKGROUND-AIM

In December 2019 SARS-CoV2 was transmitted from animal to human level in China and spreaded all over the world. Vaccinations started in December 2020. In the European Union the mRNA-based vaccines Comirnaty® (Biontech/ Pfizer) and Spikevax® (Moderna), furthermore the vector based vaccines Vaxzevria® (AstraZeneca) and Janssen COVID-19 vaccine (Johnson und Johnson) were used. Since cases with sinus venous thrombosis emerged in young women after vaccination with Vaxzevria®, this vaccine was restricted to people older than 60 years. In consequence people < 60 years, in whom the first vaccination was performed with Vaxzevria®, received a mRNA-based vaccine for second vaccination (mixed vaccination). In this study we investigated the immune response after different vaccination forms and its time course up to eight months.

METHODS

In this cross-sectional study we determined neutralizing antibodies (SARS-CoV-2 TrimericS IgG assay®, DiaSorin) and T-cell immunity (SARS-CoV-2 Interferon-gamma Release Assay Quan-T-cell®, Euroimmun) against SARS-CoV-2 in 320 people working as outpatient nursing staff (18 - 75 years) antibody levels and T-cell response within three months after the second vaccination were compared using the Kruskal-Wallis-test, the association of antibody levels and T-cell response with the time between vaccination and blood withdrawal was evaluated by multivariate analysis after adjusting for covariates.

RESULTS

Antibody levels and T-cell response were significantly or in tendency lower in people vaccinated by Vaxzevria® only compared to vaccination with mRNA-based vaccines or mixed vaccinations. In contrast to the decrease of antibody levels T-cell immunity remained stable after all forms of vaccination except for mixed vaccination with Vaxzevria® and Comirnaty®.

CONCLUSIONS

The mixed vaccination with Vaxzevria and Comirnaty provoked high antibody levels and T-cell response for three months. However in time course as well antibody levels as T-cell response declined. Thus, people with this mixed vaccination might be less protected against an infection with SARS-CoV-2 without boostering compared to other vaccination forms.

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T296

HEMATOLOGIC PROFILES AND CORRELATION BETWEEN NEUTROPHIL/ LYMPHOCYTE RATIOS WITH MARKERS OF INFLAMMATION OF COVID-19.

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BACKGROUND-AIM

COVID-19 is a pandemic with significant mortality and it is important to differentiate between severe and non-severe cases. We conducted a study to evaluate hematologic profiles with inflammation markers in COVID-19 patients and to determine the correlation of neutrophil-lymphocyte ratio (NLR) with disease severity.
The AIM. In this study, we would like to evaluate hematologic profiles and their correlation with markers of inflammation in ambulatory COVID-19 patients and to determine whether NLR might provide useful information regarding COVID-19 disease severity.

METHODS

Methods. A cross-sectional study involving ambulatory COVID-19 patients confirmed with a positive SARS-CoV-2 PCR. We calculated the NLR and divided the patients in two groups. The first one includes NLR>5 and the other includes NLR<5. The inflammatory marker C-reactive protein (CRP) and D-dimer were performed in both two groups to evaluate the severity of disease. Patients' data was analyzed using Spearman correlation.

RESULTS

Results.There were 116 patients aged 18 to 77 years with COVID-19; 31 (26.7%) have NRL>5(mean 9.56), and 85 (73.3%) <5 (mean2.52). In the first group (severe group) the PCR and D-dimer are higher than the second one (mean 62 and 1.27 vs 25 and 0.83). There is a statistically significant difference in NLR, and CRP, d-dimer between the two groups ($p < 0.001$). There is also a strong correlation between NLR and CRP d-dimer ($r = 0.698$).

CONCLUSIONS

Conclusion. In COVID-19 cases, a strong correlation between NLR and CRP, D-dimer might suggest the use of NLR to differentiate between non-severe and severe cases with a high degree of inflammation. NLR is a simple tool that can be used in resource-limited settings for triage and early referral to higher levels of care.

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T297

ACTINOBACULUM SCHAALII, A COCOBACILLUS DIFFICULT TO IDENTIFY

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BACKGROUND-AIM

INTRODUCTION
The Actinobaculum genus contains four species: A. suis, A. schaalii, A. urinale and A. massiliae. They have been related to infections, especially of the urinary tract in elderly patients, and 7 cases described so far in pediatric patients. In addition, cases of osteomyelitis, endocarditis, skin and soft tissue infection, and sepsis of urinary origin have been described. These infections can be serious, especially in immunocompromised patients.

METHODS

PRESENTATION OF THE CASE
A 13-year-old girl who consulted for a 2-week history of hip pain accompanied by dysuria. Personal history of paroxysmal supraventricular tachycardia in treatment with flecainide. Comburg test is performed, which is normal. On examination, a piloidal sinus is observed in the intergluteal line with several fistulous tracts. Empirical treatment with amoxicillin/clavulanate, cloxacillin, and cefixime was started without good response. The persistence of exudate continued so a sample is collected for culture.

RESULTS

The sample was cultured on chocolate agar plates at 37ºC and Schaedler agar plates at 37ºC in an anaerobic environment. Cultures grew a microorganism that macroscopically resembled a streptococcus, catalase negative. On Gram stain, a small, slightly curved Gram-positive coccobacillus is observed, sometimes branched and without forming spores. In order to identify the microorganism, a Beckman Microscan panel was performed, which identified Actinobaculum schaalii. The antibiogram showed sensitivity to amoxicillin/clavulanate, cefoxitin, cefuroxime, clindamycin, imipinem, pencilin, and vancomycin, and resistance to metronidazole, ciprofloxacin, and colistin.
Given the torpid evolution of the infection, he was referred for pediatric surgery. He is currently awaiting surgery to clean the wound and drain it.

CONCLUSIONS

DISCUSSION
A. schaalii infections are rare and difficult to diagnose as they require anaerobiosis for growth and many commercial systems do not include Actinobaculum spp. in your database. In addition, in skin and soft tissue infections, they can be considered as contaminants. However, we should suspect them in elderly patients, with pathologies of the urinary tract, endocarditis, osteomyelitis and soft tissue infections, who do not respond to empirical antibiotic treatment.

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T298

HUMORAL IMMUNE RESPONSE TO TWO DOSES OF AZD1222/ COVISHIELD FOLLOWED BY ONE DOSE OF PFIZER VACCINE

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BACKGROUND-AIM

With the ongoing global challenge of COVID-19 since 2020, many countries embarked upon mass vaccination campaigns to contain this pandemic. In this dire moment, intensive research led to several vaccines against SARS CoV-2 infection. In the process of developing immunity through vaccination, the vaccination regimen, which commenced as two doses of vaccine to induce a complete immune response, was later increased to 3 doses of vaccines on the expert recommendation. This study assessed the humoral immune response to 2 doses of the Covisheild vaccine with a booster dose of Pfizer vaccine for the COVID-19 infection employing virus-neutralizing antibody development against the viral spike protein the host using a cohort of health care workers.

METHODS

We conducted a descriptive cross-sectional study using 200 participants who received two doses of AZD1222 with a booster dose of the Pfizer vaccine to assess the immune response to the SARS CoV-2 vaccines. The antibody response was assessed at 4-6 weeks after each vaccine dose and just before the second and third doses using a two-site sandwiched immunoassays technique developed to detect IgG by Ortho Clinical diagnostics. Data were analyzed using SPSS-22.

RESULTS

Out of 200 participants, only 174 completed all the followed-up visits. More than half the study group (60%) was females. The mean age of the participants was 44.2 years (range 23 - 68, SD = 9.73). None of them had PCR-positive COVID-19 infection at the time of inclusion in the study, and 23.1% stated to have low risk and 56.9% intermediate risk, while the rest claimed to have a high risk of COVID-19 exposure. Fever and myalgia were the most typical symptoms (60%) following the first AZD1222 dose, while none reported having any significant symptoms following the booster doses. After the first AZD1222 dose, IgG was measured in 200 participants with a mean Ab index of 5.8 (SD = 4.3), with an 82.5% overall seroconversion and highest seroconversion observed (86.9%) among the middle-aged. Before the second AZD1222 dose was given after 12-weeks from the first dose, 182 participants were assessed for IgG levels, and the mean Ab response was 4.0 (SD = 4.8) with the persistence of seroconversion only in 64.5% ($p = 0.001$). After the second AZD1222 dose, IgG was measured in 176 participants with a mean Ab index of 9.3 (SD = 4.6) with 96.4% overall seroconversion and the highest seroconversion (98.9%) observed among the middle-aged. The increase in seroconversion after the second dose was statistically significant ($p = 0.000$). After 24-26 weeks from the second dose of AZD1222, the Ab response was assessed in 190 participants, and it was observed to reduce to a mean Ab index of 4.2 (SD = 2.36). However, the participants who had PCR positive COVID infection or significant exposure to infection had a higher Ab index of 14.87 (SD = 5.13), and they reported having a mild or asymptomatic infection. Measurement of Ab response 4 – 6 weeks after the booster dose of Pfizer revealed a mean Ab index of 20.86 (SD = 2.08). Interestingly, when paired t-test was applied to the Ab indexed of participants who had PCR confirmed or possible COVID infection, there was no significant difference between the before and after values from the Pfizer booster.

CONCLUSIONS

All participants demonstrated seroconversion irrespective of age and sex after three doses of vaccines, possibly reaching the full immune response. There is a possibility of two doses of AZD1222 providing adequate immunity to suppress the COVID infection to a subclinical state, but confirmation of this requires a more extensive study. Also, two doses of AZD1222 followed by COVID infection may reach full immunity in the individual, again confirmation of which requires further study. Since the Ab response was observed to reduce after a while from the vaccine, it is prudent to assess the long-term persistence of the Ab response for the COVID vaccines.

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T299

COVID-19 AND LABORATORY TESTING IN PATIENT DIAGNOSIS AND TREATMENT. ALBANIAN FINDINGS.

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BACKGROUND-AIM

Clinical laboratory testing is playing a critical role in efforts to control the COVID-19 pandemic, whether by diagnosing patients with the novel coronavirus or screening the general public to determine the full extent of the outbreak.As in the rest of the world in Albania in the first stage of pandemic the laboratory testing consist in PCR testing, which is the only test approved for diagnosis of COVID-19.At an early stage biochemical monitoring of COVID-19 patients through in vitro diagnostic testing is critical for assessing disease severity and progress as well as monitoring therapeutic intervention.

The aim of our study is to identify the biochemical and hematological test changes of the patients implicated in unfavorable COVID-19 progression, potentially providing important prognostic information.

METHODS

This study represent data collection from laboratory testing of 150 adult patient with mean age 54.60± 12.57 years old who have been diagnosed with novel Covid 19 virus by PCR and radiology positive test.Laboratory test panel included ; ferritine. CRP. D-Dimmer and difference in hematological parameters before and during treatment The blood sample were analyzed in patient disagreement according gender at different stages of infection. The data were statistically treated by using Descriptive statistic. MINITAB 17 software package was used for data analysis. Dynamic levels of hematological indexes were compared for two groups and for different stage of infection

RESULTS

WBC (white blood cells), neutrophil to lymphocyte ratio (NLR), D-dimer, and CRP levels of the second week of infection were significantly higher ($P < 0.05$) than the first week tes. Ferritine values were found to be higher in the first week of infection mean velue 280 ng/ml ($p<0.05$) , therefore lymphocyte number was lower representing significant NLR ratio higher in the first week of infection ($p<0.05$).

CONCLUSIONS

Our findings are in concordance with other studies. The dynamic change of hematological index , NLR and D-Dimer level can distinguish severe COVID-19 cases from the mild/moderate and severe stage, potentially providing important prognostic information.

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T300

RT-PCR DETECTION OF SARS-COV-2 USING SALIVA COLLECTION DEVICES IN PAEDIATRIC POPULATION AND PEOPLE WITH DISABILITIES

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BACKGROUND-AIM

The saliva sample collection method could replace the nasopharyngeal swab, especially in disabled people and children, where nasopharyngeal sampling is particularly difficult and not well tolerated. The aim of the study is to evaluate the use of RT-PCR molecular testing of saliva as an alternative to the nasopharyngeal swab (gold standard) for the detection of SARS-CoV-2 in the study population.

METHODS

During the period from 01/09/2021 to 31/01/2022, 3210 saliva samples were collected using the Lollisponge device (COPAN, Italy BS). These samples, once received at the Microbiology Unit of the San Bortolo Hospital in Vicenza, were processed according to the supplier's instructions to collect the saliva absorbed by the device. Suitable samples, containing sufficient material for the analysis, were processed using the RT-PCR molecular assay Allplex 2019-nCoV Assay Seegene Arrow. This assay detects 4 SARS-CoV-2 gene targets (E, S, RdRp, N). Samples that did not have sufficient saliva volume were reported as unsuitable and to be repeated.

RESULTS

Of the 3210 samples received in the laboratory, 177 (5%) were unsuitable. 3033 samples (95%) were suitable and subjected to molecular testing. The results obtained are shown in this list:

- N° of positive: 18
- N° of Negative: 3015

CONCLUSIONS

This type of sample collection facilitates the operator in the management of the subjects taken into consideration in this study, and the patients themselves were more willing to accept this collection method.

From the pre-analytical point of view, this type of device requires more time and manual skills than the nasopharyngeal swab. It should also be noted that 5% of the samples considered yealded insufficient material to perform the exam and required a second sample. This was not the case with the nasopharyngeal swabs. However, saliva remains a suitable collection method for pre-school children and those with certain types of severe disability.

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T301

INFECTION CONTROL IN GAZA DURING THE CORONA PANDEMIC "COVID19" 2019-2022

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Background-Aim: The importance of infection control and limiting the spread is important in maintaining the health of societies, and medical laboratories are one of the most important elements in the follow-up procedures for controlling infection in countries and revealing the source and type of infection. In Gaza, from the beginning of the pandemic on 2019 to 2022 until this moment, we have followed up the epidemiological situation in one of the non-governmental and governmental health institutions centers, and we touched on several issues in the challenges that affected me positively and negatively at the level of infection control. The efforts made by the health staff and the follow-up from the Laboratories Department continued to conduct swabs, take samples according to contacts, and follow up on the cases throughout the Gaza Strip. The matter was dependent on the home quarantine of the affected and their follow-up. AIM: Identifying the challenges that Gaza faces in light of the pandemic and what are the advantages that have positively affected safety and reduced the number of affected.

Methods: This retrospective study was designed to study cases and controls using data held in the governmental and non-governmental electronic medical records. Where this study included numbers of people infected with the Corona virus during a period of three months and it was at the height of the pandemic. The study dealt with methods of medical treatment and health interventions. Identify the tools used in infection control in health institutions; Identify the challenges faced by the health staff. Taking into account the elements of increasing numbers and their differences in the peak and spread of the virus random samples previously registered in 100 sample the electronic archive were taken from health staff in governmental and non-governmental health institutions and ways of using infection control elements were compared, as it became clear that there is a lack of awareness and preventive education in those health institutions

Results and Conclusions: Firstly, the study was divided into two models in governmental and non-governmental health institutions to study the challenges. Through this model, the difference in the use of the infection control system and the number of infections within those health institutions was identified, whether in laboratory devices and their development or in laboratory solutions and health staff. Secondly, assisting university educational institutions with information about the activities carried out on laboratory research on the virus, which plays an important role in exploring risks and reducing the severity of the virus inside Gaza. Thirdly, identifying the tools used and which have proven their effectiveness in confronting the spread of the virus, which lies in following the elements of infection control through awareness and health education about the necessary measures to reduce the infection rate. Fourth, the use of the surveillance system in the management of infection control by tracking epidemics around the world.