

W001

Microbiology - Infectious diseases including COVID 19

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

G. Ameya³, D. Alelign², M. Siraj², F. Fenta¹

¹Arba Minch College of Health Science

²Arba Minch University

³Kotebe Metropolitan University

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

C. Lopes¹, T. Reis¹, R. Ribeiro¹, J. Oliveira-Silva², R. Batista-Silva², G. Marques¹, L. Araujo¹, I. Antunes², F. Rodrigues¹

¹Department of Clinical Pathology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

²Department of Occupational Health, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

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=21x10³, IQR= 13.5-33.1 x10³ AU/mL), 97.6% presented a robust humoral response (>4160 AU/mL), whereas at three months (median=3.2x10³, IQR= 2.0-5.1 x10³ AU/mL) it decreased by 6.5-fold to 35.1% and then by 3.0-fold to 3.3% at 6 months (median=1.0x10³, IQR= 0.65-1.7 x10³ AU/mL). The Friedman's test revealed that there was a statistically significant difference in SARS-CoV-2 IgG throughout the follow up [$\chi^2(3)=8652.4, P<0.0001$], 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, R²=0.049), T2 (P<0.0001, R²=0.573) and T3 (P<0.0001, R²=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.

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

I.M. Portell Rigo², C. García Pérez⁴, S.J. Guardia Alés², M.P. Luzón García³, C. Avivar Oyonarte¹, M.M. Palanca Giménez³

¹Biotechnology Department, Poniente Hospital Public Health Agency. El Ejido. Almería, Spain.

²Clinical Analysis Unit; Biotechnology Department, Poniente Hospital Public Health Agency. El Ejido. Almería, Spain.

³Microbiology Unit. Biotechnology Department, Poniente Hospital Public Health Agency. El Ejido. Almería, Spain.

⁴Microbiology Unit. Microbiology Department. Virgen de la Victoria Hospital. Málaga. Spain.

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 false 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, false negatives for immunosuppression) or positive up to 18 mm (possible false 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.

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

I.M. Portell Rigo², S. Martinez Martin², L. Martinez Carreras², M.I. Cabeza Barrera³, C. Avivar Oyonarte¹, M.M. Palanca Giménez³

¹Biotechnology Department, Poniente Hospital Public Health Agency. El Ejido. Almeria, Spain.

²Clinical Analysis Unit; Biotechnology Department, Poniente Hospital Public Health Agency. El Ejido. Almeria, Spain.

³Microbiology Unit. Biotechnology Department, Poniente Hospital Public Health Agency. El Ejido. Almeria, Spain.

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

S. Bérnago Vázquez¹, M. Mico García¹, C. González Fernández¹, L. González García¹, M. Sala Grau¹, M. Prado Ramos¹, L. Mares Rueda¹, J. Trapé¹

¹Laboratory Medicine, Althaia- Xarxa Assistencial Universitaria de Manresa, Manresa

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 (TaqPath™ 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.

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GOOD CONSENSUS IN SARS-COV-2 EQA SCHEMES

H. Berghäll², M. Järvenpää², M. Waris¹, V. Mäntynen³, J. Pelanti²

¹Dept of Virology, University of Turku, Turku, Finland

²Labquality, Helsinki, Finland

³Nordlab, Oulu, Finland

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 B.1.1.7 and B.1.351 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 B.1.351 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.

