Niger. J. Physiol. Sci. 39 (December 2024): 177 – 183
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Review article

Review of Immune-Metabolic Studies and Re-purposed Treatments of Nigerian COVID-19 Patients: A Pointer to Mild, Gender- and Age-Based Status of Admitted Patients

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Summary: When Severe Acute Respiratory Human Coronavirus 2 (SARS-hCOV 2) infection began in December 2019, detailed knowledge about the virus was lacking. This included non-availability of anti-viral treatment or vaccine, no knowledge of virus-human interaction, and lack of prognostic factors for stages of illness among others. A publication in Nigerian Journal of Physiological Sciences (2020). 35: 20-25 titled "Immune Responses During Human Coronavirus Infection: Suggestions For Future Studies" adduced investigations into immune parameters of COVID-19 patients so as to throw more light on the immunopathogenesis of SAR-CoV-2 infection, in order to create avenue for the development of vaccines or herd immunity. This present publication is a review of studies carried out on COVID-19 patients in one Infectious Diseases Center (I.D.C), Ibadan, Nigeria as a response to the gaps in knowledge raised in above mentioned publication. Cummulatively, immune-metabolic studies from this IDC revealed mild, age- and sex-dependent status of COVID-19 in patients admitted into this center. Thus, explaining the basis for the effectiveness of adopted re-purposed drugs (chloroquine or hydroxychloroquine, zinc, vitamins C and D and or antibiotics), physiotherapy and nutritional support used for the management of admitted COVID-19 patients. Also, this paper vindicated that inflammation was heightened during SARS-CoV 2 infection; therefore therapeutic interventions to control the inflammatory processes, oxidative stress, antibodies against structural and non-structural proteins or blocks receptor sites were proposed. In addition, development of herd immunity and efficacy of COVID-19 vaccines (Astrazeneca and Moderna) were elucidated in general population. However, study to determine host genetic factors in hCoV infection was lacking. This review concluded that interdisciplinary collaborative approach will be useful in the management of future emerging or re-emerging infection.

Keywords*: Antibodies, Herd-immunity, Inflammation, Phagocytes, Re-purposed drugs, Vaccines.*

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Manuscript received- August 2024; Accepted: October 2024

DOI: https://doi.org/10.54548/njps.v39i2.X

INTRODUCTION

The causative agent (SARS-Cov 2) of coronavirus disease 2019 (COVID-19) was discovered in January 2020 to be betacoronovirus in the same subgenus as Severe Acute Respiratory Syndrome Coronavirus 2 (WHO, 2020). For its invasion, it was established that SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) as entry receptors on host cells (Hoffmann *et al*., 2020). The host innate immune system detects dsRNA (a Pathogen-Associated Molecular Patterns, PAMPs) of CoV using Pattern Recognition Receptors (Toll-like receptors, mannose receptor, scavenger receptor; mannose-binding lectin and C-reactive protein), follow by NF-kB activation which promotes the synthesis of type I IFNs and other proinflammatory cytokines (Schneider *et al*., 2014). IL-1, IL-6, IL-8, TNF-β and MCP-1 produced by infected cells in response to CoV infection attract, retain and activate lymphocytes and leukocytes to the site of infection (Edem and Arinola 2015). This stage is associated with high fever, hypoxemia and progression to pneumonia-like symptoms despite progressive decline in virus titers (Peiris *et al*., 2003). Two immune lung cells (alveolar macrophages and nerve/airway associated-macrophages), four types of blood immune cells (antibody-secreting cells, follicular helper T cells, activated CD4+ T cells and CD8+ T cells) and immunoglobulin M and IgG antibodies activate immunity to CoV (Fehr and Perlman, 2015). Macrophages present viral peptides to B- and T- lymphocytes leading to adaptive immunity. For easy understanding, this review is separated into the following sections, viz: humoral cellular and humoral adaptive immune responses-, metabolic responses- and repurposed treatment responses- of COVID-19 patients one I.D.C, Nigeria.

Humoral innate immune responses of COVID-19 patients in Ibadan: The first study (Arinola *et al*., 2021c)

on hospitalised COVID-19 patients in an IDC, Ibadan, Nigeria provided demographic features of these patients. 58% of these patients were between age 18 and 35 years, 48.1% were employees of private establishments, and 64.1% were males. High proportion (84.3%) of the patients spent less than 14 days on admission with no mortality as at June 2020. The findings of this study emphasised the importance of gender and age on therapeutic strategies.

Transmission of SARS-CoV 2 was through aerosols; breathe through nostrils though ocular infection was also assumed (Arinola, 2020). To prevent nasal SARS-CoV 2 entry, the wearing of nose-mask was instituted during COVID-19 pandemic. Following successful SARS-CoV 2 entry into the nostrils of host, innate factors such as nasal cilia filteration, mucus trapping action and muco-ciliary movements prevented further spread. The ingestion òf SARS-CoV 2 into the gut was likelihood, which caused the detection of CoV 2 particles in the faeces. Moreoever, SARS-CoV 2 might also be ejected through sneezing causing further viral spread. The failure of these initial host innate functions to block the entrance of the virus resulted to attachment and invasion of host cell by CoV 2, followed by viral replication and production of genomic dsRNA in the cytoplasm. Recognition and sensitisation of Pattern Recognition Receptors (especially TLR-3) by dsRNA and NF-kB activation promoted the synthesis type I IFNs and other proinflammatory cytokines (Jiang *et al*., 2020). Also, on the adaptive aspect of immune response, the processed CoV 2 in the macrophages and its peptide presented to T cells leading to T cell activation and production of cytokines, complement factors, acute phase proteins and respiratory burst factors (McKechnie and Blish, 2020).

Respiratory burst function, which is the release of reactive oxygen species from leucocytes, is one of the innate immune mechanisms to prevent the establishment of intracellular pathogens in the host. A study (Arinola *et al*., 2021f) in Nigerian COVID-19 patients showed that mean plasma levels of nitric oxide (NO), myeloperoxidase (MPO) and hydrogen peroxide (H2O2) were significantly decreased while SOD was significantly increased in COVID-19 patients at admission compared with control. In male COVID-19 patients above 40yrs of age, the level of MPO was significantly increased compared with MPO level in female COVID-19 patients below 40yrs of age. The study concluded that respiratory burst function as an innate immune response was involved in the immuno-pathogenecity of SARS-CoV 2; and that MPO might explain differential prevalence of COVID-19 among gender and age groups.

Another study (Akinwumi *et al*., 2021) determined the prognostic value of some cellular inflammatory cells and their indices in relation to duration of hospital admission, gender, and age of Nigerian COVID-19 patients. This longitudinal and case–control study determined blood cell components (total white blood cells (TWBC), neutrophil, lymphocyte, monocyte, and platelet) and inflammatory indices (neutrophil lymphocyte ratio [NLR], lymphocyte monocyte ratio [LMR], platelet lymphocyte ratio [PLR], derived NLR [DNLR], and systemic immune inflammatory index [SII]. The mean platelet count and PLR were significantly lower while TWBC counts were significantly increased in COVID-19 patients compared with control. The mean neutrophil count, PLR, and SII were significantly lower while mean lymphocyte count was significantly higher in COVID-19 patients aged <40 years compared with patients aged ≥40 years. This study concluded that inflammatory response is a phenomenon in COVID-19 patients especially in patients ≥40 years of age and that this inflammation persist till discharge, though gender had no influence on cellular inflammatory indices of COVID-19 patients.

Acute phase proteins are sensitive systemic markers of inflammation, nutritional status and tissue damage while COVID-19 is an inflammatory disease with tissue damaging effects. It was thus assumed that high sensitive C-reactive protein (a marker of inflammation), albumin and prealbumin (markers of nutritional status) could be prognostic of COVID-19 disease among Nigerian patients (Arinola *et al*., 2022). High sensitive C-reactive protein (hsC-RP) level was significantly higher in newly admitted COVID-19 patients compared with discharged COVID-19 patients or COVID-19 free control ($p<0.05$). The mean values of plasma hs C-RP, albumin and prealbumin in most COVID-19 patients (89%, 100% and 91% respectively) were within normal reference ranges. hsC-RP were significantly increased in newly admitted COVID-19 patients who are females, above 40years of age and in COVID-19 patients with hospital admission above 10days ($p<0.05$ in each case). The study also found higher plasma hsCRP level in female than male COVID-19 patients or COVID-19 patients above 40years compared with those below 40years of age. Therefore, plasma hs C-RP level was concluded to be a useful prognostic marker of COVID-19 and that low grade inflammation due to host C-RP and viral dsRNA existed in COVID-19 patients. The high hsCRP in COVID-19 patients was implicated in the cardiovascular episode of poorly managed COVID-19 patients.

It was documented that co-infection of malaria and COVID-19 existed and that malaria reduced the severity of COVID-19 (Anyanwu 2021), but the mechanisms of malaria parasiteamia on SARS-CoV 2 susceptibility and severity remained unclear. A study (Arinola *et al*., 2021b) that determined the cellular and humoral factors of oxidative burst (aspect of innate immunity) in COVID-19 patients in IDC, Ibadan, Nigeria reported that the level of plasma nitric oxide was significantly higher in co-infection of COVID-19 and malaria (CoV+P) compared with COVID-19 without malaria (CoV-P) or control. Myeloperoxidase enzyme activity was significantly higher in CoV+P compared with CoV-P. Blood cell counts, hydrogen peroxide, superoxide dismutase and catalase were similar in all groups. In summary, raised levels of NO and MPO were produced by phagocytic cells in CoV+P patients. Taken together, the findings indicated that raised NO in CoV+P might have regulated the adverse cardiovascular episodes of MPO in CoV+P patients. Another longitudinal study (Akinwumi *et al*., 2023) using biochemical nutritional markers (albumin, prealbumin and total cholesterol) and nutritional indices [Controlling Nutritional Status (CONUT) score and Prognostic Nutritional Index (PNI)] classified Nigerian COVID-19 patients at IDC, Ibadan as mild. Also, in this study, it was found that most COVID-19 patients (89.5%) were well-nourished based on Prognostic Nutritional Index. Reports indicated that immune complexes (ICs) might play active roles in COVID-19 immunopathology (Ankerhold *et al*., 2022; Kolb *et al*., 2023), but the changes in circulating

IC (CIC) level during the course of COVID-19 was not determined. Plasma level of CIC in Nigerian adults with SARS-CoV-2 infection was followed up from diagnosis till discharged from IDC, Ibadan (Arinola *et al*., 2021c). The mean CIC level was higher in COVID-19 patients at diagnosis compared with the control. However, there was no significant difference in the mean plasma levels of CIC at discharge compared with the level at diagnosis in COVID-19 patients. It was concluded from this study that the plasma level of CIC might be involved in the immunopathology of COVID-19; COVID-19 was thus concluded to be an immune complex disease.

The presence of cytokine storm syndrome as a characteristic of COVID-19 patients was exploited in the formulation of management strategies of these patients. A study investigated the effect of repurposed management on serum Th1 pro-inflammation cytokine (IFN-γ) and Th2 anti-inflammation cytokine (IL-4) in Nigerian COVID-19 patients (Arinola *et al*., 2023a). The mean values of IFN-γ and IL-4 were significantly higher in COVID-19 patients at admission compared with discharged COVID-19 patients whereas IFN-γ:IL-4 ratio was significantly higher in discharged COVID-19 patients compared with admitted COVID-19 patients. Significantly higher proportion of COVID-19 patients at discharge had IFN-γ within the normal reference ranges compared with COVID-19 patients at admission whereas the proportions of COVID-19 patients at discharge and COVID-19 patients at admission having IL-4 within the normal reference ranges were the same. The study concluded that the repurposed treatment suppressed pro-inflammation cytokine (IFN-γ) in most discharged COVID-19 patients.

Humoral adaptive immune responses of COVID-19 patients in Ibadan: Dearth of specific therapy for COVID-19 despite its pandemicity called for a clear understanding of antibody based protective immunity following natural infection with SARS-CoV 2. This was expected to give knowledge on effectiveness of vaccines or convalescent plasma against SARS-CoV-2 and possibility of development of herd immunity. To provide these informations, the following studies were carried out on COVID-19 patients from IDC, Ibadan, Nigeria. Arinola (2021) reported that CovIgG was positive in none (0)% and 20% of COVID-19 patients at admission and at discharge respectively while CovIgM was positive in 54% and 69% of COVID-19 patients at admission and at discharged respectively. The level of CovIgG was significantly higher in COVID-19 patients at discharge compared with the level at admission while the level of CovIgM was insignificantly reduced in COVID-19 patients at discharge compared with the level at admission. The data strongly suggested that plasma from recovered patients might give opportunity for the development of effective antibody-based therapies to treat COVID-19 patients. Using cassette system lateral flow immunoassay (Arinola *et al*., 2021d), anti-SARS 2 Cov-IgG and -IgM antibodies were not detected in all COVID-19 patients either at diagnosis or discharge, 7.1% COVID-19 patients were detected for the combination of anti-CovIgG and anti-CovIgM antibodies at discharge. Also, anti-SARS 2 -IgG and -IgM antibodies were more prevalent in COVID-19 patients at discharge than those newly diagnosed, thus not all plasma from all COVID-19 patients should be consider for plasma therapy. This result had implications for vaccine development, vaccine efficacy and serological surveys.

The importance of IgA in mucosal immune system might open a novel approach in therapeutic settings against COVID-19. A study (Fasasi *et al*., 2023) among Nigerian COVID-19 patients showed that the mean value of serum IgA was significantly increased in COVID-19 patients at admission or COVID-19 patients at discharge compared with the level in corresponding control. The mean value of serum IgA was similar in COVID-19 patients at admission compared with COVID-19 patients at discharge, thus concluded that serum IgA could be a useful biomarker to differentiate patients with COVID-9 from un-infected controls. The major importance was that IgA was protective in COVID-19 patients and might be one of the neutralizing antibodies stimulated by COVID-19 vaccines.

Neutralizing antibodies (NAbs) play critical roles in blocking viral infections, viral clearance during acute infection or controlling disease progression during chronic phase. CovIgG which was found to have neutralizing activity (Wu *et al*., 2020) and NAbs in the plasma of convalescent COVID-19 patients were successfully used in the passive antibody therapy to treat severe cases of SARS-CoV-2 infection (Duan *et al*., 2020). But, the rapidly declining CoV specific antibodies from 6 months created challenge on the duration of COVID-19 antibody based vaccine and convalescence plasma therapy (Wu *et al*., 2020).

Apart from the pandemicity of COVID-19, availability and accessibility to molecular testing was also limited. Thus, to provide epidemiologic data and to contain outbreaks, a number of diagnostic testing methods and kits were developed for COVID-19 (Yang *et al*., 2020). The widely use diagnosis test for COVID-19 was the nucleic acid amplification tests (NAATs) which detected the presence of the viral genome and current infection (Jacofsky *et al*., 2020). Another testing method was the serological test which detected the anti-SARS-CoV 2 antibodies (mainly IgG, IgM and IgA) produced by the immune system, but this was not suitable for very early diagnosis as there is a time lag (6 – 7 days) for antibody production to viral antigens (Jacofsky *et al*., 2020). There were a number of point-of-care tests for SARS-CoV 2 diagnosis however; the reliability of these test kits was poorly documented. The sensitivity and specificity of two serological test kits for COVID-19 diagnosis in Nigeria using a commercially available Rapid Diagnostic Test (RDT) and enzyme linked immunosorbent assay (ELISA) was carried out (Arinola *et al*., 2021d). The sensitivity and specificity of the test kits were determined using ROC while the Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), Diagnostic Odds Ratio (DOR) and accuracy were calculated as appropriate. Twenty eight (59.6%) of the study participants had positive PCR result. ELISA and RDT identified 20 (42.6%) and 13 (27.7%) participants respectively as having COVID-19. ELISA had a better sensitivity performance, NPV, PLR, DOR and accuracy than the RDT while the RDT had a better specificity performance than ELISA. The proportion of participants with positive SARS-CoV-2 IgM antibody identified using ELISA was significantly higher compared with RDT. In

contrast, the proportion of participants with positive SARS-CoV-2 IgG antibody identified using RDT was significantly higher compared with ELISA. The importance of this study was that ELISA had a better sensitivity for SARS-CoV-2 Spike-protein specific antibodies than the RDT. However, combination of RDT and ELISA for SARS-COV-2 antibodies might be useful for population COVID-19 screening.

Metabolic responses of COVID-19 patients in Ibadan: ACE is an integral receptor on almost all organs (Du *et al*., 2020) and it is needed for SARS-CoV 2 host invasion, therefore its presence served as the basis for multi-organ dysfunction of COVID-19 patients. Apart from multi-organ presence of ACE 2, the presence of viral RNA in the blood coupled with viral replication in the intestine made it plausible that SARS-CoV 2 could enter the portal circulation to reach the liver. The attempt by hepatic Kupffer cells to clear the virus initiated an inflammatory response. Also inflammatory mediators from the intestine enter the portal system and sinusoids (Jose and Manuel, 2020) which might have been responsible for the observed liver- and renal- dysfunctions in Nigerian COVID-19 patients (Arinola *et al*., 2021e; 2023, Alonge and Arinola, 2022). Therefore, full understanding of metabolic pathways of SARS-CoV 2 could facilitate therapeutic insights for COVID-19 management. High proportions of COVID-19 patients had values of the liver (59%-96%) and renal (43%-97%) function test parameters within the normal reference intervals. Similarly, high proportions of COVID-19 patients had values of lipid profile (71%-86%) within the normal reference intervals. The infrequent alteration in lipid metabolism as well as liver and renal functions suggests mild COVID-19. It could be concluded from this study that renal and liver dysfunctions as well as lipid dysmetabolism are uncommon in patients with mild COVID-19.

Angiotensin Converting Enzyme-2 is known to affect renal functions, vasoconstriction and fluid homeostasis (Du *et al*., 2020). The receptor of this ACE also attaches to SARS-CoV 2, thus the impact of SARS-CoV-2 infection on renal function parameters is worth investigating. Plasma obtained from whole blood samples Nigerian COVID-19 patients were analysed for albumin, urea, creatinine, Na, K, Cl and HCO3. It was observed that 57.1%, 37.8%, 32.7%, 28.1%, 18.7%, 17.8% and 3.4% of newly diagnosed COVID-19 patients had values of Cl, creatinine, albumin, Na, K, HCO3 and urea respectively outside the reference ranges. While 43.3%, 4.7%, 2.5%, 2.5%, 2.0%, 1.7% and 1.0% of COVID-19 patients had values of Cl, creatinine, Na, K, albumin, Urea and HCO3 respectively above the reference ranges. Of all admitted patients, 33.1%, 30.7%, 25.6%, 16.8%, 16.3%, 13.8% and 1.7% had creatinine, albumin, Na, HCO3, K, Cl and urea values respectively below reference ranges. The changes in some renal function parameters of newly diagnosed COVID-19 patients portend that renal failure is possible in poorly managed COVID-19 patients and this has immunopathologic implications.

LDH is an enzyme involved in energy production by conversion of lactate to pyruvate and it is present in almost all body cells with highest levels in heart, liver, lungs, muscles, kidneys and blood cells (Lu *et al*., 2018). LDH is also an indicator of acute or chronic tissue damage, and inflammation (Sepulveda, 2013). There is close relation between LDH organ distribution, hypoxia, immune dysregulation with COVID-19 pathogenesis, it is thus reasonable to determine the level of serum LDH as prognostic factor of COVID-19. Changes in LDH activity from admission through discharge were determined in Nigerian SARS-CoV-2 patients with a view to determining its prognostic properties (Onifade *et al*., 2023). The mean serum LDH activity was significantly higher in COVID-19 patients at admission and at discharge compared with the controls. However, the mean LDH activity was slightly lower in COVID-19 patients at discharge compared with the activity at admission. It was concluded from the study that LDH activity is elevated in COVID-19 patients and changes in its activity during the course of COVID-19 management could provide clinical information on patient's response to therapy. The values of activities of LDH in our COVID-19 patients within normal reference range indicated that majority of COVID-19 patients in our study had mild COVID-19. This observation corroborates previous reports which classified COVID-19 in patients from the same IDC as mild (Alonge *et al*., 2022, Arinola *et al*., 2022). This study suggested the possible use of LDH inhibitors as metabolic checkpoint in the management of COVID-19 patients.

Clinical trial of IDO inhibitor or uses of micro-nutrient supplements during management of diseases is commonly done without having adequate basis for the practise. Tryptophan (Trp) is an essential amino acid needed for T-lymphocyte function, and indoleamine-2,3-dioxygenase (IDO) is a potent immunoregulatory molecule that catalyses the rate-limiting step of Trp degradation in the kynurenine (Kyn) pathway (Ünüvar *et al*., 2019). Mean IDO activity in COVID-19 patients was significantly higher compared with corresponding control. The data suggested that IDO inhibitor might be necessary in COVID-19.

Repurposed treatment responses of COVID-19 patients in Ibadan: SARS-CoV-2 shares almost 80% of the genome with SARS-CoV (Lu *et al*., 2020). Therefore, the immune-metabolic attributes of earlier covid viruses were assumed similar to those of SARS-CoV-2 making it easy to formulate a repurposed treatment for COVID-19, which included chloroquine or hydroxychloroquine, zinc, vitamins C and D and or antibiotic(s) designed by the Case Management Team of the Oyo State COVID-19 Task Force (Alonge *et al*., 2022). Physiotherapy and nutritional support for these patients were also included. See Table below. This repurposed drug treatment also arose as a result of no known specific drugs or vaccine developed to manage or prevent COVID-19 (Wu *et al*., 2020).

National Institute of Health (NIH) and Food and Drug Administration (FDA) recommended, remdesivir and convalescent blood products as promising potentials for COVID-19 treatment apart from the uses of chloroquine, hydroxychloroquine, favipiravir, ivermectin, and colchicines (Hossen *et al*., 2020). Usage of albendazole antihelminthic drug (Onifade and Arinola, 2020), plant extracts with antiviral potentials (Attah *et al*., 2021) and protease inhibitors (Olubiyi *et al* 2020) were also proposed.

Table 1:

Medication used by COVID-19 patients at an IDC, Ibadan, Nigeria.

Medications	Groups
	COVID-19 Patients
Vitamin D (1,000 iu)	1,000 iu twice daily for 3 weeks
Vitamin C (1,000 mg)	1,000 mg twice daily for 3 weeks
Zn (20mg)	100 mg daily for 3 weeks
Azithromycin	500 mg daily for 3 days
Hydroxychloroquine	400 mg on day 1 and 200 mg daily for 3 more days
Chloroquine (As an alternative to Hydroxychloroquine)	500 mg on day 1 and 250 mg daily for 3 more days

Santos *et al* (2020) reported that therapeutics for COVID-19 includes camostatmesylate, remdesivir, favipiravir, tocilizumab, baricitinib, convalescent plasma, and humanized monoclonal antibodies. Other treatment options for COVID-19 included therapeutics targeting the spike protein attachment to human cells (Yang *et al*., 2020). The repurposing of chloroquine and hydroxychloroquine for treatment of SARS-CoV-2 infection was premised on the anti-inflammatory properties of these antimalarial which may be beneficial to the patients if there is underlying malaria parasitaemia and also to reduce pre-empted occurrence of inflammatory cytokine storm which is associated with SARS-Cov-2 infection (Yang *et al*., 2020). Meo *et al* (2020) proposed that chloroquine and hydroxychloroquine are beneficial in the management of COVID-19 through inhibition of the receptor binding by the virus, inhibition of membrane fusion by the virus and immune modulation leading to a decrease in cytokine release. The use of vitamin D in the management of COVID-19 patients hinged on the autocrine function (Arinola and Edem, 2020; Rahamon *et al*., 2021); vitamin C has immunomodulatory and antioxidant capacity, zinc regulates the number and function of immune cells (macrophages, neutrophils, dendritic cells, mast cells, T cells and B cells) (Haase and Rink, 2007). Immunomodulatory and anti-inflammatory functions of vitamin D, vitamin C and Zn made them appropriately supportive in the management of COVID-19 patients. Also, in this environment (Nigeria), a randomised trial (ClinicalTrials.gov ID: NCT04459286) was conducted by Fowotade *et al*, (2022) where mild to moderate COVID-19 patients received standard of care (SoC) or SoC plus a 14-day course of nitazoxanide (1,000 mg b.i.d.) and atazanavir/ritonavir (300/100 mg od) and followed through day 28. Nitazoxanide co-administered with atazanavir/ritonavir was found to be safe but not better than standard of care in treating COVID-19 in this trial. The HIV protease inhibitor, atazanavir (boosted with ritonavir), blocks pro-inflammatory cytokine production and has been shown to inhibit the major protease enzyme required for viral polyprotein processing during coronavirus replication (Fintelman-Rodrigues *et al*., 2020). Additionally, tizoxanide is inactivated by glucuronidation and atazanavir is a well-known inhibitor (Zhang *et al*., 2005). Hence, atazanavir was expected to enhance tizoxanide exposure when used in combination with nitazoxanide.

Conclusion: Knowledge gained from these studies could be applied against emerging or re-emerging diseases.

Further Studies: *Future studies should develop strategies that differentiate immune responses during vaccination from active SARS-CoV 2 infection, host genetics during COVID-19 and sequelae of post-COVID-19 experience.*

Limitations: *(a). As a monocentric review, it may be difficult to generalise results to other regions of varying epidemiological characteristics, (b). data on pre-admission herbal medicines or other drugs used as self-medication before developing COVID-19 were not available, (c). most of the further studies can only be done on archived samples.*

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