Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Microbial Pathogenesis 158 (2021) 104975

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Detection of SARS-CoV-2 virus using an alternative molecular method and evaluation of biochemical, hematological, inflammatory, and oxidative stress in healthcare professionals

Carla Marcelino Trassante [a], Victor dos Santos Barboza [a], Liziane dos Santos Rocha [a], Paulo Maximiliano Correa [b], Cristiane Luchese [c], Ethel Antunes Wilhelm [c], Claudio Martin Pereira de Pereira [d], Matheus Dellaméa Baldissera [e], Virginia Cielo Rech [f], Janice Luehring Giongo [a], Rodrigo de Almeida Vaucher [a,*]

[a] Laboratório de Pesquisa Em Bioquímica e Biologia Molecular de Micro-organismos (LaPeBBioM), Grupo de Pesquisa Em Bioquímica e Biologia Molecular de Micro-organismos, Centro de Ciências Químicas, Farmacèuticas e de Alimentos (CCQFA), Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil
[b] Curso de Farmácia, Centro de Ciências Químicas, Farmacèuticas e de Alimentos (CCQFA), Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil
[c] Laboratório de Pesquisa Em Farmacologia Bioquímica (LaFarBio), Centro de Ciências Químicas, Farmacèuticas e de Alimentos (CCQFA), Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil
[d] Laboratório de Lipidômica e Bio-orgânica, Centro de Ciências Químicas, Farmacèuticas e de Alimentos (CCQFA), Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil
[e] Programa de Pós-Graduação Em Farmacologia, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[f] Programa de Pós-Graduação Em Nanociências, Universidade Franciscana (UFN), Santa Maria, RS, Brazil

ARTICLE INFO

Keywords:
Health professionals
RT-LAMP
RT-PCR
SARS-CoV-2
Oxidative stress
Inflammation

ABSTRACT

In early December 2019, an outbreak of coronavirus disease 2019 caused by a new strain of coronavirus (SARS-CoV-2), occurred in the city of Wuhan, Hubei Province, China. On January 30, 2020, the World Health Organization (WHO) declared the outbreak a public health emergency of international concern. Since then, frontline healthcare professionals have been experiencing extremely stressful situations and damage to their physical and mental health. These adverse conditions cause stress and biochemical, hematological, and inflammatory changes, as well as oxidative damage, and could be potentially detrimental to the health of the individual. The study population consisted of frontline health professionals working in BHU in a city in southern Brazil. Among the 45 participants, two were infected with the SARS-CoV-2 virus and were diagnosed using immunochromatographic tests such as salivary RT-LAMP and qRT-PCR. We also evaluated biochemical, hematological, inflammatory, and oxidative stress markers in the participants. The infected professionals (CoV-2-Prof) showed a significant increase in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, lactic dehydrogenase, lymphocytes, and monocytes. In this group, the levels of uric acid, triglycerides, leukocytes, neutrophils, hemoglobin, hematocrit, and platelets decreased. In the group of uninfected professionals (NoCoV-2-Prof), significant increase in HDL levels and the percentages of eosinophils and monocytes, was observed. Further, in this group, uric acid, LDH, triglyceride, and cholesterol levels, and the hematocrit count and mean corpuscular volume were significantly reduced. Both groups showed significant inflammatory activity with changes in the levels of C-reactive protein and mucoprotein. The NoCoV-2-Prof group showed significantly elevated plasma cortisol levels. To our kowledge, this study is the first to report the use of the RT-LAMP method with the saliva samples of health professionals, to evalute of SARS-CoV-2.

1. Introduction

The emergence of viral diseases poses a serious threat to global public health. Several viral epidemics have emerged in the past few decades. On December 31, 2019, the Chinese Health Authority alerted the World Health Organization (WHO) [1] to several cases of pneumonia

* Corresponding author. Graduate Program in Biochemistry and Bioprospecting, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil.
E-mail address: rodvaucher@hotmail.com (R. de Almeida Vaucher).

https://doi.org/10.1016/j.micpath.2021.104975
Received 25 December 2020; Received in revised form 11 May 2021; Accepted 13 May 2021
Available online 19 May 2021

of unknown etiology in the city of Wuhan in Hubei province, China [2]. The pathogen causing the new disease was named SARS-CoV-2 coronavirus, and the WHO named the disease coronavirus disease 2019 (COVID-19) [3]. On December 14, 2020, 4.24 million confirmed cases and 294,046 deaths worldwide caused by COVID-19 were reported [1]. SARS-CoV-2 was classified as a 2B group beta-coronavirus with high similarity to two bat-derived coronavirus strains, with more than 96% identity. The genome of the new virus comprises a 5′ untranslated region (UTR), replicase complex (ORF1ab), Spike (S gene), E, M, and N genes, and 3′UTR [4]. Since the outbreak of SARS-CoV-2 infection began, the recommended gold standard assay is polymerase chain reaction with real-time reverse transcription (RT-qPCR). This technique has played an important role in the clinical diagnosis and investigation of suspected cases, with numerous commercially standardized kits and recommendations from the CDC and WHO [5]. However, alternative methods have been proposed for the detection of SARS-CoV-2, including loop-mediated isothermal amplification (LAMP), developed by Notomi et al. [6]. RT-LAMP was performed under isothermal conditions of 65 °C for the amplification of genes such as the ORF1ab, and the spike (S), envelope (E), and nucleocapsid genes from SARS-CoV-2, and the results were obtained within 15–40 min [7–13]. The RT-LAMP technique can detect the virus in both throat and nasopharyngeal swab samples, with a detection limit ranging from 5 to 10 copies of RNA and with a 99%–100% agreement with RT-qPCR [10–12]. In addition, the results of RT-LAMP can be assessed by visual colorimetric evaluation, making result acquisition faster and more convenient than that of RT-qPCR [10]. The use of this method for screening SARS-CoV-2 by healthcare professionals has not been reported to date. Health professionals face great demand for work, as well as risks to their physical integrity, and are frequently exposed to contagion by SARS-CoV-2. Therefore, they must be monitored, and the RT-LAMP method emerges as an alternative for the detection of SARS-CoV-2 because its operation is relatively simple and low-cost, and it has a shorter execution time compared to that of RT-qPCR [9]. The physical and psychological impact of stress experienced by health professionals during the pandemic, as well as the fear of becoming infected with SARS-CoV-2 during the performance of professional activities, have been the subject of recent studies on the health conditions of these individuals [14]. Protocols with behavioral tests, as well as the evaluation of laboratory parameters capable of assisting in such investigations, are also important tools for understanding the results of such studies worldwide [12]. Biochemical, hematological, coagulatory, and oxidative damage markers stand out among the investigated laboratory parameters, in addition to numerous inflammatory markers, particularly cytokines, which can be detected in cases of SARS-CoV-2 infection [15–17]. We believe that the study of these markers combined with the detection of SARS-CoV-2 by a fast and less expensive technique could be an interesting tool to assist professionals to assess their condition. Thus, the aim of this study was to assess the condition of stress in health professionals in southern Brazil by investigating the presence of SARS-CoV-2 in the saliva of the individuals using the RT-LAMP technique, along with an evaluation of the biochemical, hematological, and inflammatory markers as well as oxidative damage in such professionals and a control group of non-professionals.

2. Material and methods

2.1. Study population

A prospective, cross-sectional study was conducted, with 90 convenience samples collected from frontline health professionals (45 individuals) in the fight against COVID-19 in Basic Health Units (BHUs) in the municipality of Pinheiro Machado, in the State of Rio Grande do Sul (RS), and samples from the control group (45 samples) composed of healthy individuals who do not work in the health field. A questionnaire was administered to collect socio-demographic data, in addition to closed questions directly related to the work of professionals during the pandemic. The questions also addressed pre-existing diseases and the use of medications. The inclusion criteria were: health professionals who perform their activities in at least one of the BHUs in the town of Pinheiro Machado/RS. Exclusion criteria were: health professionals and individuals in the control group who did not sign the ICF and/or who had already been diagnosed with COVID-19 within three months before the beginning of the study.

2.2. Samples

Saliva samples were collected from individuals in the non-professional health and control groups to detect SARS-CoV-2 using the RT-LAMP method. All health professionals and individuals in the non-professional control group were tested after nasopharyngeal swab collection by qRT-PCR at the Laboratório Central do Rio Grande do Sul (LACEN/RS) for the detection of SARS-CoV-2. After an overnight fast, blood samples were collected from all subjects by venous puncture into Vacutainer® (BD Diagnostics, Plymouth, UK) tubes in anticoagulants with EDTA and sodium citrate. The citrated plasma and serum tubes were centrifuged at 2500×*g* for 15 min at 4 °C. All collections were performed at the Rita de Cássia Laboratory, Pinheiro Machado/RS, and the Laboratory of Biochemistry Research and Molecular Biology of Microorganisms (LaPeBBioM), Universidade Federal de Pelotas (UFPel). All collection and transportation of samples used in the study followed the protocols recommended by the Center for Disease Control and Prevention [18]. This study protocol (4.124.248) was approved by the local research ethics committee, and the volunteers who participated in the study signed a free prior informed consent form.

2.3. Extraction of viral RNA

Viral RNA of individuals' saliva was extracted using the proteinase K method, as proposed by Chantal et al. [19], with a few modifications: Approximately 0.5 mL of the subjects' saliva was collected in sterile falcon tubes and sent immediately to the laboratory to begin the extraction of the viral RNA. Then, 50 μL of saliva was transferred to a microtube, with 6.25 μL of proteinase K (20 mg/mL) added (Ludwig Biotecnologia, Porto Alegre, Brazil), 50 μL of buffer containing 10x TBE (Ludwig Biotecnologia, Porto Alegre, Brazil) and 1% Tween 20 (Sigma-Aldrich, St. Louis, MO, U.S.A.). The tubes were incubated for 1 min at room temperature, and then for a further 4 min in a thermocycler at 55 °C. Subsequently, the tubes were inactivated at 95 °C for 30 min. After this procedure, the material was used to detect SARS-CoV-2 using the RT-LAMP method. Samples from nasopharyngeal swabs as well as the positive control (inactivated SARS-CoV-2, kindly provided by Dr. Edison Durigon of the University of São Paulo (USP) were extracted using MagMax™ core nucleic acid purification kit (Applied Biosystems), according to the manufacturer's instructions. After extraction, the RNAs were quantified using a NanoDrop® spectrophotometer (Thermo Scientific, Waltham, MA). A concentration of approximately 10 ng RNA was used to perform RT-LAMP and RT-qPCR.

2.4. RT-LAMP

The RT-LAMP reaction was performed on saliva samples from the professional and non-professional group, as proposed by Park et al. [13], with a few modifications. Briefly, a reaction mixture with a final volume of 25 μL and made using the WarmStart® Colorimetric LAMP 2X Master Mix kit (New England Biolabs, Inc.) according to the manufacturer's instructions, was used. Oligonucleotides for the N gene (Nsp3_1–61) and actin genes were synthesized. The concentrations used in each Nsp3_1–61 oligonucleotide reaction were: 1.6 μM of F3 (5′ GGAATTTGGTGCCACTTC 3′) and B3 (5′ CTATTCACTTCAATAGTCTGAACA 3′), 0.4 μM of FIP (5′ CTTGTTGACCAACAGTTTGTTGACTTCAACCTGAAGAAGAGCAA 3′) and BIF (5′

CGGCAGTGAGGACAATCAGACACTGGTGTAAGTTCCATCTC 3′), and 0.4 μM LF (5′ CGGCAGTGAGGACAATCAGACACTGGTGTAAGTTCCATCTC 3′) and LB (5′ TCAAACAATTGTTGAGGTTCAACC 3′). The actin gene was used as an endogenous control, and the concentrations applied in each reaction of the oligonucleotide ACT were: 0.4 μM of primers F3 (5′ AGTACCCCATCGAGCACG 3′) and B3 (5′ AGCCTGGATAGCAACGTACA 3′), 0.4 μM of FIP (5′ GAGCCACACGCAGCTCATTGTATCACCAACTGGGACGACA 3′) and BIF (5′ CTGAACCCCAAGGCCAACCGGCTGGGGTGTTGAAGGTC 3′), and 0.4 μM of LF (5′ TGTGGTGCCAGATTTTCTCCA 3′), and LB (5′ CGAGAAGATGACCCAGATCATGT 3′), respectively. After the reaction mixtures were prepared, the tubes were incubated in a thermal block at 65 °C for 30 min, and then using a colorimeter.

2.5. qRT-PCR

RNA samples extracted from the nasopharyngeal swabs of the healthcare professionals and control groups were evaluated by qRT-PCR, as described by Corman et al. [20]. Briefly, a reaction of 25 μL of final volume was used, with the following volumes added to the 1x concentrated master mix: 5 μL of sample RNA, 12.5 μL of 2 × reaction buffer, 1 μL of SuperscriptTM III One-Step with PlatinumTM Taq DNA Polymerase (Invitrogen, Darmstadt, Germany), 0.4 mM of each dNTP, 0.4 μL of 50 mM $MgSO_4$ solution (Invitrogen), 1 μg of non-acetylated bovine albumin (Roche), and DEPC-treated water. Primers and probes used were: E-Sarbeco-F (5′ ACAGGTACGTTAATAGTTAATAGCGT 3′), E-Sarbeco-R (5′ ATATTGCAGCAGTACGCACACA3′), E-Sarbeco-P1 (5′-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ 3′), Rp-SARSr-F (5′ GTGARATGGTCATGTGTGGCGG 3′), RdRp-SARSr-R (5′ CARATGTTAAASACACTATTAGCATA 3′) and RdRp-SARSr-P1 (5′-FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ 3′). The cycling conditions were: 55 °C for 10 min for reverse transcription, followed by 95 °C for 3 min and 40 cycles of 95 °C for 15 s and 58 °C for 30 s. The reactions were performed on Real-time OneStep® equipment (Applied Biosystems, USA).

2.6. Biochemical measurements

Serum from individuals in the health professional and non-professional groups were initially used to assess the presence of anti-SARS-CoV-2 IgG/IgM antibodies using the immunochromatographic method (Wondfo Biotech Co. Ltd., China). Levels of uric acid, albumin, ALT, AST, creatinine, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, glucose, total globulins, lactic dehydrogenase, total proteins, C reactive protein, and triglycerides were assessed using standard methods on a Cobas MIRA® automated analyzer (Roche Diagnostics, Basel, Switzerland). Colorimetric mucoprotein was identified using the Labtest commercial kit (Minas Gerais, Brazil), as instructed by the manufacturer. Serum cortisol was determined by immunoenzymatic assay (ELISA). Cortisol level (Diagnostics Biochem Canada Inc) was quantified according to the manufacturer's recommendations. Microplate reading was performed on a microplate reader (TP-Reader, Thermo plate, China) with a length of 450 nm. FRAP, total oxidation status (TOS), and total antioxidant capacity (TAC) concentrations were determined on the Cobas MIRA®, described previously by Erel [21,22]. The ratio of total peroxide (TOS) to the TAC gave the oxidative stress index (OSI), an indicator of the degree of oxidative stress [23].

2.7. Hematologic analysis

Hematological parameters were assessed in whole blood collected in tubes containing EDTA (Vacutainer®) using an ABX Micros 60® automated hematology system (HORIBA Medical, Japan). Total leukocytes (WBC), total erythrocytes (RBCs), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT) were determined. Blood smears were fixed in methanol and stained with Instant-Prov (NewProv®) staining to determine the differential WBC count. At least 200 WBCs were counted in differential WBC determinations.

2.8. Anticoagulant activity

Anticoagulant activity was determined based on thromboplastin partial actived time (aTTP) and prothrombin time (TP), which indicate the intrinsic and extrinsic coagulation pathways, respectively, in citrated plasma [24]. The patient samples were then centrifuged at 800×*g* for 10 min aTTP and TP were determined using monochannel coagulometer (Clotimer, Brazil) with commercial kits (Biotécnica, Varginha, Minas Gerais, Brazil), following the manufacturer's instructions.

2.9. Statistical analysis

Data are expressed as the mean ± SD for duplicates or triplicates for each experimental point. Data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test when needed, using GraphPad Prism 4.0 software.

3. Results

Table 1 shows the results from the socio-demographic questionnaire and closed questions directly related to the professionals' performance during the COVID-19 pandemic, the existence of pre-existing diseases, and the use of medications.

Table 2 shows the results of the immunological and molecular tests performed for the detection of SARS-CoV-2 in the study individuals.

RT-LAMP (Fig. 1) detected the presence of SARS-CoV-2 in two saliva samples from the tested health professionals (channels 10 and 11). Due to this fact, the health professionals were subdivided into NoCoV-2-Prof and CoV-2-Prof during the course of the study. Results were confirmed

Table 1
Socio-demographic data of the individuals studied.

	NoCoV-2-Prof	CoV-2-Prof	No-Prof
Gender (%)			
Female	86.7	100	77.9
Male	13.3	0	22.1
Age (years)			
18 to 35	57.8	100	35.6
Above 35	42.2	0	64.4
Education			
High school	54.6	0	73.3
University education	45.4	100	26.7
Profession			
Community agent/endemic	24.4	–	–
Doctors	8.9	50	–
Nurses/Nursing technicians	31.1	–	–
Other health professionals	35.6	50	–
Pre-existing diseases			
Some type of pre-existing disease	45.1	50	41.3
Respiratory disease	21.4	–	12.7
Hypertension	16.7	–	19
Heart disease	4.8	–	3.14
Diabetes	7.2	–	16
Depression	4.8	50	7.86
Use of medicines			
Contraceptive	32.6	50	22.4
Losartan	10.9	–	8.8
Hydrochlorothiazide	7.2	–	7.8
Omeprazole	7.2	–	5.5
Clonazepan	5.4	–	5.5
Fluoxetine	3.6	25	3.3
Metformin	5.4	–	7.8
Budsonide	5.4	–	7.8
Others	22.3	25	31.1
Total (n = 90)	**100% (43)**	**100% (2)**	**100% (45)**

Table 2
Immunological and molecular tests performed in the study.

Test	Result	NoCoV-2-Prof (n = 43)	CoV-2-Prof (n = 2)	No-Prof (n = 45)
Immunochromatographic test	Positive IgG/IgM	–	(2/90)	–
	Negative	(43/90)	–	(45/90)
RT-LAMP (Saliva)	Positive	–	(2/90)	–
	Negative	(43/90)	–	(45/90)
qRT-PCR	Detected	–	(2/90)	–
	Inconclusive	–	–	–
	Not detected	(43/90)	–	(45/90)

by qRT-PCR, indicating that RT-LAMP is a reproducible and reliable method. In addition, all negative results for RT-LAMP were also confirmed by qRT-PCR and presented similar results; that is, the presence of SARS-CoV-2 virus was not detected in the individuals' nasopharyngeal swab samples. The serology results also showed the same pattern, with only NoCoV-2-Prof group IgG/IgM class antibodies detected after 15 days of viral infection.

Table 3 shows the results of biochemical analysis. A significant reduction in the levels of uric acid and triglycerides can be seen in the CoV-2-Prof group when compared to No-Prof individuals. There was a significant increase in the levels of ALT, AST, HDL, and LDH in the CoV-2-Prof group compared with that in the No-Prof group. Significant increase in HDL levels was observed in the NoCoV-2-Prof group compared to that in the No-Prof group. There was a significant decrease in the levels of uric acid, LDH, triglycerides, and VLDL in the NoCoV-2-Prof group compared to the No-Prof group.

When assessing anti-inflammatory activity, a significant increase in mucoprotein levels can be seen in the NoCoV-2-Prof group (Fig. 2A) compared to the No-Prof group. A nonsignificant reduction in mucoprotein was observed in the CoV-2-Prof group compared to the No-Prof control. The levels of C-reactive protein showed a significant increase only in the CoV-2-Prof group compared to the No-Prof group (Fig. 2B).

The professionals' stress assessment was validated by the detection of serum cortisol levels. A significant increase in circulating cortisol levels was observed in the NoCoV-2-Prof group compared to the cortisol levels of the individuals in the No-Prof group (Fig. 3).

Oxidative stress was assessed using FRAP, TOS, TAC, and the OSI assay. Fig. 4A shows the levels of antioxidants in the serum for the groups evaluated using the FRAP assay. Significant decrease in antioxidant levels can be observed in the NoCoV-2-Prof group compared to the No-Prof group. Fig. 4B shows the results of TOS levels. A significant increase in TOS levels was observed in the NoCoV-2-Prof and CoV-2-Prof groups compared to the No-Prof group. Fig. 4C shows TAC levels. There was a significant increase in TAC levels in the NoCoV-2-Prof group compared to the No-Prof group. Fig. 4D shows the results of the OSI.

Table 4 shows the results of the hemograms. Significant reduction in the values of the WBC, neutrophils, hemoglobin, hematocrit, and platelets was observed in the CoV-2-Prof group compared with the No-Prof group. There was a significant increase in lymphocyte and monocyte values in the CoV-2-Prof group compared to those in the No-Prof group. A significant increase in the values of eosinophils and erythrocytes was observed in the NoCoV-2-Prof group compared to the No-Prof group. There was a significant decrease in hematocrit and MCV values in the NoCoV-2-Prof group compared with that in the No-Prof group.

Regarding coagulation proteins, a significant decrease in the prothrombin time (PT) activity was observed in the NoCoV-2-Prof group compared to the No-Prof control (Fig. 5A). For the activated partial prothrombin time (aTTP), no significant differences were observed between the groups (Fig. 5B).

4. Discussion

Study participants were divided into three groups based on the results initially observed in the RT-LAMP method: NoCoV-2-Prof, CoV-2-Prof and No-Prof. This result was important, as it is the first report on the use of the RT-LAMP technique to test health professionals using saliva. Although the method was previously reported by Park et al. [13], using commercially available reagents, the RT-LAMP presented in this study

Table 3
Biochemical parameters evaluated in the study.

Tests	NoCoV-2-Prof	CoV-2-Prof	No-Prof	P value
Uric Acid (mg/dL)	**5.05 ± 2.23** **	**4.15 ± 0.92** *	7.41 ± 1.55	**0.0001**
Albumin (mg/dL)	4.28 ± 1.34	4.05 ± 0.35	4.54 ± 1.06	0.4562
ALT (U/L)	15.62 ± 8.01	**51.5 ± 23.3** *	42.8 ± 62.31	**0.0222**
AST (U/L)	22.7 ± 9.4	**105 ± 21.21** **	39.49 ± 39.45	**0.0001**
Creatinine (mg/dL)	1.15 ± 0.16	1.09 ± 0.06	1.24 ± 0.29	0.1570
Total cholesterol (mg/dL)	213.07 ± 47.25	216 ± 12.72	195 ± 39.02	0.1237
Glucose (mg/dL)	83.98 ± 57.9	74.5 ± 9.19	80.28 ± 27.7	0.9077
Total globulins (g/dL)	4.23 ± 1.64	6.15 ± 1.05	4.15 ± 1.69	0.2535
HDL (mg/dL)	**91.5 ± 25.7** **	**92 ± 23.7** **	64.4 ± 15.9	**0.0001**
Lactic dehydrogenase (U/L)	**270.5 ± 64.16** **	**515 ± 91.92** **	343.9 ± 80.88	**0.0001**
LDL (mg/dL)	97.15 ± 42.91	101 ± 36.7	96.07 ± 38.15	0.9195
Total proteins (g/dL)	8.51 ± 1.1	10.2 ± 0.71	8.67 ± 1.37	0.1347
Triglycerides (mg/dL)	**122.1 ± 82.18** *	**115 ± 21.21** *	173.1 ± 92.49	**0.0296**
Urea (mg/dL)	36.14 ± 9.63	30.3 ± 13.44	39.6 ± 13.44	0.1626
VLDL (mg/dL)	**24.43 ± 16.44***	**23 ± 4.24***	34.6 ± 18.5	**0.0296**

Data are expressed as mean ± SD of the **NoCoV-2-Prof** and **CoV-2-Prof** groups compared to the **No-Prof (control)**. *p < 0.05; **p < 0.01; ***p < 0.001 compared to control.

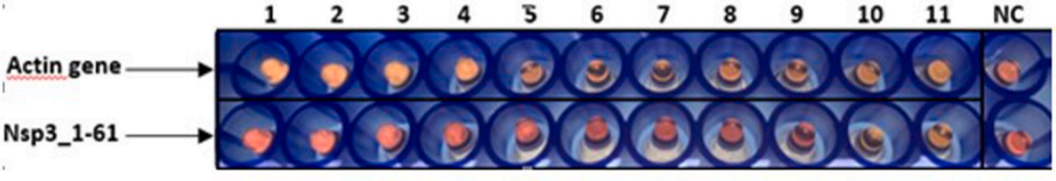

Fig. 1. Results of RT-LAMP in saliva samples for the study subjects. Channel 1 to 9: negative result for the detection of SARS-CoV-2. Channel 10 and 11: positive result for SARS-CoV-2. Pink reaction: no amplification; Yellow reaction: amplification. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

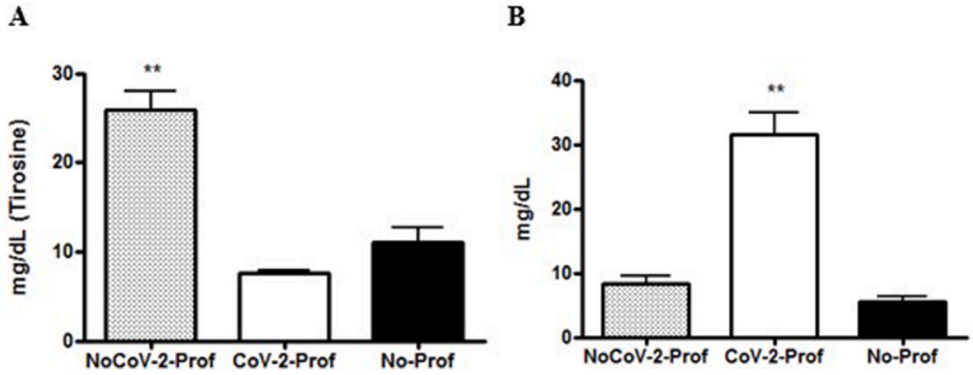

Fig. 2. Anti-inflammatory activity with levels of mucoproteins (**A**) and C-reactive protein (**B**). Data are expressed as mean ± SD of the NoCoV-2-Prof and CoV-2-Prof groups compared to the No-Prof (control). *p < 0.05; **p < 0.01; ***p < 0.001 compared to control.

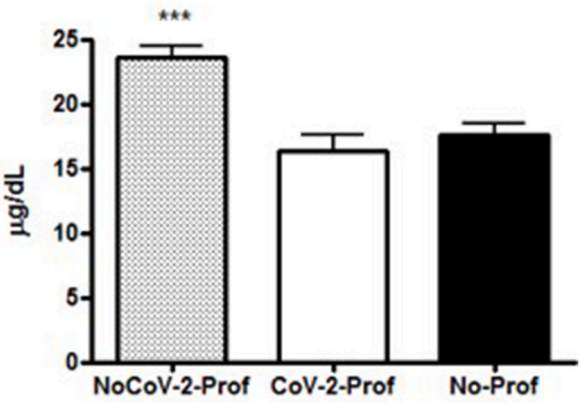

Fig. 3. Individuals' stress assessed by plasma cortisol levels. Data are expressed as mean ± SD of the NoCoV-2-Prof and CoV-2-Prof groups compared to the No-Prof (control). *p < 0.05; **p < 0.01; ***p < 0.001 compared to control.

showed interesting advantages over qRT-PCR. Some recent studies using RT-LAMP aimed at standardizing the method to detect viral RNA for the diagnosis of SARS-CoV-2 in nasopharyngeal samples [13,25–28]. Dao et al. [29] tested a two-color RT-LAMP assay protocol to detect SARS-CoV-2 viral RNA using a 300-primer set specific for the N gene. Yan et al. [10] developed an RT-LAMP assay that contained specific primers for the SARS-CoV-2 *orf1ab* and *S* genes.

SARS-CoV-2 transmission is mainly mediated by saliva droplets [16]. The FDA has approved saliva as a source of virus detection. Ben-Assa et al. [29] applied the RT-LAMP method to nasal swab samples and self-collected saliva samples. Tests were performed on 186 samples from suspected patients, and the results, as well as thsoe from our study, were compared with those obtained using qRT-PCR. However, among the samples tested, only 3 were saliva. Two of them confirmed the results for SARS-CoV-2, and another was a suspected patient with a negative result. Tests of saliva samples from patients confirmed by RT-LAMP and RT-qPCR that 2 subjects were positive, while one negative case was confirmed as negative by qRT-PCR. Our results are consistent with those reported by Ben-Assa et al. [29], showing that RT-LAMP is a fast, low-cost, and safe method for the screening of SARS-CoV-2 in healthcare professionals. A study reported by Nagura-Ikeda et al. [30] evaluated self-collected saliva, with a median collection time of three days after receiving their first positive qRT-PCR results. Self-collected saliva at initial-stage symptoms proved to be an alternative option for diagnosing SARS-CoV-2. However, no study has yet tested a group of health professionals using the RT-LAMP method to detect SARS-CoV-2 in the saliva.

Among the 45 individuals health professionals who participated in the study, we observed a diversity of job functions at the BHUs. Depending on their roles, individuals may be more exposed to the risk of infection with SARS-CoV-2. Nurses and nursing technicians were exposed in greater numbers (31.1%), while doctors (8.9%) and community workers (24.4%) had lower exposure. Of individuals exposed, 45.1% had pre-existing disease, with a prevalence of respiratory disease (21.4%), followed by hypertension (16.7%). Diabetes affected 7.2% of this population. Heart disease and depression were each present in 4.8% of participants. During this study, 2 professionals (one doctor and one dentist) had a detectable result for SARS-CoV-2 and ended up comprising the CoV-2-Prof group. Johnstone and Turale [31] reported that nurses and nurse technicians could be the professionals most affected by the virus during the pandemic. Our results showed that, although there was a greater number of professionals with this role in the NoCoV-2-Prof group, there were no cases of infection with the SARS-CoV-2 virus in this group during the study.

The pandemic caused by the new coronavirus has had several psychological effects worldwide. Previous psychiatric illnesses have been exacerbated. In this study, one psychological impact less reported by individuals was stress (4.7%), a result that was contrary to the increased levels of plasma cortisol observed significantly in the group NoCoV-2-Prof compared to No-Prof. Blackman [32] recently reported on emotional exhaustion of health professionals during the pandemic, with effects on the behavior of such individuals. For example, 74.4% of professionals stated that they had some change in their professional routine. Da Silva and Neto [33] found that the psychological suffering of health professionals can be associated with the uncertainty of a safe workplace. Moore et al. [34] showed that 35% of frontline professionals in the United Kingdom needed support but did not feel able to ask for help. Furthermore, 64% reported feeling anxious during the April 2020 peak of the pandemic.

Stress is the response of the body to any non-specific demand related to emotional tension and pressure [35]. Stress conditions can activate different metabolism response routes and cause oxidative damage in these individuals. Prolonged emotional pressure in distressing periods, such as the pandemic or chronic stress, can lead to a wide spectrum of physical and psychological illnesses [36]. Such situations can cause physiological changes, increasing the level of certain biochemical, hematological, coagulation, and inflammatory markers.

In this study, a significant reduction in uric acid values was observed in the CoV-2-Prof group. A significant decrease in the levels of uric acid and triglycerides was observed in the NoCoV-2-Prof group. Qin et al. [37] reported that in severe cases of COVID-19, extremely low levels of uric acid were detected because of the disease. Trinder et al. [38] showed that changes in lipids correlate with the severity of the infection; that is, the more severe the infection, the greater the changes in lipid and lipoprotein levels. Alvarez [39] and Sahin and Yldiz [40] reported that

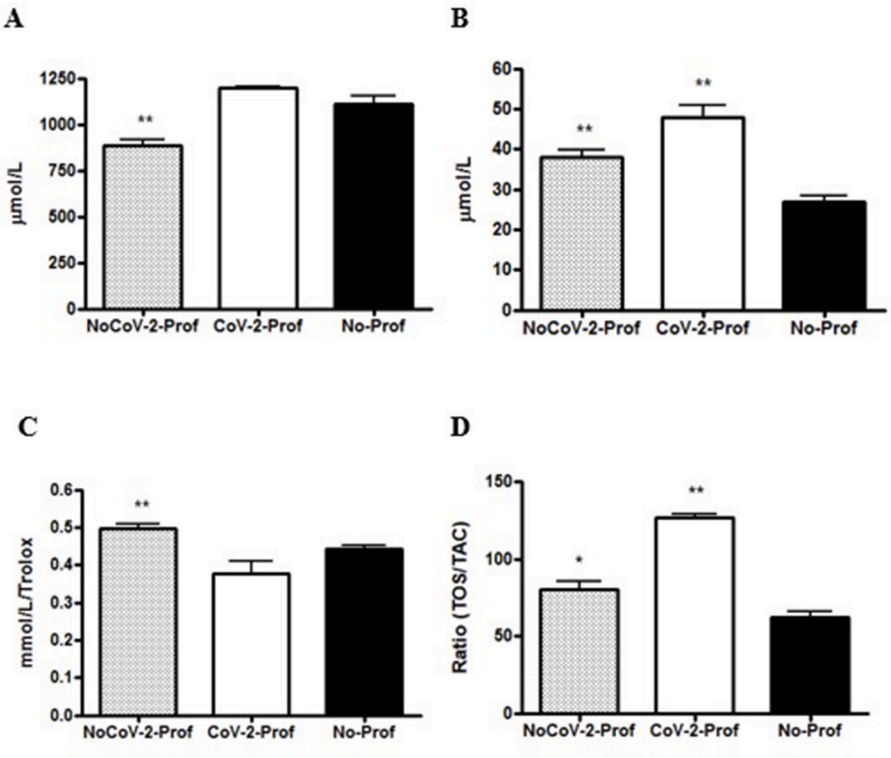

Fig. 4. Antioxidant activity and oxidative stress of the individual groups belonging to the study. FRAP (A), TOS (B), TAC (C) and OSI (D). Data are expressed as mean ± SD of the NoCoV-2-Prof and CoV-2-Prof groups compared to the No-Prof (control). *p < 0.05; **p < 0.01; ***p < 0.001 compared to No-Prof (control).

Table 4
Blood count values of the groups evaluated.

	NoCoV-2-Prof	CoV-2-Prof	No-Prof	P
WBC (x 10^3/µL)	7.62 ± 3.3	**2.8 ± 1.41** **	6.41 ± 1.8	**0.0052**
Rods (%)	0.7 ± 0.72	0	0.5 ± 0.56	0.1292
Neutrophil (%)	68 ± 10.7	**39.0 ± 2.12** ***	67 ± 4.9	**0.0001**
Eosinophil (%)	**2.0 ± 0.68** **	**2 ± 1** **	1.0 ± 0.80	0.0043
Basophil (%)	0.3 ± 0.4	0	1.0 ± 0.31	0.9082
Lymphocyte (%)	24 ± 3.9	**45.0 ± 3.53** ***	26 ± 3.4	**0.0001**
Monocyte (%)	5.0 ± 1.30	**15 ± 1.41** ***	4.5 ± 3.1	**0.0001**
RBC (x 10^6/µL)	**4.75 ± 0.63** **	3.9 ± 0.19	4.23 ± 0.38	**0.0003**
Hb (g/dL)	13 ± 0.81	**10.9 ± 0.56** *	14.2 ± 1.56	**0.0415**
Ht (%)	**34.8 ± 12.9** *	**34.3 ± 1.83** *	37.1 ± 3.3	**0.0365**
MCV (fl)	**82.7 ± 13.5** **	85 ± 2.82	87.8 ± 3.8	**0.0094**
MCH (pg)	33.0 ± 12.9	29.3 ± 0.91	32.7 ± 3.4	0.7709
MCHC (g/dL)	33.8 ± 13.4	34.4 ± 1.69	37.6 ± 3.1	0.7463
PLT (x 10^3/µL)	311 ± 113.4	**125 ± 21.21** *	274.3 ± 65.9	**0.0126**

Data are expressed as mean ± SD of the **NoCoV-2-Prof** and **CoV-2-Prof** groups compared to the **No-Prof (control)**. *p < 0.05; **p < 0.01; ***p < 0.001 compared to control.

triglyceride levels may be elevated or inadequatein cases of viral infection. Feingold et al. [41] also showed that in patients with COVID-19 infections, serum trigyceride levels were variable, both high and low. Wang et al. [42] found that the levels of serum triglycerides were important influencing factors for recovery from SARS-CoV-2 infection. However, there are no data available in the literature regarding the measurement of these parameters in health professionals who work during the pandemic for a concrete comparison of results.

A significant increase in ALT, AST and HDL levels in the CoV-2-Prof group was observed in this study. There was also a significant increase in HDL values and a significant decrease in urea, triglycerides and VLDL levels in the NoCoV-2-Prof group. Changes in liver function tests have been reported in up to 40% of COVID-19 patients [43–45]. The suggested mechanism for liver damage involves the presence of angiotensin II receptors (ACE2) and transmembrane serine protease 2 (TMPRSS2) in cholangiocytes and hepatocytes, suggesting that the damage to liver function is caused by viral cytopathic effects [46,47]. In addition, other mechanisms may be involved, such as autoimmune damage, hypoxic hepatitis, and drug-induced liver damage [48]. There is association of the severity of COVID-19 with liver damage, and some meta-analyses indicate that alteration of these liver enzymes can be used as a prognostic marker for the severity of COVID-19 [49,50] Shao et al. [47] conducted a study on 98 mild COVID-19 cases in patients at the Wenzhou Central Hospital in Wenzhou, China, and their results suggest that hepatobiliary complications are prevalent in patients with mild COVID-19.

Thus, our results are inconsistent with the data in the literature. Regarding HDL dosage, the infected professionals had significantly increased values compared to the control group, differing from the results in the literature, which show a decrease in total LDL and/or HDL cholesterol levels in patients infected with COVID-19. In most studies, the severity of the disease is higher the greater the decrease in LDL and/or HDL levels [41]. Decreased serum HDL cholesterol levels are associated with the severity of COVID-19 infection. Infected patients had drastically reduced concentrations of serum total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol [51]. As the professionals had a satisfactory recovery, HDL levels may have remained higher than those reported in the literature for the most severe cases of COVID-19 in hospitalized patients. In these patients, the levels of total cholesterol, LDL, and HDL may be decreased [32,33]. A study by Scalsky et al. [52] reported that high HDL levels are associated with a reduced risk of positive testing for SARS-CoV-2, a result opposite to the one we obtained, since this parameter was high in infected individuals. Li et al. [53] reported that a high level of lactic acid dehydrogenase (LDH) was significantly associated with severe COVID-19 on admission. Patients with risk factors such as older age, hypertension, and high LDH levels required careful observation and early intervention to prevent the worsening of COVID-19. According to

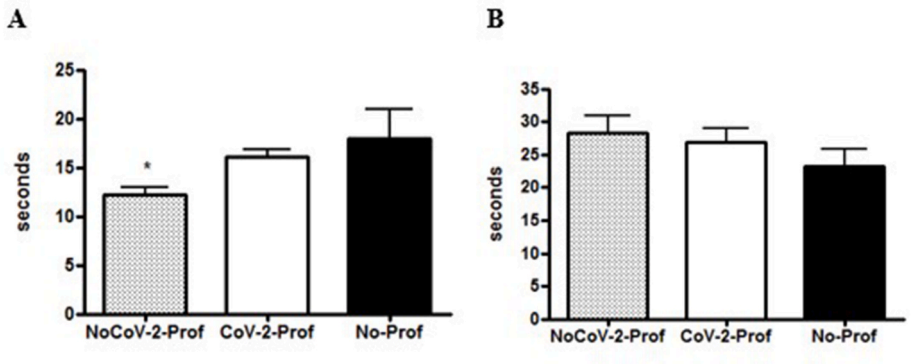

Fig. 5. Evaluation of proteins from the intrinsic and extrinsic pathways of coagulation in the groups. TP (A) and aTTP (B). Data are expressed as mean ± SD of the NoCoV-2-Prof and CoV-2-Prof groups compared to the No-Prof (control). *p < 0.05; **p < 0.01; ***p < 0.001 compared to No-Prof (control).

Li et al. [50], Zhang et al. [54], and Wu et al. [55], a high level of LDH is a factor for COVID-19 severity. Wang et al. [42] found in their results that 40% of patients infected had high LDH. The CoV-2-Prof group showed a significant increase in LDH compared to the control group, but did not have a severe form of the disease. Erez et al. [56] reported that the LDH of 398 individuals was recognized as a marker for severe prognosis in several diseases, including cancer and infection.

Inflammatory markers were evaluated in this study. A significant increase in mucoprotein (MUC) levels was demonstrated in the NoCoV-2-Prof group, and a non-significant reduction in mucoprotein was observed in the CoV-2-Prof group. There was no data in the current literature to compare these results, since it is the first report on the use of mucoproteins to assess the acute inflammatory process in health professionals working during the pandemic. CRP levels increased significantly in the CoV-2-Prof group. We attribute this increase to the SARS-CoV-2 viral infection in this group of professionals. Guan et al. [44] showed elevated C-reactive protein (CRP) levels in 60.7% of patients evaluated with COVID-19. Lippi, Plebani, and Henry [57] reaffirm this hypothesis, reporting that one of the most frequent laboratory alterations in patients with COVID-19 is an increase in CRP by 75–93%. These data are in agreement with those described in the present study. Li et al. [53] observed that, approximately 7–14 days after the onset of initial symptoms, an increase in the clinical manifestations of the disease begins. This occurs in parallel with a pronounced systemic increase in inflammatory mediators and cytokines, which can be characterized as a "cytokine storm." Higher CRP levels are associated with unfavorable aspects of COVID-19, such as the development of Acute Respiratory Discomfort Syndrome (ARDS), higher levels of troponin-T, myocardial injury, and death [55] Several studies have established that the hyper-inflammatory response induced by SARS-CoV-2 is one of the main causes of illness severity and death in infected patients.

It is known that chronic stress can stimulate the conserved transcriptional response to adversit through the sympathetic nervous system, leading to the induction of proinflammatory cytokines and the suppression of genes involved in the production of antibodies and interferons, causing vulnerability to viral infections. Proinflammatory cytokines induce chronic inflammation and generate ROS, thus producing an unbalanced oxidative stress response [58]. Oxidative stress results from an imbalance in the generation of oxidizing compounds and the performance of antioxidant defense systems [59]. Several respiratory viruses induce unregulated ROS formation due to increased recruitment of inflammatory cells at the site of infection [60]. Among the markers of oxidative stress, total antioxidant capacity (TAC), total oxidative state (TOS), and oxidative stress index (OSI) can act as important tools for investigating oxidative stress in organisms [21,22,61]. In addition, FRAP in the serum has been determined as an indirect method to assess antioxidant capacity. We observed a significant decrease in the level of antioxidants in the NoCoV-2-Prof group, significant increase in TOS levels in the NoCoV-2-Prof and CoV-2-Prof groups. A significant increase in TAC levels in the No-CoV-Prof group, and a significant increase in OSI levels in the NoCoV-2-Prof and CoV-2-Prof groups. Current research findings and reports have suggested that oxidative stress plays an important role in SARS-Cov-2 infections. The absence or reduction of oxidative stress would have a significant beneficial effect during the initial stage of viral infection, preventing the binding of viral proteins to host cells [59]. Derouiche [64] reports that oxidative stress affects repair mechanisms and the immune control system, which is one of the main events in the inflammatory response. This fact leads to oxidative stress that may be linked to a greater propensity to be infected with COVID-19, in addition to being a factor that increases the severity of the virus, especially in patients with chronic diseases. All reported markers used to assess the level of oxidative stress in groups of individuals were reported in an unprecedented manner. Thus, we do not have the data from the literature for comparison.

Regarding the frequent hematological changes in patients with COVID-19, it is known that total leukocyte count demonstrates considerable variation, sometimes appearing high or decreased, but lymphopenia was evident. There was also a decrease in hemoglobin level [57]. The number of leukocytes in the CoV-2-Prof group was reduced. The hemoglobin in this group was also reduced, corroborating the data previously mentioned. The neutrophil count in the CoV-2-Prof group was decreased. Varim et al. [63] reported that individuals with severe COVID-19 had an increase in neutrophils compared to patients who had mild disease. Higher neutrophil value is related to poor prognosis. Neutrophils play a vital role in our immune defenses, eliminating invading microorganisms. Common causes of neutropenia include autoimmune diseases, drug reactions, chemotherapy, and hereditary disorders [66]. Although further research is required on the underlying etiology, several factors can contribute to COVID-19-associated lymphopenia. Lymphocytes have been shown to express the ACE2 receptor on their surface [65,67], and SARS CoV-2 can directly infect these cells, ultimately leading to cell lysis. In addition, the cytokine storm is characterized by markedly increased levels of interleukins and tumor necrosis factor alpha (TNFα), which can lead to apoptosis in lymphocytes [68,69]. Substantial cytokine activation may also be associated with atrophy of lymphoid organs [70].

Huang et al. [7] and Wang et al. [42] highlighted an association between lymphopenia and the need for care in the ICU. Wu et al. [55] showed an association between lymphopenia and the development of acute respiratory distress syndrome (ARDS). Lymphopenia, excessive activation of the inflammatory cascade, and cardiac involvement are characteristics of COVID-19, and have a high prognostic value. However, our understanding of the underlying mechanisms is still limited [71]. Professionals in the CoV-2-Prof group had increased lymphocyte values, contrary to the literature data. He et al. [70] comments that the

effect of lymphocytes in mild cases of COVID-19 (as in the case of the two individuals in the infected group) remains unclear. Monocytes were also increased in the CoV-2-Prof group. The effect of monocytes in mild COVID-19 cases is unclear.

In line with our study, Zhou et al. [71] found significantly increased circulating proportions of CD14 and CD16 monocytes in peripheral blood of 33 patients hospitalized with COVID-19, and this percentage was highly increased in COVID-19 patients with acute respiratory distress syndrome. The platelet count in the CoV-2-Prof group was reduced. Platelet count is a simple, inexpensive, and easily available biomarker, which is why it was quickly adopted as a potential biomarker for COVID-19 patients [72]. It has been reported that the number of platelets was significantly reduced in patients with COVID-19, and even lower in non-survivors compared to survivors [72,73]. In the evaluation of coagulation proteins, a significant decrease in activity was observed in the prothrombin time (PT) of the NoCoV-2-Prof group and activated partial prothrombin time (aTTP). However, it is known that coagulation disorders are observed relatively frequently among patients with COVID-19, especially among those with severe disease with increased clotting times due to deficiency in the production by the liver [73].

5. Conclusions

The psychological damage that the COVID-19 pandemic has caused to humanity is indisputable. One particularly affected group is that of health professionals who work daily to face the pandemic. RT-LAMP can be an alternative molecular method for screening SARS-CoV-2 in the saliva samples collected from these professionals. We consider it a fast, safe, and less expensive method than qRT-PCR. We emphasize that our study is the first to report the use of the RT-LAMP method with saliva samples from health professionals. Health care professionals have also experienced chronic stress during the pandemic, a fact confirmed here by the increase in plasma cortisol levels and the alteration of some biochemical, hematological, inflammatory, and oxidative stress markers.

Funding

Current research was funded by Foundation of Amparo Research of Rio Grande do Sul (FAPERGS) [20/2551-0000263-2].

CRediT authorship contribution statement

Carla Marcelino Trassante: Methodology, Writing – original draft. **Victor dos Santos Barboza:** Methodology, Writing – original draft. **Liziane dos Santos Rocha:** Methodology, Writing – original draft. **Paulo Maximiliano Correa:** Methodology, Writing – original draft. **Cristiane Luchese:** Investigation, Formal analysis, Writing – review & editing. **Ethel Antunes Wilhelm:** Investigation, Formal analysis, Writing – review & editing. **Claudio Martin Pereira de Pereira:** Formal analysis, Resources. **Matheus Dellaméa Baldissera:** Investigation, Formal analysis, Writing – review & editing. **Virginia Cielo Rech:** Validation, Formal analysis, Resources. **Janice Luehring Giongo:** Conceptualization, Investigation, Validation, Formal analysis. **Rodrigo de Almeida Vaucher:** Conceptualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rodrigo de Almeida Vaucher reports was provided by Federal University of Pelotas. Rodrigo de Almeida Vaucher reports a relationship with Federal University of Pelotas that includes: employment.

Acknowledgments

Thanks to the National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES) and the Federal University of Pelotas for supporting the scholarships to students.

References

[1] World Health Organization, Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected. https://www.who.int/docs/default-source/coronaviruse/clinical-management-of-novel-cov.pdf, 2020. Accessed on August 23, 2020.

[2] H. Lu, C.W. Stratton, Y.W. Tang, Outbreakof pneumonia of unknown etiology in Wuhan, China: the Mystery and the miracle, J. Med. Virol. 92 (4) (2020 Abr) 401–402, https://doi.org/10.1002/jmv.25678.

[3] A.E. Gorbalenya, S.C. Baker, Coronaviridae Study Group of the International Committe on Taxonomy of Viruses. The species *Severe acute respirato ry syndrome-related coronavirus*: classifying 2019-nCoV and naming it SARS-CoV-2, Nat. Microbiol. (2020) 536–544, https://doi.org/10.1038/s41564-020-0695-z.

[4] P.E. Yang, X. Wang, COVID-19: a new challenge for human beings, Cell. Mol. Immunol. 17 (5) (2020) 555–557, https://doi.org/10.1038/s41423-020-0407-x.

[5] A. Ganguli, A. Mostafa, J. Berger, M. Aydin, F. Sun, E. Valera, B.T. Cunningham, W. P. King, R. Bashir, Rapid isothermal amplification and portable detection system for SARS-CoV-2, bioRxiv [Preprint] 21 (2020) 2020, https://doi.org/10.1101/2020.05.21.108381, 05.21.108381.

[6] T. Notomi, Loop-mediated isothermal amplification of DNA, Nucleic Acids Res. 28 (12) (2000) E63, https://doi.org/10.1093/nar/28.12.e63, 15.

[7] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, B. Cao, Clinical characteristics of patients infected with the novel coronavirus 2019 in Wuhan, China, *Lancet.* 15 de fevereiro de 395 (2020) 497–506, https://doi.org/10.1016/S0140-6736(20)30183-5, 10223.

[8] L.E. Lamb, S.N. Bartolone, E. Ward, M.B. Chanceler, Rapid detection of new coronavírus/Servere Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2) by isotermal amplification mediated by reverse trancriotion, PloS One 15 (6) (2020) 234682, https://doi.org/10.1371/joutnal.pone.0234682.

[9] A. Mohammed, E.C. Rohaim, S. Irem, V. Julianne, E. Manar, Mohammad Q. A. Khalifa, B. Mahmoud, C.P. Aurore, B.A. Manoharanehru, T. Mukunthan, S. C. Nouman, S. Ravinder, B. Amy, B. Peter, H. Wendy, B. Judy, B. Joe, Hayley, C. Wilkinson, J. Williams, E. Whittingham-Dowd, M.H. Shaw, B. Lisa, D. B. Michelle, L.R. Roberto, B. Wamadeva, F. Anil, M. Muhammad, Loop-mediated isothermal amplification assisted by artificial intelligence (AI-LAMP) for rapid detection of SARS-CoV-2, Viruses (2020), https://doi.org/10.3390/v12090972.

[10] C. Yan, J. Cui, L. Huang, B. Du, L. Chen, G. Xue, S. Li, W. Zhang, L. Zhao, Y. Sun, H. Yao, N. Li, H. Zhao, Y. Feng, S. Liu, Q. Zhang, D. Liu, J. Yuan, Rapid and visual detection of the new coronavirus 2019 (SARS-CoV-2) by a loop-mediated reverse transcription of isothermal amplification, Clin. Microbiol. Infect. 26 (6) (2020 Jun) 773–779, https://doi.org/10.1016/j.cmi.2020.04.001.

[11] W. Yang, Xiaofei Dang, Q. Wang, Mingjie Xu, Qianqian Zhao, Yunying Zhou, Huailong Zhao, Li Wang, Yihui Xu, Jun Wang, Han Shuyi, Min Wang, Fenyan Pei, Yunshan Wan, Rapid detection of SARS-CoV-2 Using the reverse transcription RT-LAMP method, MedRxiv (2020), https://doi.org/10.1101/2020.03.02.20030130.

[12] Yinhua Zhang, Nelson Odiwuor, Jin Xiong, Luo Sun, Raphael Ohuru, Wei, N. A. Hongping Nyaruaba, Rapid molecular detection of SARS-CoV-2 (COVID-19) RNA virus using colorimetric LAMP, MedRXiv (2020), https://doi.org/10.1101/2020.02.26.20028373.

[13] G.S. Park, K. Ku, S. Baek, S.J. Kim, S.I. Kim, B. Kim, J.S. Maeng, Development of isotherm amplification assays mediated in a reverse transcription loop aiming at severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), J. Mol. Diagn. 22 (6) (2020) 729–735, https://doi.org/10.1016/j.jmoldx.2020.03.006.

[14] K. Tsamakis, E. Rizos, A.J. Manolis, S. Chaidou, S. Kympouropoulos, E. Spartalis, D. A. Spandidos, D. Tsiptsios, A.S. Triantafyllis, COVID-19 pandemic and its impact on mental health of healthcare professionals, Exp. Ther. Med. 19 (6) (2020) 3451–3453, https://doi.org/10.3892/etm.2020.8646.

[15] J.J. Zhang, X. Dong, Y.Y. Cao, Y.D. Yuan, Y.B. Yang, Y.Q. Yan, C.A. Akdis, Y. D. Gao, Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China, Allergy 75 (7) (2020) 1730–1741, https://doi.org/10.1111/all.14238.

[16] B.M. Bakadia, B.O.O. Boni, A.A.Q. Ahmed, G. Yang, The impact of damage to oxidative stress induce of by enviromental stressors in COVID-19, Life Sci. 926 (2020) 264–118653, https://doi.org/10.1016/j.lfs.2020.118653.

[17] F. Tekos, Z. Skaperda, N. Goutzourelas, D.S. Phelps, J. Floros, D. Kouretas, The importance of redox status in the frame of lifestyle approaches and the genetics of the lung innate immune molecules, SP-A1 and SP-A2, on differential outcomes of COVID-19 infection, Antioxidants 9 (9) (2020 Aug 25) 784, https://doi.org/10.3390/antiox9090784.

[18] Center For Disease Control And Prevention, Interim infection prevention and control recommendations for patients with known or patients under investigation for 2019 novel coronavirus (2019-nCoV) in a healthcare setting, 2020. Disponível em, https://www.cdc.gov/coronavirus/2019-ncov/hcp/infection-control.html. Acess in: dez. 2020.

[19] B.F. Chantal, A.E. Vogels, C.A. Watkins, D.E. Harden, J.S. Brackney, W. Jianhui, et al., SalivaDirect: a simplified and flexible platform toenhance SARS-CoV-2 testing capacity. medRxiv [Preprint]. this version posted September 28, 2020, https://doi.org/10.1101/2020.08.03.20167791.

[20] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K.W. Chu, T. Bleicker, S. Brünink, J. Schneider, et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Euro Surveill. 25 (3) (2020 Jan 23) 2000045, https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

[21] O. Erel, A new automated colorimetric method for measuring total oxidant status, Clin. Biochem. 38 (12) (2005) 1103–1111, https://doi.org/10.1016/j.clinbiochem.2005.08.008.

[22] O. Erel, A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation, Clin. Biochem. 37 (2004) 277–285.

[23] A. Aycicek, O. Erel, A. Kocyigit, Increased stress in infants exposed to passive smoking, Eur. J. Pediatr. 164 (2005) 775–778.

[24] M.M. Cerveira, H.S. Vianna, E.M.K. Ferrer, B.N. Rosa, C.M.P. Pereira, M. D. Baldissera, L.Q.S. Lopes, V.C. Rech, J.L. Giongo, R.A. Vaucher, Bioprospection of novel synthetic monocurcuminoids: antioxidant, antimicrobial, and in vitro cytotoxic activities, Biomed. Pharmacother. 133 (January 2021) 111052, https://doi.org/10.1016/j.biopha.2020.111052.

[25] W.E. Huang, B. Lim, C.C. Hsu, D. Xiong, W. Wu, Y. Yu, H. Jia, Y. Wang, Y. Zeng, M. Ji, H. Chang, X. Zhang, H. Wang, Z. Cui, RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2, Microb. Biotechnol. 13 (4) (2020 Jul) 950–961, https://doi.org/10.1111/1751-7915.13586.

[26] B. Schermer, F. Fabretti, M. Damagnez, C.V. Di, E. Heger, S. Arjune, N.A. Tanner, T. Imhof, M. Koch, A. Ladha, J. Joung, J.S. Gootenberg, O.O. Abudayyeh, V. Burst, F. Zhang, F. Klein, T. Benzing, R.U. Müller, Rapid test Sars-cov-2 on primary material based on a new multiplex assay RT-LAMP, PloS One 15 (11) (2020), e0238612, https://doi.org/10.1371/journal.pone.0238612, 2.

[27] Y.H. Baek, J. Um, K.J.C. Antigua, J.H. Park, Y. Kim, S. Oh, Y.I. Kim, W.S. Choi, S. G. Kim, J.H. Jeong, B.S. Chin, H.D.G. Nicolas, J.Y. Ahn, K.S. Shin, Y.K. Choi, J. S. Park, M.S. Song, Development of a reverse transcription-loop-mediado amplificação isothermal como um método de detecção precoce rápido para sar romances-cov-2, Micróbios. Emerg. Infectam. 9 (1) (2020) 998–1007, https://doi.org/10.1080/22221751.2020.1756698.32306853.

[28] T.V.L. Dao, K. Herbst, K. Boerner, M. Meurer, L.P. Kremer, D. Kirrmaier, A. Freistaedter, D. Papagiannidis, C. Galmozzi, M.L. Stanifer, S. Boulant, S. Klein, P. Chlanda, D. Khalid, M.I. Barreto, P. Schnitzler, H.G. Kräusslich, M. Knop, S. Anders, A colorimetric RT-LAMP assayand LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples, Sci. Transl. Med. 12 (556) (2020 12), eabc7075, https://doi.org/10.1126/scitranslmed.abc7075.

[29] N. Ben-Assa, R. Naddaf, T. Gefen, T. Capucha, H. Hajjo, N. Mandelbaum, L. Elbaum, P. Rogov, D.A. King, S. Kaplan, A. Rotem, M. Chowers, M. Szwarcwort-Cohen, M. Paul, N. Geva-Zatorsky, Direct on-the-spot detection of SARS-CoV-2 in patients, Exp. Biol. Med. 245 (14) (2020) 1187–1193, https://doi.org/10.1177/1535370220941819.

[30] M. Nagura-Ikeda, K. Imai, S. Tabata, K. Miyoshi, N. Murahara, T. Mizuno, M. Horiuchi, K. Kato, Y. Imoto, M. Iwata, S. Mimura, Ito, K. Tamura, Y. Kato, ClinicalEvaluationof self-collected saliva by Quantitative reverse transcription-PCR (RT-qPCR), direct RT-qPCR, reverse transcription-loop-mediated isothermal amplification, and a rapid antigen test to diagnose COVID-19, J. Clin. Microbiol. 24 (9) (2020) 20–e 01438, https://doi.org/10.1128/JCM.01438-20, 58.

[31] M.J. Johnstone, S. Turale, Nurses' experiences of ethical preparedness for public heal the emergencies and healthcare disasters: a systematic review of qualitative evidence, Nurs. Health Sci. 16 (1) (2014) 67–77, https://doi.org/10.1111/nhs.12130.

[32] J.S. Blackman, A psychoanalytic view of reactions to the coronavírus pandemic in China, J. Psicoanal. (2020), https://doi.org/10.1057/s11231-020-09248-w.

[33] da Silva, F. C.; Neto, M. L. R. Psychological effects caused by the COVID-19 pandemic in health professionals: a systematic review with meta-analysis. Prog. Neuropsychopharmacol. Biol. Psychiatr..2020Jan 10;104:110062. doi: 10.1016/j.pnpbp.2020.110062.

[34] C. Moore, J. e Kolencik, Acute depression, extreme anxiety, and prolonged stress among COVID-19 frontline healthcare workers, Psychosociol. Iss. Hum. Res. Manag. 8 (1) (2020) 55–60, https://doi.org/10.22381/PIHRM8120209.

[35] G. Giacomello, A. Scholten, M.K. Parr, Current methods for stress marker detection in saliva, J. Pharmaceut. Biomed. Anal. 30 (191) (2020) 113604, https://doi.org/10.1016/j.jpba.2020.113604.

[36] G. Russell, S. Lightman, The human stress response, Nat. Rev. Endocrinol. 15 (2019) 525–534, https://doi.org/10.1038/s41574-019-0228-0.

[37] C. Qin, H. Minghan, Z. Ziwen, L. Yukun, Alteration of lipid profile and value of lipids in the prediction of the length of hospital stay in COVID-19 pneumonia patients, Food Sci. Nutr. 27 (11) (2020) 6144–6152, https://doi.org/10.1002/fsn3.1907, 8.

[38] M. Trinder, K.R. Genga, H.J. Kong, L.L. Blauw, C. Lo, X. Li, M. Cirstea, Y. Wang, P. C.N. Rensen, J.A. Russell, K.R. Walley, J.H. Boyd, L.R. Brunham, Cholesteryl Ester Transfer Protein influences high-density lipoprotein levels and survival in sepsis, Am. J. Respir. Crit. Care Med. 1 (7) (2019) 854–862, https://doi.org/10.1164/rccm.201806-1157OC.30321485, 199.

[39] C. Alvarez, A. Ramos, Lipids, lipoproteins and apoproteins in serum during infection, Clin. Chem. 32 (1 Pt 1) (1986) 142–145.

[40] F. Sahin, P. e Yildiz, Distinct biochemical changes in pulmonary tuberculosis and pneumonia, Arch. Med. Sci. 9 (2013) 656–661.

[41] Feingold, K. R. Lipid and lipoprotein levels in patients with COVID-19 infections. In: Feingold KR, Anawalt B, Boyce A, et al., eds. *Endotext*. 2020. South Dartmouth (MA): MDText.com, Inc.; November 15, 2020.

[42] L. Wang, Q. Zhang, X. Zhao, H. Dong, C. Wu, F. Wu, B. Yu, J. Lv, S. Zhang, G. Wu, S. Wu, X. Wang, Y. Wu, Y. Zhong, Low level of high density lipoprotein correlates with the severity of COVID-19 patients: an observational study, Lipídios Saúde Dis. 19 (1) (2020 7) 204, https://doi.org/10.1186/s12944-020-01382-9.32892746.

[43] W.E. Huang, B. Lim, C.C. Hsu, D. Xiong, W. Wu, Y. Yu, H. Jia, Y. Wang, Y. Zeng, M. Ji, H. Chang, X. Zhang, H. Wang, Z. Cui, RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2, Microb. Biotechnol. 13 (4) (2020 Jul) 950–961, https://doi.org/10.1111/1751-7915.13586.

[44] W. Guan, Z. Ni, Y. Yu Hu, M. Hu, W. Liang, C. Ou, J. He, L. Liu, H. Shan, C. Lei, D. Hui, Bin Du, M. Lan-Juan, G. Zeng, K. Yuen, R. Chen, T. Wang, P. Chen, J. Xiang, S. Li, J. Wang, Z. Liang, Y. Peng, L. Wei, Clinical features of coronavirus disease 2019 in China, New England J. (2020), https://doi.org/10.1056/NEJMoa2002032.

[45] Z. Chen, Jijia Hu, Z. Zhang, J. Shan, S. Han, D. Yan, R. Zhuang, B. Hu, H. Zhang, Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial, N. Engl. J. Med. 382 (2020) 1708–1720, https://doi.org/10.1056/NEJMoa2002032, 2020.

[46] C.J. Pirola, S. eSookoian, SARS-CoV-2 virus and liver expression of host receptors: putative mechanisms of liver involvement in COVID-19, Liver Int. 40 (8) (2020) 2038–2040, https://doi.org/10.1111/liv.14500.

[47] T. Shao, Y. Tong, S. Lu, A.J. Jeyarajan, F. Su, J. Dai, J. Shi, J. Huang, C. Hu, L. Wu, X. Dai, Z. Cheng, J. Yan, P. Huang, Tian, S. Li, R.T. Chung, D. Chen, γ-GlutamyltransferaseElevations are frequent in patients with COVID-19: a clinical epidemiologic study, Hepatol. Commun. 11 (12) (2020) 1744–1750, https://doi.org/10.1002/hep4.1576, 4.

[48] J. Sun, X. Deng, X. Chen, J. Huang, S. Huang, Y. Li, J. Feng, J. Liu, G. He, Incidence of adverse drug reactions in COVID-19 Patients in China: an active monitoring study by hospital pharmacovigilance System, Clin. Pharmacol. Ther. 108 (4) (2020) 791–797, https://doi.org/10.1002/cpt.1866.

[49] M.A. Hundt, Y. Deng, M.M. Ciarleglio, M.H. Nathanson, J.K. Lim, Abnormal liver tests in COVID-19: a retrospective observational cohort study of 1,827 patients in a major US hospital network, Hepatology 72 (4) (2020) 1169–1176, https://doi.org/10.1002/hep.31487.32725890.

[50] M. Parohan, S. Yaghoubi, A. Seraji, Liver injury is associated with severe coronavírus disease 2019 (COVID-19) infection: a systematic review and meta-analysis of retrospective studies, Hepatol. Res. 50 (8) (2020) 924–935, https://doi.org/10.1111/hepr.13510.

[51] X. Hu, D. Chen, L. Wu, G. He, W. Ye, High-density lipoprotein cholesterol is associated with the severity of COVID-19 infection, Clin. Chim. Acta 510 (2020) 105–110, https://doi.org/10.1016/j.cca.2020.07.015.

[52] R.J. Scalsky, K. Desai, Y.J. Chen, J.R. O'Connell, J.A. Perry, C.C. Hong, Baseline and risk SARS-CoV-2 cardiometabolic profiles in the UK Biobank, medRxiv (2020), https://doi.org/10.1101/2020.07.25.20161091.

[53] T. Li, H. Lu, W. Zhang, Clinical observation and treatment of patients with COVID -19, Emerg. Microb. Infect. 1 (2020) 687–690.

[54] J.J. Zhang, X. Dong, Y.Y. Cao, Y.D. Yuan, Y.B. Yang, Y.Q. Yan, C.A. Akdis, Y. D. Gao, Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China, Allergy 75 (7) (2020) 1730–1741, https://doi.org/10.1111/all.14238.

[55] C. Wu, X. Chen, Y. Cai, Risk factors associated with acute respiratory discomfort syndrome and death in patients with coronavirus disease 2019 Pneumonia in Wuhan, China, JAMA Intern. Med. 180 (7) (2020) 934–943, https://doi.org/10.1001/jamainternmed.2020.0994.

[56] A.M.D. Erez, S. Tal, M.D. Assaf, M. Perl-Laufer, Z. Joseph, O. Shental, H. Gur-Guzner, Diagnostic and prognostic value of very high sum lactate dehydrogenase in hospitalized medical patients, Isr. Med. Assoc. J. 16 (2014) 439–443.

[57] G. Lippi, M. Plebani, B.M. Henry, Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis, Clin. Chim. Acta 506 (2020) 145–148, https://doi.org/10.1016/j.cca.2020.03.022.

[58] B.M. Bakadia, B.O.O. Boni, A.A.Q. Ahmed, G. Yang, The impact of damage to oxidative stress induced by environmental stressors in COVID-19, Life Sci. 926 (2020) 264–118653, https://doi.org/10.1016/j.lfs.2020.118653.

[59] S. Suhail, J. Zajac, C. Fossum, H. Lowater, C. McCracken, N. Severson, B. Laatsch, A. Narkiewicz-Jodko, B. Johnson, J. Liebau, S. Bhattacharyya, S. Hati, Role of oxidative stress on SARS-CoV (SARS) e SARS-CoV-2 (COVID-19) infection: a review, Protein J. 39 (6) (2020) 644–656, https://doi.org/10.1007/s10930-020-09935-8.

[60] Delgado-Roche, L.; Mesta, F. Oxidative stress as key player in severe acute respiratory syndrome coronavirus (SARS-CoV) *Infection*. Arch. Med. Res.. 2020 (5): 384-387. doi: 10.1016/j.arcmed.2020.04.019.

[61] M.M. Tarpey, D.A. Wink, M.B. Grisham, Methods for detection of reactive metabolites of oxygen and nitrogen: *in vitro* and *in vivo* consideration, Am. J. Physiol. 286 (2004) R431–R444.

[63] C. Varim, Neutrophil count to albumin ratio como um novo preditor de mortalidade em pacientes com COVID-19 ınfection, Rev. Assoc. Med. Bras. 2 (Suppl 2) (2020) 77–81, https://doi.org/10.1590/1806-9282.66.S2.77.32965361, 66Suppl.

[64] A.S. Zerghan, U. Achaarya, Cyclic neutropenia, in: Stat Pearls Treasure Island (FL), Stat Pearls Publishing; 2020, 2020, 32491328.de 2020.

[65] H. Xu, L. Zhong, J. Deng, J. Peng, H. Dan, X. Zeng, T. Li, Q. Chen, High expression of the 2019 -nCoV ACE2 receptor on oral mucosa epithelial cells, Int. J. Oral Sci. 12 (1) (2020) 8, https://doi.org/10.1038/s41368-020-0074-x.

[66] S. Aggarwal, S. Gollapudi, S. Gupta, Increased TNF-alpha-induced apoptosis induced by lymphocytes from elderly humans: changes in the expression of the TNF-alpha receptor and activation of caspases, J. Imunol. 162 (4) (1999) 2154–2161.

[67] S. Singh, A. Sharma, S.K. e Arora, High-producing haplotype (CAG) of the -863C/A, −308G/A and -238G/A polymorphisms in the TNF - alpha gene promoting region associated with increased lymphocyte apoptosis in HIV-infected individuals - 1 subtype C in northern India, PloS One 9 (5) (2014), e98020.

[68] J.F. Chan, A.J. Zhang, S. Yuan, V.K. Poon, C.C. Chan, A.C. Lee, W.M. Chan, Z. Fan, H.W. Tsoi, L. Wen, R. Liang, J. Cao, Y. Chen, K. Tang, C. Luo, J.P. Cai, K.H. Kok, H. Chu, K.H. Chan, S. Sridhar, Z. Chen, H. Chen, K.K. To, K.Y. Yuen, Simulation of the clinical and pathological manifestations of coronavírus disease 2019 (COVID-19) in a golden syrian hamster model: implications for disease pathogenesis and transmissibility, Clin. Infect. Dis. 71 (9) (2020) 2428–2446, https://doi.org/10.1093/cid/ciaa325, 3.

[69] U. Akhmierov, E. e Marban, COVID-19 and the heart, Circ. Res. 126 (10) (2020), https://doi.org/10.1161/CIRCRESAHA.120.317055. Epub 2020 Apr 7.

[70] S. He, S. Tang, L. e Rong, A discrete stochastic model of the COVID-19 outbreak: forecast and control, Math. Biosci. Eng. 17 (4) (2020) 2792–2804, https://doi.org/10.3934/mbe.2020153.

[71] J. Zhou, H. Chu, C. Li, B.H. Wong, Z.S. Cheng, V.K. Poon, T. Sun, C.C. Lau, K. K. Wong, J.Y. Chan, J.F. Chan, K.K. To, K.H. Chan, B.J. Zheng, K.Y. Yuen, Active replication of Middle East respiratory syndrome coronavirus and aberrant induction of inflammatory cytokines and chemokines in human macrophages: implications for pathogenesis, J. Infect. Dis. 1 (9) (2014) 1331–1342, https://doi.org/10.1093/infdis/jit504, 209.

[72] D. Khurana, S.A. Deoke, Thrombocytopenia in critically patients: clinical and laboratorial behavior and its correlation with short-term outcome during hospitalization, Indian J. Crit. Care Med. 21 (12) (2017) 861–864, https://doi.org/10.4103/ijccm.IJCCM_279_17.

[73] A. Ganji, I. Farahani, B. Khansarinejad, A. Ghazavi, G. Mosayebi, Increase dexpression of CD8 marker on T-cells in COVID-19 patients, Blood Cells Mol. Dis. 83 (2020) 102437, https://doi.org/10.1016/j.bcmd.2020.102437.