Discovering Associations between Clinical Parameters and Ex-Haled Air Spectral Characteristics of COVID-19 Patients

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Abstract. The associations of the exhaled air IR absorption spectra with clinical parameters characterising the state of COVID-19 patients were studied at the time of their hospitalization. The clinical parameters included standard blood tests characteristics and the presence of comorbidities. The exhaled air IR absorption spectra were measured by laser photo-acoustic gas-analyzer based on a tunable CO_2 laser. The measured absorption spectra analysis was based on their splitting on subgroups in dependence on clinical parameters values and the further comparison of these subgroups with the same for healthy participants using principal component analysis. In the result, informative clinical parameters, which allowed distinguishing exhaled air absorption spectra from COVID-19 patients and healthy participants, were established. Revealed correspondence of changes in exhaled air spectral characteristics of COVID-19 patients and clinical parameters demonstrates that pathological processes occurring in the body of COVID-19 patients and success of their therapy can be revealed not only by the results of laboratory tests but also by the exhaled air spectral analysis.

Keywords: COVID-19 disease; absorption spectra of exhaled air; laser photo-acoustic spectroscopy; volatile organic compounds; principal component analysis.

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1 Introduction

The problem of COVID-19 disease and its complications is still urgent. Computed tomography (CT) has emerged as a primary diagnostic tool for COVID-19. However, the X-ray images often resemble those of other lung diseases (such as bacterial pneumonia, tuberculosis, influenza), and these findings typically appear after the onset of major symptoms. The key challenge for doctors is early disease identification to prevent complications, ideally before significant symptoms develop [1]. Therefore, the standard methods for detecting COVID-19 have become nucleic acid amplification tests: reverse transcription polymerase chain reaction (RT-PCR), rapid RT-PCR (rRT-PCR), reverse transcription-coupled loop-mediated isothermal Amplification (RT-Lamp), pooled RT- PCR; next generation sequencing (NGS); antigen tests; antibody tests. These diagnostic methods are time consuming, need reagents for test-systems, high-tech

equipment and room appropriating the virus-level safety requirements [2–6].

Additionally, spectroscopic methods for detecting COVID-19 (such as Raman and infrared spectroscopy, fluorescence techniques) have been investigated. Collecting biological samples (saliva, plasma, nasopharyngeal swabs, fecal samples, etc.) for these studies requires invasive procedures performed by qualified personnel. Furthermore, the analysis often necessitates pre-treatment and can pose safety risks [7].

Typically, the virus enters through the epithelium of the upper respiratory tract, followed by the activation of pathophysiological processes in cells and corresponding immune response. As a consequence, the products of varying metabolism appear in tissue, blood, lymph and other biofluids [8−12]. It is a base of using routine blood tests for COVID-19 diagnostics and success of its therapy. The level of lymphocytes, leukocytes, mean corpuscular hemoglobin, basophils, eosinophils, C-reactive protein, total bilirubin, D-dimer in the blood can be used as the COVID-19 biomarkers [8, 9]. However, blood tests are invasive and time consuming.

COVID-19 volatile molecular biomarkers are also products of shifted metabolism. They can excrete from the body through the lungs to exhaled air [12−14]. Gas chromatography and mass spectrometry have emerged as effective tools for analyzing exhaled breath. Studies have identified several volatile organic compounds (VOCs) as potential markers for COVID-19. For example, the COVID-19 acute phase is characterized by the following volatile biomarkers measured in the exhaled air: 2,3-butanedione, aldehyde, 2, 8-dimethylundecane, n-propyl acetate; ethanal, 2-butanone, methanol, octanal, isoprene, heptanal, propanal, propane; methylpent-2-enal, 2,4-octadiene 1-chloroheptane, nonanal; butanoate, butyraldehyde, isopropanol; alcohol, acetone, carbon monoxide and octanal, nonanal, heptanal, decane, tridecane and 2-pentylfuran [13]. Four specific VOCs (methylpent-2-enal, 2,4-octadiene 1-chloroheptane, and nonanal) showed promise as COVID-19 biomarkers, demonstrating a sensitivity (Se) of 90% and a specificity (Sp) of 94% in distinguishing COVID-19 patients from ARDS patients [15] In Ref. [16], the following volatile organic compounds (VOCs) allowed differentiating COVID-19 patients from healthy humans with accuracy of 81.2%: nitric oxide, butene, methanethiol, acetaldehyde, heptanal, ethanol, methanol-water cluster and propionic acid. An increase in the content of ethyl butanoate in exhaled air is also a feature of COVID-19 [17]. Seven VOCs (benzaldehyde, 1-propanol, 3,6-methylundecane, camphene, beta-cubebene, iodobenzene, and an unidentified compound) allowed detecting PCR-positive COVID-19 patients with the following classification efficiency metrics: 0.84 area under the curve (AUC), 68% Se, 85% Sp [18].

In addition to VOCs, viral RNA can also be detected in exhaled breath [19]. However, the presence of viral RNA in exhaled air has not been conclusively linked to viral load [15, 19]. Therefore, individuals who have previously had COVID-19 or are asymptomatic carriers may be identified as false positive due to the presence of viral RNA in their exhaled breath.

The advantages of detection of COVID-19 presence or its complications through control of specific VOC profile are noninvasiveness and operativity. However, similar COVID-19 molecular biomarker patterns are highly variable [20–22]. For example, acetaldehyde considered as a potential COVID-19 feature [16] has high concentrations in other infection diseases [23]. For better validation of COVID-19 breath test, a comparative study of blood and exhaled air parameters for COVID-19 patients is useful.

Among acceptable tools for breath tests, laser photo-acoustic spectroscopy (LPAS) looks attractive because it does not require frequent calibration, is less costly, and the short measurement time [24–27]. LPAS is based on the registration by a microphone of an acoustic wave appearing in a measurement cell with a studied gas sample when an amplitude-modulated laser radiation is absorbed by the sample.

The work aims to establish correlations between spectral characteristics of exhaled air measured with LPAS and blood parameters for COVID-19 patients.

The study considered 2 hypotheses: (i) SARS-CoV-2 causes changes the molecular composition of blood and exhaled air through metabolic pathways, which are partly overlapped, (ii) presence of comorbidity, observed respiratory failure and severity of the condition at the time of hospitalization of COVID-19 patients influences on the molecular composition of blood and exhaled air.

2 Materials and Methods

Initially, the study involved 35 COVID-19 patients. The diagnosis was confirmed by PCR positive test result. Five patients were excluded from the study due to the lack of completeness of clinical data. Thus, the target group consisted of 30 COVID-19 patients. The control group was recruited in equal numbers (30 participants). Inclusion criteria for the study were the following: verified SARS-CoV-2, age 18 years or older. Exclusion criteria for the control group were: age under 18 years, presence of respiratory symptoms, lung and respiratory diseases at the time of the study. The study was conducted in accordance with the principles of the Declaration of Helsinki, with the Nuremberg Code, legislative of the Russian Federation and approved by the Ethics Committee of Tomsk State University (registration No. 5, November 19, 2020). All participants signed an informed consent for participation in the study.

The average age of the target group was 65.5 (60.3, 72.5) years, the control group – 52.0 (30.0, 60.0) years. The patient's condition was assessed by the attending physician according to the set of observed parameters included in the database: demographic characteristics − age; clinical − presence of concomitant diseases (diabetes (D), myocardial damage (MD), hypertonic disease (HD)), the presence and level of respiratory failure (RF: I, II), the results of general blood analysis (erythrocyte sedimentation rate (ESR), color index (CI), red blood cells (RBC), haemoglobin (HGB), white blood

cells (WBC), lymphocytes (LYM), monocytes (MONO), neutrophils (NEU), platelets (PLT)) and biochemical blood tests (total bilirubin (TBIL), total protein (TP), glucose (GLU), fibrinogen (FG), alanine transaminase (ALT), aspartate transaminase (AST), urea (UREA), creatinine (CREAT)), as coagulation analysis (prothrombin time (PT), activated partial thromboplastin time (aTTP), international normalised ratio (INR), prothrombin index (PI)). A description of the blood characteristics used in the study is presented in Table S1. Reference values of blood parameters are shown in Table 1.

Among hospitalized COVID-19 patients, the ratio of mild to severe coronavirus infection was 15/15, and no moderate severity was observed. COVID-19 disease severity and outcome are known to depend on many factors: age [28, 29], viral load [30], socioeconomic status [31], but an important one is the presence of concomitant diseases [32, 33]. The concomitant pathologies in the target group were as follows: solely diabetes was observed in 1 case, 4 patients had comorbid conditions with myocardial damage and hypertension (Fig. 1). In turn, hypertension was recorded in 12 patients, among whom myocardial lesions occurred in 6 cases. Artificial lung ventilation was not required as part of respiratory support because the 14 patients had respiratory failure I, II degree, the rest had no respiratory failure.

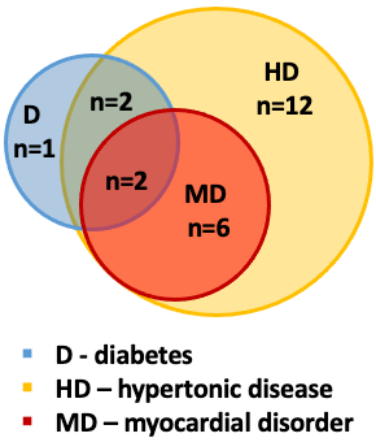


Fig. 1 Comorbidities in COVID-19 patients(D − diabetes, HD − hypertonic disease, MD − myocardial damage).

A breath test was conducted in the morning on an empty stomach in both groups. Before the breath sampling, multiple mouth rinses with water were performed. The first 100−250 ml portion of breath was quietly exhaled into the surrounding space, and the rest one was collected into a disposable 1000 ml container (Fig. 2). The safety of breath samples collection from COVID-19 patients was provided as follows. The medical staff used special personal protective equipment in the "red zone". A special antibacterial-viral electrostatic filter with a gas sampling port (model 4333/711BSSA, China) was attached to the input tube of the container. This filter provides protection against 99.999% of pathogens, including SARS-CoV-2. After breath sample collection the filter was rejected and input tube closed. After that the filter was utilized according to the "red zone" safety rules. The container with a probe was disinfected using an antiseptic liquid (95% ethyl and 95% isopropyl alcohols with equal proportion) and moved to a "green zone". Breath sample absorption spectra were measured in 4 h after its collection. The laser photo-acoustic gas-analyzer "LaserGasAnalyser-3" (LGA-3, Novosibirsk, Russian Federation) with CO_2 laser tunable in the 9.2−10.6 μm spectral range was used. The block-scheme of LGA-3 is shown in Fig. 3 [34].

Fig. 2 Assembled breath sampler.

Three spectral scans for every probe were recorded. Thus, 90 absorption spectra for each group of participants were measured. Venous blood sampling was carried out in the morning on an empty stomach in 3 vacutainers in the following volume according to the National Standard of the Russian Federation: 4−5 ml for biochemical analysis, 2−3 ml for general analysis, 2−3 ml for coagulation study. The study of blood samples was carried out within 4 h after collection according to the storage standards.

In the data analysis, the Shapiro-Wilk criterion, the principal component analysis, the calculation of pairwise distances between points in the space of principal components using Euclidian metric were applied.

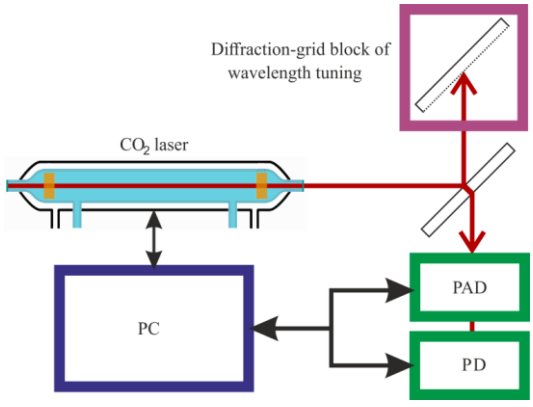


Fig. 3 The block-scheme of LGA-3: PAD is a photo-acoustic detector, PD is a pyrodetector, PC is a personal computer.

Table 1 The intervals of quantitative clinical parameter values corresponding to the "normal" human state [35, 36].

Blood value	**ALT (U/l)**	**aPTT (s)**	**AST (U/l)**	**CI (Units of account)**	**CREAT (μmol/l)**	**ESR (mm/h)**	**FG (g/l)**
Limit of the norm	31.0-45.0	21.0-36.0	31.0-45.0	0.85-1.15	53-115	1.0-30.0	2.0-4.0
Blood value	**GLU (mmol/l)**	**HGB (g/dL)**	**INR**	**LYM (10^3/μL)**	**MONO (10^3/μL)**	**NEU (10^3/μL)**	**PI (%)**
Limit of the norm	3.3-5.5	120.0-160.0	0.8-1.2	25.0-35.0	2.0-6.0	47.0-67.0	80-110
Blood value	**PLT (10^3/μL)**	**PT (s)**	**RBC (10^6/μL)**	**TBIL (μmol/l)**	**TP (g/l)**	**UREA (mmol/l)**	**WBC (10^3/μL)**
Limit of the norm	180.0-320.0	11.0-16.0	3.7-5.5	7.9-19.9	65.0-85.0	2.6-7.3	4.0-9.0

3 Results

COVID-19 patients were split on subgroups according to the following criteria:

− the blood characteristic values relatively to the reference intervals presented in Table 1: "below normal", and "above normal" (the state "normal" was absent for COVID-19 patients);

− "presence" or "absence" of concomitant disease (D, HD, MD);

− "mild" or "severe" severity;

− "absence" or "I", "II" degree of respiratory failure.

After that, a EAS absorption spectrum of a definite COVID-19 patient was labeled depending on a definite clinical parameter and its gradation. Measured absorption spectra of the exhaled air samples (EASs) from the target and control groups are shown in Fig. S1 (see Supplementary File). The analysis of the relation between two sets of EAS absorption spectra and clinical parameters was conducted in the principal component (PC) space [37–39]. The first three PCs were taken into account because they provided 98% of explained variance of absorption spectra dataset (Fig. S2, Supplementary File). The example of similar COVID-19 patients EAS absorption spectra splitting on three subgroups in dependence on the ALT parameter grade in PC space is shown in Fig. 4.

Euclidean metric was used in PC space for quantitative assessing the relation between the EAS absorption spectra and clinical parameters:

$$d_{\alpha c} = \sum_{i=1}^{3} \sqrt{(PC_i^{\alpha} - PC_i^{c})^2}. \qquad (1)$$

Here and below, Greek subscript symbols are used for enumeration of healthy participants, Latin symbols numerate COVID-19 patients. For healthy participants, intragroup pair distances $d_{\gamma\mu}$ ($\gamma \neq \mu$) for every clinical parameter were calculated in the same manner as in Eq. (1). Then, the probability distribution f of $d_{\alpha\beta}$ values ($\alpha \neq \beta$) was calculated. The same was done for $d_{\alpha c}$values. Probability distributions f for the intragroup pair distances $d_{\alpha\beta}$ and intergroup pair distances d_αc were approximated by Gaussian curves corresponding to the normal distribution. For all cases, the deviations of the probability distributions from the Gaussian curve were less than 5% (see Table S2). The protocol of data processing is shown in Fig. 5. The probability distributions f for the intragroup pair distances and intergroup pair distances are shown in Figs. 6–10. For low occurrence of the studied trait in the subgroup (number of measurements was less than 9), the Gaussian-shape probability distribution curve cannot be constructed. It was the case of RBC, PLT, TP, WBC – "value exceeding normal", PI – "normal", INR, MONO, ALT, AST, UREA, CREAT, TBIL – "below normal". These data are not considered below.

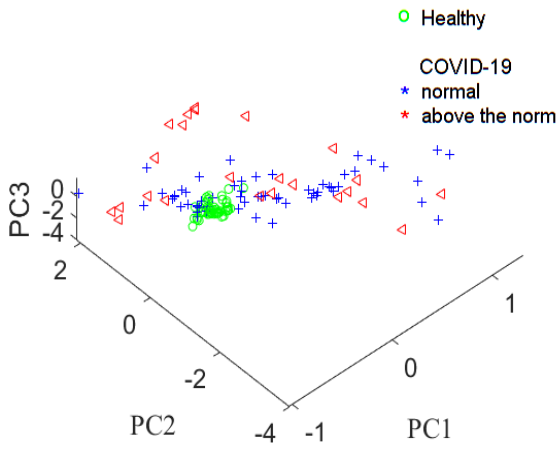
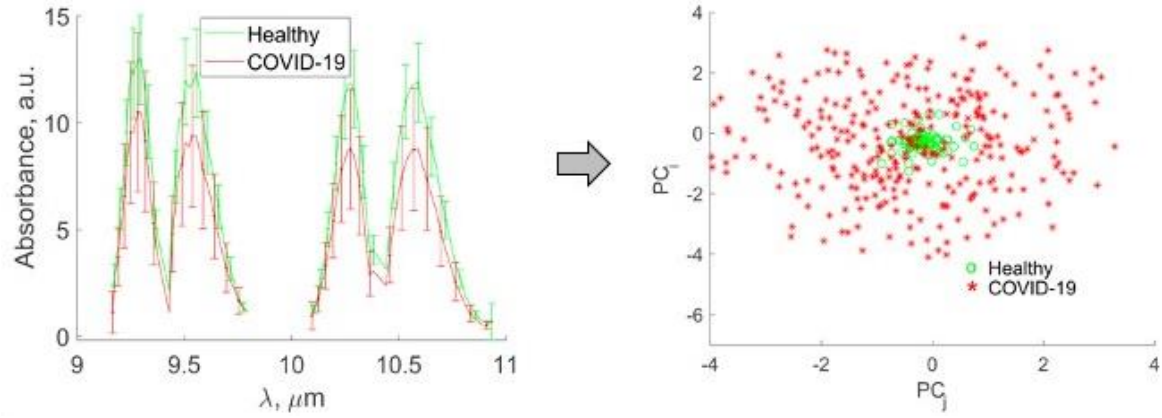


Fig. 4 The example of the COVID-19 patients EAS absorption spectra split on three subgroups in dependence on the ALT parameter grade.

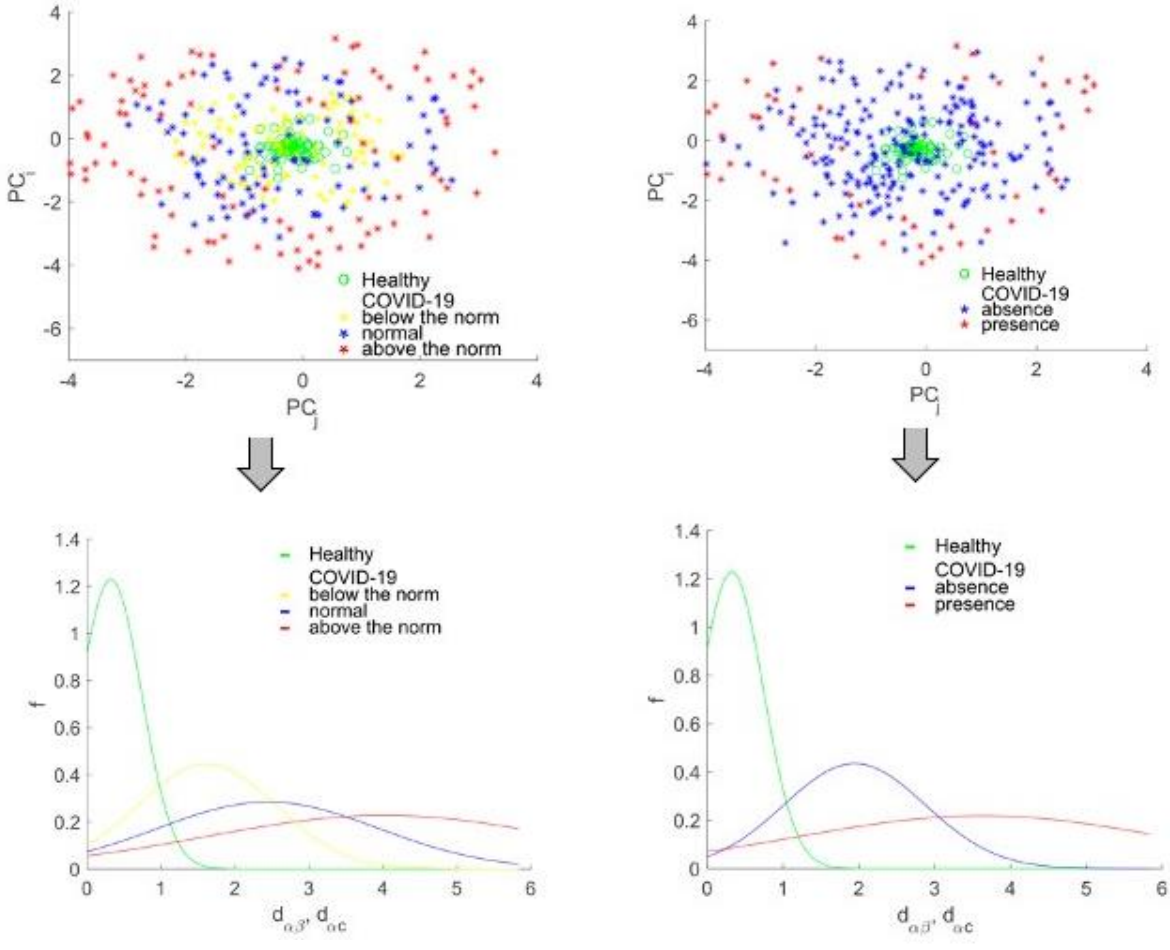


(a)

Dividing patients into subgroups

*COVID-19				
A quantitative parameter			A qualitative parameter	
*below the norm	*normal	*above the norm	*absence	*presence

(b)



(c) (d)

Fig. 5 The protocol of data processing. (a) EAS absorption spectra. EAS absorption spectra in PC space. (b) Classification of indicators characterizing the condition of patients with COVID-19. (c) EAS absorption spectra in PC space (quantitative clinical parameters).Probability distributions for the intragroup and intergroup pair distances. (d) EAS absorption spectra in PC space (qualitative clinical parameters). Probability distributions for the intragroup and intergroup pair distances.

For a quantitative description of the constructed probability distributions, the following characteristics were introduced (Fig. 11):

(i) mode (Mo) - the most frequently encountered parameter value;

(ii) S - the area under the distribution curve for data from COVID-19 patients;

(iii) Sr − the area under the curve, which characterizes the proportion of data from COVID-19 patients, which are not overlapped with data from healthy participants;

(iv) (S-Sr) − the area under the curves, which characterizes the proportion of data from COVID-19 patients, which are overlapped with data from healthy participants;

(v) Sr/S − the parameter which characterizes the separability of COVID-19 patients from healthy participants.

The relations among the EAS absorption spectrum shape and the presence of a comorbidity, the variability of blood characteristics relatively the norm are shown in Table 2.

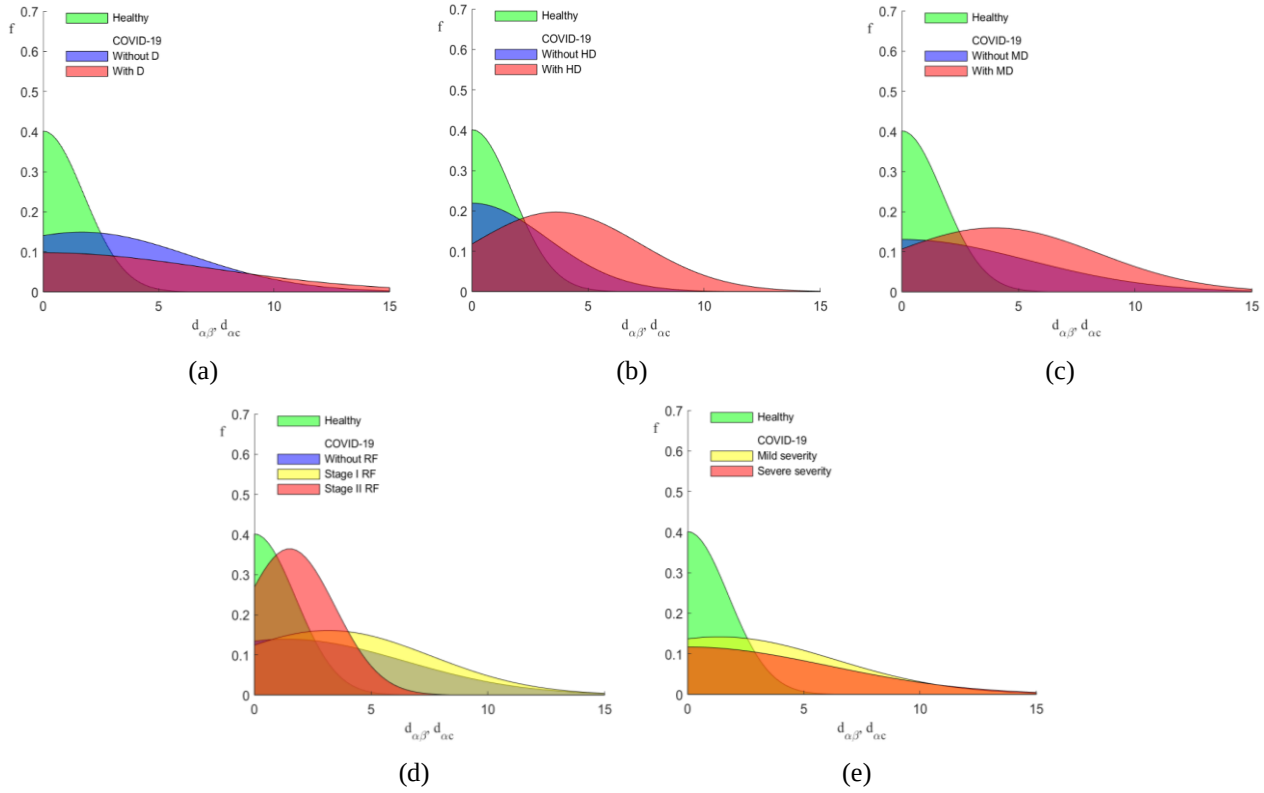


Fig. 6 Probability distributions of pairwise distances between points in the principal components space for the following complications presence: (a) diabetes (D), (b) hypertonic disease (HD), (c) myocardial damage (MD), (d) respiratory failure (RF), (e) severity.

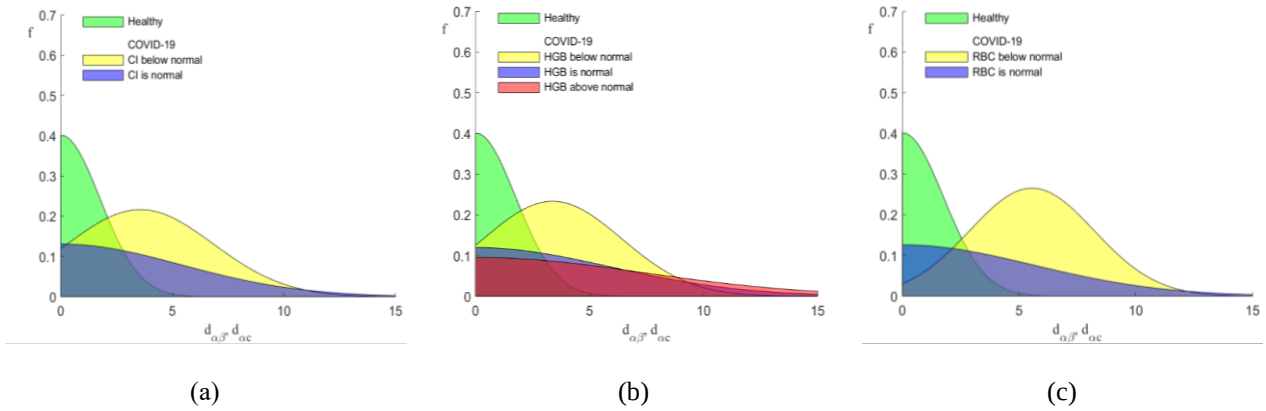


Fig. 7 Probability distributions of pairwise distances between points in the principal components space for the following clinical parameters: (a) color index (CI), (b) hemoglobin (HGB), (c) red blood cells (RBC).

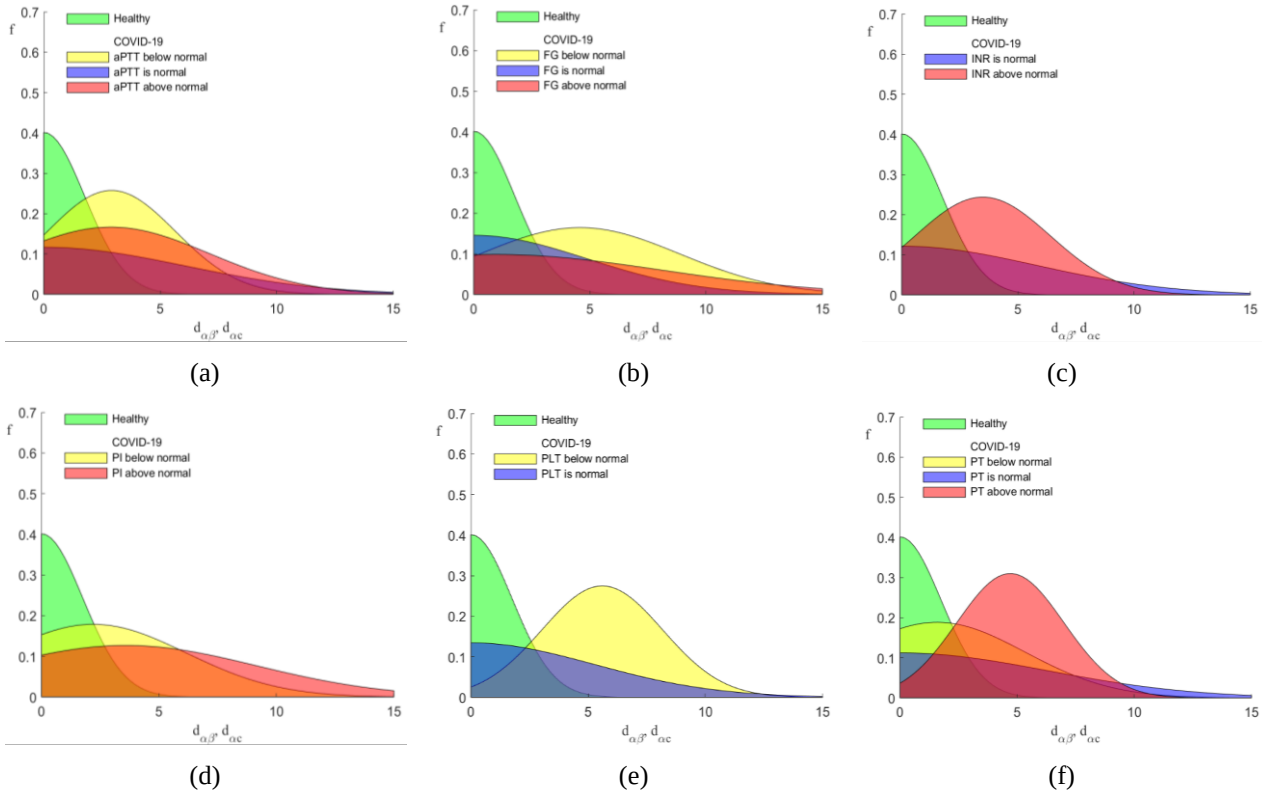


Fig. 8 Probability distributions of pairwise distances between points in the principal components space for the following parameters: (a) activated partial thromboplastin time (aPTT), (b) fibrinogen (FG), (c) international normalized ratio (INR), (d) prothrombin index (PI), (e) platelets (PLT), (f) prothrombin time (PT).

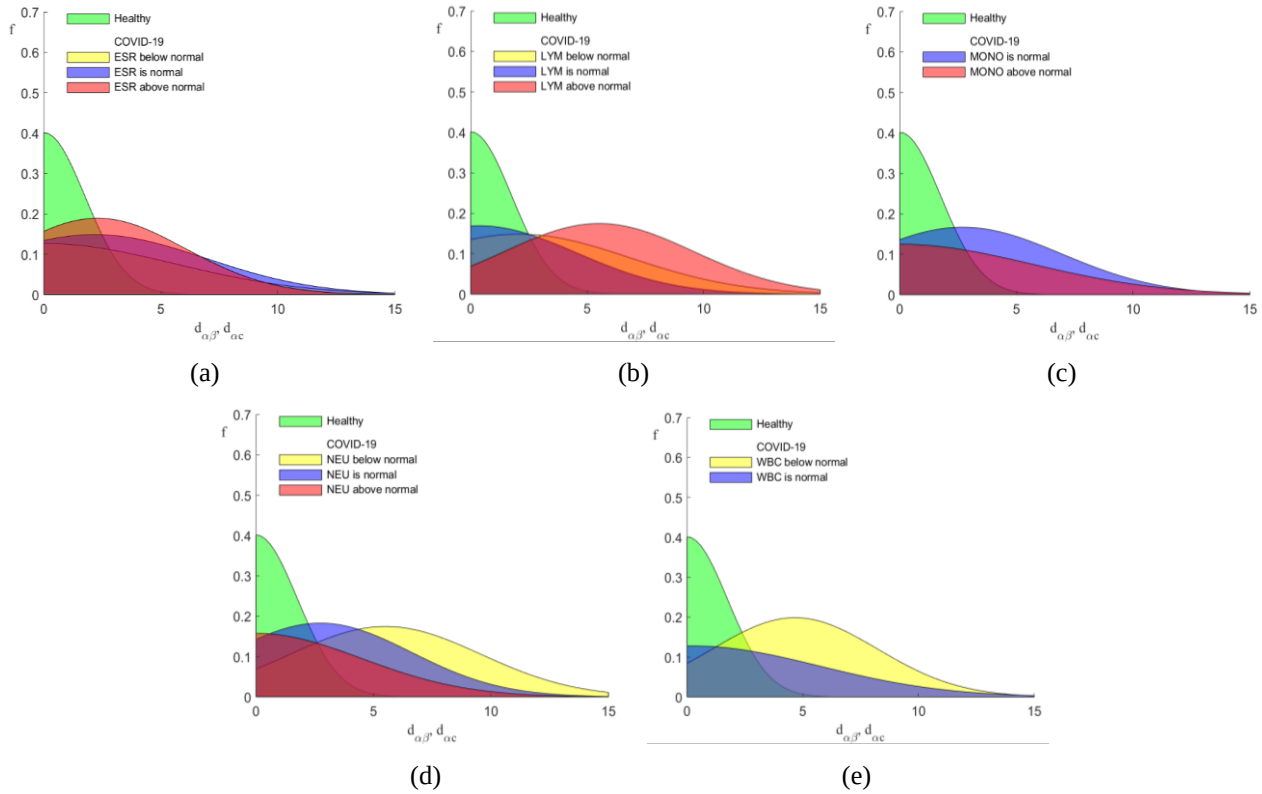


Fig. 9 Probability distributions of pairwise distances between points in the principal components space for the following parameters: (a) erythrocyte sedimentation rate (ESR), (b) lymphocytes (LYM), (c) monocytes (MONO), (d) neutrophils (NEU), (e) white blood cells (WBC).

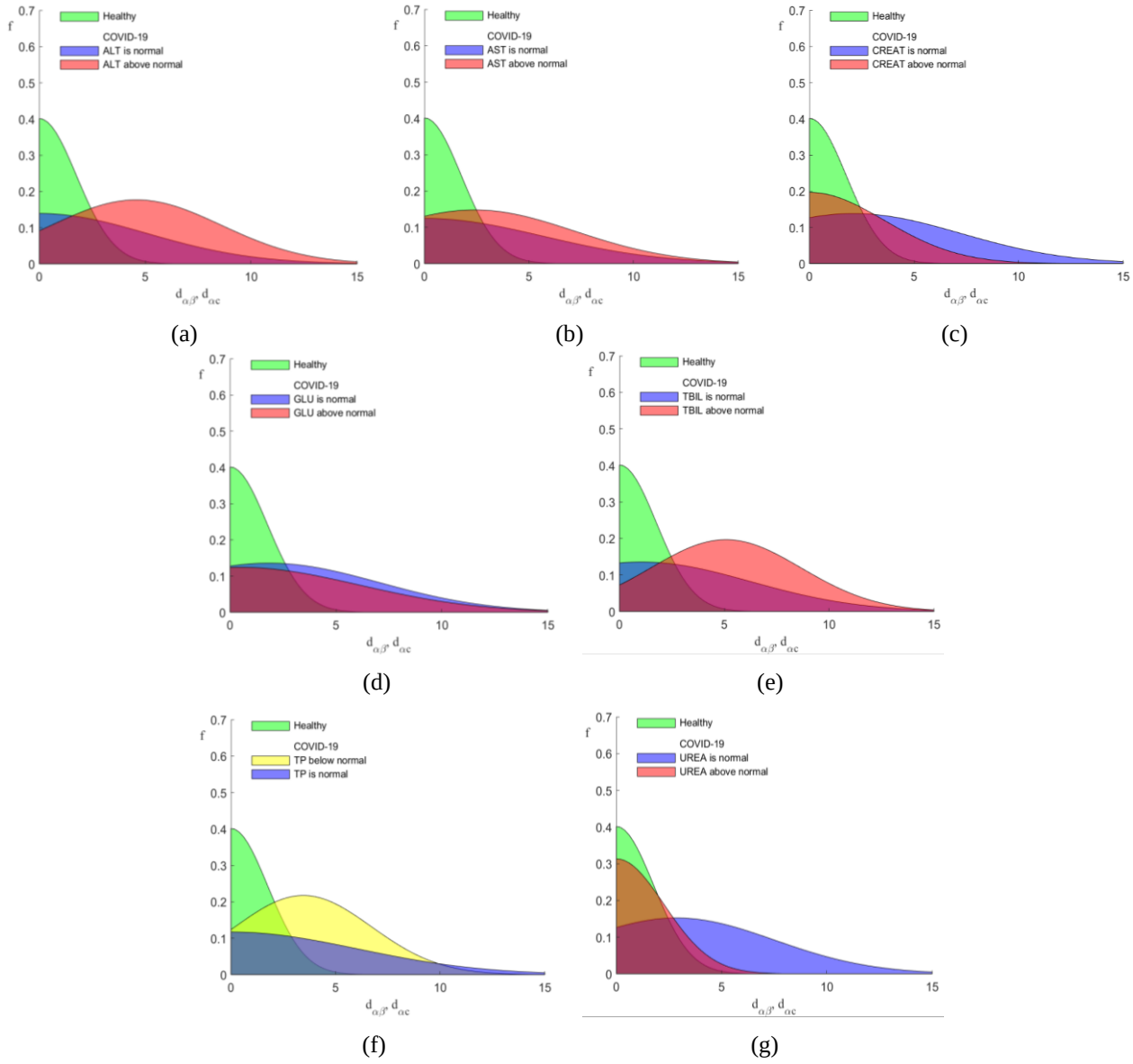


Fig. 10. Probability distributions of pairwise distances between points in the principal components space for the following parameters: (a) alanine transaminase (ALT), (b) aspartate transaminase (AST), (c) creatinine (CREAT), (d) glucose (GLU), (e) total bilirubin (TBIL), (f) total protein (TP), (g) urea (UREA).

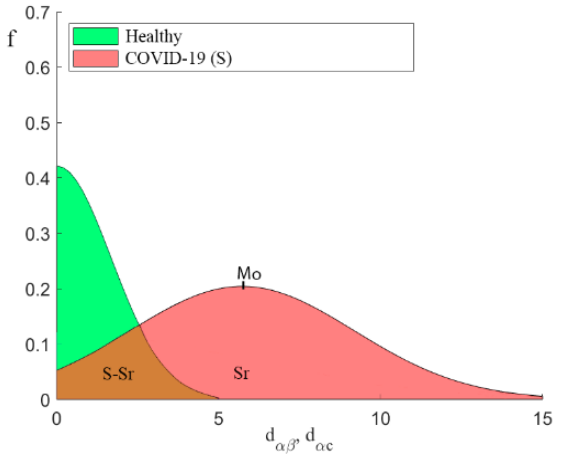


Fig. 11 Introduction of new quantitative characteristics to describe the distribution.

The following characteristics turned out to be informative: PI, PT and INR, FG. Definitely, there are associations of the EAS absorption spectra with below norm values of TP, RBC, CI, HGB and high levels of ALT, AST.

4 Discussion

The associations of the EAS absorption spectra with RBC, CI, and HGB values below norm. Indicated the presence of hypoxia in COVID-19 patients in the result of microvascular lesions of the lungs and micro-hemorrhages [9, 10, 12, 16, 20, 21]. The presence of hypertension or myocardial damage causes change in the EAS composition. Such cardiovascular disorders exacerbate the severity of the patient's condition and increase the likelihood of an unfavorable outcome [31–33, 70, 74].

Table 2 The associations of the EAS absorption spectra with the blood parameters in COVID-19 patients.

Value	Mo	Sr/S	Blood parameters associated with COVID-19 disease	References
			ALT	
Below normal	–	–	It was not typical.	Not found in the literature
Above normal	4.60	0.71	There is a direct correlation of this parameter with COVID-19 disease severity. Is used to assess target organs (liver, heart, nervous tissue, skeletal muscles).	[8, 40–45]
			AST	
Below normal	–	–	It was not typical.	Not found in the literature
Above normal	2.40	0.59	There is direct correlation of this parameter with COVID-19 disease severity, is used to assess target organs (liver, kidneys, heart, skeletal muscles, pancreas).	[8, 41, 44, 45]
			CI	
Below normal	2.80	0.55	It is observed with hemolytic anemia, there is a risk of poor outcome.	[46, 47]
Above normal	–	–	It was not typical.	Not found in the literature
			FG	
Below normal	4.60	0.70	It is less common, observed due to consumption of coagulopathy.	[48–51]
Above normal	1.00	0.60	It is observed more frequently, is a consequence of ARDS and venous thromboembolism. There is a correlation of this parameter with disease severity.	[52–54]
			HGB	
Below normal	3.40	0.58	It is observed with anemia, there is a correlation of this parameter with high risk of onset of adverse outcome.	[46, 55, 56]
Above normal	0.00	0.57	Hemolytic anemia is rare in COVID-19.	[57, 58]
			INR	
Below normal	-	-	It is observed less frequently, indicates the presence of bleeding.	[48]
Above normal	3.40	0.64	It is observed more often. There is a correlation of this parameter with the severity of the disease, is related to onset of high adverse outcome risk.	[59]
			PI	
Below normal	2.20	0.55	It was met less frequently and indicates the presence of bleeding.	[60]
Above normal	3.60	0.67	It was met more frequently. Is an indicator of thrombocytopenia consumption. Thrombosis increases the adverse outcome risk onset.	[49, 61]
			PT	
Below normal	1.60	0.50	It was met with thrombosis, indicates the development of ARDS and of a high probability of developing pneumonia. It is an important indicator for assessing the disease severity.	[62–64]
Above normal	4.80	0.77	It was met rarely, indicates the presence of bleeding, increases the adverse outcome risk.	[55, 65]
			RBC	
Below normal	5.60	0.81	It is observed less frequently. Is an indicator of the disease severity, microvascular damage, bleeding.	[49, 52]
Above normal	–	–	It was not typical.	Not found in the literature
			TP	
Below normal	3.40	0.61	Hypoproteinemia may indicate renal, cardiac and hepatic insufficiency, diseases of the pancreas, as well as the presence of a characteristic inflammatory process in the lung tissues.	[66–69]
Above normal	–	–	It was not typical.	Not found in the literature

Table 2 Cont.

			HD	
Presence	3.60	0.65	It was met frequently, is associated with a burdening of the comorbid background, indicates the development of ARDS. There is a correlation with severity of the disease, is a factor of adverse outcome risk.	[70, 71–73]
			MD	
Presence	4.00	0.67	It was met frequently, is associated with a burdening of the comorbid background. There is a correlation with severity of the disease. It is a factor of adverse outcome risk.	[70, 71]

The found association of the EAS absorption spectra with high levels of ALT, AST, and low TP values indicated a change in the EAS composition of exhaled air in the result of the virus cytopathic effect and subsequent toxic damage of the main organs [47], hypercytokinemia [44, 45], ischemic injury [46, 47], and drug toxicity, etc. [48, 49].

Supposed that with an increase in the degree of respiratory failure, the composition of exhaled air would differ more from healthy participants (due to hyperventilation, hypoxemia and hypercapnia [75]).

The correlation between the severity of the condition and the change in the composition of the exhaled air [76–78] was not confirmed, it is possible that this is due to difficulty in breathing in a seriously ill patient due to a decrease in his vital functions or the development of compensatory mechanisms (use of oxyhemoglobin reserves and increase in cardiac output); [79], the high the coefficient of solubility of molecular CO_2 during diffusion through the air-blood barrier [80].

5 Conclusions

The conducted analysis of the probability distributions of pairwise distances in the PC space revealed a correspondence between the EAS absorption spectra from COVID-19 patients and the following medical and biological parameters: the presence of MD and HD in the patient's history. Among blood parameters, RBC, CI and HGB, PI, PT, FG, INR, ALT, AST, TP demonstrate correlation with spectral features of the EAS absorption spectra.

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Institutional Review Board Statement

The study was conducted in accordance with the principles of the Declaration of Helsinki, with the Nuremberg Code, legislative of the Russian Federation and approved by the Ethics Committee of Tomsk State University (registration No. 5, November 19, 2020). All participants signed an informed consent for participation in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Disclosures

The authors declare no conflicts of interest.

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