Advance Publication by J-STAGE

Japanese Journal of Infectious Diseases

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Received: May 31, 2023. Accepted: November 29, 2023.
Published online: December 28, 2023.
DOI:10.7883/yoken.JJID.2023.211

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.

DYSREGULATED LEUKOTRIENE METABOLISM IN COVID-19 PATIENTS

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Running Title: Dysregulated Leukotriene Metabolism in COVID-19

Keywords: COVID-19; cysteinyl leukotriene; 5-lipooxygenase; cysteinyl leukotriene receptor; 5-LO activating protein

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Abstract

The aim of this study was to examine leukotriene metabolism in COVID-19. A total of 180 people were included in the study. Of these, 60 were healthy controls, 60 were patients who needed intensive care unit (ICU), and 60 were patients who did not need intensive care (non-ICU). Serum levels of 5-lipoxygenase (5-LO), 5-LO activating protein (ALOX5AP) and cysteinyl leukotriene (CYSLT) were measured and mRNA expressions of 5-LO, ALOX5AP and cysteinyl leukotriene receptor 1 (CYSLTR1) were investigated. As compared to the control group, both non-ICU and ICU groups had lower levels of 5-LO and mRNA expression. ICU patients had lower levels of 5-LO and mRNA expression compared with non-ICU patients. The expression of CYSLTR1 mRNA was higher in patients compared to healthy controls. CYSLTR1 mRNA expression was found to be higher in ICU group than in non-ICU group. CYSLT levels were higher in the control group compared to both non-ICU and ICU patients. Due to the higher expressions of CYSLTR1 in patients than control group, selective leukotriene receptor blockers can be used as a treatment option. CYSLTR1 expressions were also higher in ICU group than non-ICU group. Thus, CYSLTR1 mRNA expression could be a promising biomarker of COVID-19 severity.

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Introduction

COVID-19 has devastating health and socioeconomic impacts, leading to the loss of millions of peoples (1). Although vaccination programs have been widely implemented in developed and developing countries, they do not provide absolute prediction (2). COVID-19 biomarker research has explored various markers to assess disease severity and progression. However, challenges and limitations exist, such as the absence of a consensus on universally predictive biomarkers across diverse populations and setting.(3). Therefore, understanding the disease's pathogenesis and finding appropriate biomarkers to know the severity of the patient's condition is crucial in mitigating its harmful health consequences.

While cytokine storm has been identified as a significant factor in the progression of COVID-19 (4), little is currently known about changes in cytokines beyond well-known proinflammatory markers like interleukins. Eicosanoids are essential signaling molecules that play a crucial role in various physiological processes, such as inflammation, and coagulation (5). Leukotrienes (LTs) are a subgroup of eicosanoids, primarily synthesized by myeloid cells (6). After the activation of myeloid cells, arachidonic acid is released and oxidized by the action of both 5-lipooxygenase proteins (5-LO) and 5-LO activating protein (ALOX5AP) (FLAP) to produce, leukotriene C4 (LTC4) and leukotriene D4 (LTD4) and leukotriene E4 (LTE4) called cysteinyl leukotrienes (CYSLT) (Fig 1.) These LTs bind to specific receptors, namely cysteinyl leukotriene receptor 1 (CYSLTR1) and cysteinyl leukotriene receptor 2 (CYSLTR2). CYSLTR1 mRNA is expressed in the bronchial smooth muscle and myeloid lineage. Activation of this receptor results in airway smooth muscle contraction, smooth muscle proliferation, plasma exudation, and mucus hypersecretion (7, 8).

Previous report has indicated associations between leukotrienes and certain lung diseases (9). However, there is a limited number of studies exploring the LT pathway in COVID-19 patients. In our previous comprehensive untargeted metabolomics analyses we identified significant abnormalities in metabolites such LT and glutathione in COVID-19 patients. This led us to propose that investigating the LT pathway could provide valuable insights into the underlying mechanisms and potential treatment approaches for COVID-19.

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In this study, we would like to compare leukotriene-related molecules (mRNAs, proteins, and lipids) in blood of healthy controls, and ICU and non-ICU patients of COVID-19. Accordingly, this study aimed to investigate the role of key biomarkers, including 5-LO, ALOX5AP, CYSLT, and the mRNA expressions of 5-LO, ALOX5AP, and CYSLTR1, in healthy controls, intensive care unit (ICU) and non-ICU patients. The study assessed differences in these biomarkers between the groups and their associations with disease severity and progression. Consequently, we believe that this study presents a significant and exciting opportunity to enhance our understanding of the association between LT metabolism and COVID-19 patients. By shedding light on this aspect, we hope to contribute valuable insights that could potentially lead to advancements in the management and treatment of COVID-19.

Materials and methods

Study population

In this study, a total of 180 participants were enrolled, comprising three groups: 60 patients in the ICU with severe COVID-19 symptoms, 60 non-ICU patients with milder COVID-19 symptoms, and 60 healthy individuals serving as controls. The clinical and laboratory data of the patients were carefully documented at the time of their hospital admission and are presented in Table 1. Patients taking anti-inflammatory drugs were excluded from the study. The criteria in the biological variation study of EFLM were taken into consideration in the determination of healthy controls (10). To ensure ethical standards, the study received approval from the Sivas Cumhuriyet University Local Ethical Committee (2020-12-03).

Sample collection

Blood samples were collected on the first day of admission to the hospital. Patients taking anti-inflammatory drugs were excluded from the study. Samples were collected from each participant into serum tubes and ethylenediaminetetraacetic acid containing tubes (Becton Dickinson, UK). The serum tubes were allowed to clot before centrifugation. After centrifugation at 4 °C for 15 min at 3500 rpm; the serum was aliquoted and immediately frozen at −80 °C.

Biochemical analyses

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The serum 5-LO (Sunglong Biotech, Zhejiang, China), ALOX5AP (Sunred Biological Technology, Shanghai, China) and CYSLT (Sunglong Biotech, Zhejiang, China) levels were measured using commercial ELISA kit. Serum ferritin and troponin measurements were performed by using the immunoassay method (Roche Cobas e601, Mannheim, Germany). Serum glucose, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and albumin levels were measured using colorimetric methods (Roche Cobas c702, Mannheim, Germany). Complete blood count analysis was performed using an autoanalyzer (Sysmex XN1000, Japan). C-reactive protein (CRP) and D-Dimer measurements were performed using the turbidimetric method (Roche Cobas c702, Mannheim, Germany).

RNA isolation

Blood samples were transferred to RNA-free eppendorf tubes (250 µL/sample) and mixed with Trizol (750 µL). After a 3-5 minute incubation at room temperature, a chloroform:isoamylalcohol mixture (49:1; 200 µL) was added. The samples were then centrifuged (15 minutes, 12.000 xg, +4°C) and the supernatant transferred to a clean RNase-free tube. Isopropanol (99%) was added to the supernatant, followed by a 10–20-minute ice incubation. After centrifugation (15 minutes, 12.000 xg, +4°C), the pellets were washed with 75% ethanol and centrifuged (5 minutes, 7.500 xg). The alcohol was removed, and the pellets were resuspended in 20 µL of RNase-free water. The obtained RNA was stored at -80°C until cDNA synthesis.

cDNA synthesis

The total cDNA of the products was synthesized using M-MLV Revertase (Invitrogen): For this reaction, 5 µg RNA isolate and 1µL Oligo(dT), 1µL (10 mM) dNTP were mixed, the final volume was made up to 12µL with distilled water and then heated at 65 ºC. It was incubated for 5 minutes. After the incubation, 4 µL of 5X First-Strand buffer, 0.1 M DTT and 1 µL of RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 U/µL) were added to the mixture, which was quickly placed on ice, and incubated at 3 ºC for 5 minutes. 1 µL (200 U) of M-MLV Reverse transcriptase enzyme was added to the mixture and then incubated at 37 ºC for 50 minutes followed by incubation at 70 ºC for 15 minutes for enzyme inactivation.

Real-Time PCR

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Q-PCR was performed with a Bio-Rad CFX96 using SYBR green SYBR™ Green Master Mix CFX (Applied Biosystem) according to the manufacturer protocol. To increase the accuracy, duplicate qPCR was performed. The ΔCT method were normalized was used to evaluate the relative expression levels of mRNA. CT values obtained by the Q-PCR method according to the reference gene according to the 2-ΔΔCT method (11). Normalized expression value and fold-changes of each gene between analyses, control and study groups were determined. Primers designed to determine the expression levels of the 5-LO, ALOX5AP and CYSLTR1 genes were 5-LO.1_F: 5'-ACAAGCCCTTCTACAACGACT-3', 5-LO.1_R: 5'-AGCTGGATCTCGCCCAGTT-3' ALOX5AP1_F: 5'-TCAGCGTGGTCCAGAATGG-3', ALOX5AP1_R: 5'-GCAAGTGTTCCGGTCCTCT-3', CYSLTR1_F:5'-AAGTCCGTGGTCATAACCTTGT-3', CYSLTR1_R: 5'-TCTGGGTACATAAGTCACGCT-3', ActinH_F: 5'-CACCATTGGCAA TGAGCGGTTC-3', ActinH_R: 5'-AGGTCTTTGCGGATGTCCACGT5-3'.

Statistical analyses

Shapiro-Wilk's test were applied to assess the data normality. To compare the clinical parameters among control, non-ICU, and ICU patient groups, a Kruskal Wallis-H Test and one-way analysis of variance (ANOVA) were used for continuous variables, while Fisher-Freeman-Halton test was used for categorical variables. Tukey and Tamhane T^2 tests were used for multiple comparisons. The relationship between continuous variables was assessed by Spearman correlation analysis. Both crude and age, gender adjusted binary logistic regression analysis were applied to identify the risk of ALOX5AP, 5-LO and CYSLT levels, ALOX5AP, 5-LO and CYSLTR1 mRNA expressions in the intensive care requirement in COVID-19 patients. Receiver-operating characteristic curve (ROC) analysis was used to assess the diagnostic effect of ALOX5AP, CYSLTR1, and 5-LO on LT metabolism. A p value of <0.05 was considered statistically significant. All analyses were conducted using R 4.2.0 (www.r-project.org) software.

Results

Demographic variables and laboratory findings of the study population was given in Table 1. Both non-ICU and ICU groups had lower levels of 5-LO protein, its mRNA, and CYSLT. Furthermore, the levels of 5-LO and its mRNA is especially lower in ICU patients than in non-ICU patients. On the other hand, CYSLTR1

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mRNA expression was higher in patients, especially in ICU group, than in healthy controls. Box plot of 5-LO, ALOX5AP and CYSLT concentrations and 5-LO, ALOX5AP and CYSLTR1 CT values were given in Fig 2. Taking these results into account, we think that abnormalities in the regulation of leukotriene metabolism could be a pivotal factor in the molecular processes underlying the disease. Besides, these results indicate that 5-LO protein, its mRNA, and CYSLR1 mRNA could be promising biomarkers of COVID-19 severity.

In ELISA results, only 5-LO were statistically significant in predicting ICU in COVID-19 patients with an AUC of 0.678. ALOX5AP, 5-LO and CYSLTR1 mRNA expressions were found to be statistically significant with AUC values of 0.619, 0.639 and 0.643, respectively. Statistical diagnostic measurements of different biomarkers for ALOX5AP, 5-LO and CYSLT in predicting ICU were given in Table 2. For patients with 5-LO level $\leq$ 868.55, we obtained a 92% sensitivity and 62% specificity. For mRNA expressions with ALOX5AP $\leq$ 9.45, 5-LO > 9.97 and CYSLTR1 $\leq$ 3.61 cut-off values, the sensitivity statistics were 57%, 35%, and 82%, while the specificity statistics were 70%, 88%, and 47%, respectively. ROC curves were generated for ALOX5AP, 5-LO and CYSLT levels and ALOX5AP, 5-LO and CYSLTR1 mRNA expressions in predicting the intensive care requirement were given in Fig 3. After adjusting for age and gender variables, a one-unit increase in 5-LO level led to a 21.40-fold ICU risk in COVID-19 patients (p<0.05). The age and gender adjusted odds ratios were 2.62, 6.88, and 3.77 for ALOX5AP, 5-LO and CYSLTR1 mRNA expressions, respectively (p<0.05) (Table 3).

Non-ICU and ICU patients were combined, and the correlation analysis were performed between ALOX5AP, 5-LO and CYSLT levels, ALOX5AP, 5-LO and CYSLTR1 mRNA expressions and laboratory parameters. We found statistically significant correlation between 5-LO and D-dimer (*rho*=-0.197, p<0.05), albumin (*rho*=0.216, p<0.05), troponin (*rho*=-0.213, p<0.05), CRP (*rho*=-0.229, p<0.05), ferritin (*rho*=-0.188, p<0.05), monocyte (%) (*rho*=0.259, p<0.05), neutrophile (%) (*rho*=-0.212, p<0.05) and lymphocyte (%) (*rho*=0.278, p<0.05). A statistically significant correlation was present between ALOX5AP mRNA expression level and glucose (*rho*=-0.212, p<0.05), albumin (*rho*=0.285, p<0.05), neutrophile (*rho*=-0.197, p<0.05), and lymphocyte (*rho*=0.216, p<0.05); between 5-LO mRNA expression level and AST (*rho*=0.184, p<0.05), ferritin (*rho*=0.201, p<0.05); between CYSLTR1 mRNA expression level and troponin (*rho*=-0.253, p<0.05).

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Discussion

Arachidonic acid is a polyunsaturated fatty acid, and its metabolites, named eicosanoids, play different roles during the inflammatory process (12). Although there are conflicting results regarding the direction of changes, dysregulations in arachidonic acid level and related metabolites have been reported in the metabolomic analyses of COVID-19 patients (13-15). In the present study, statistically significant differences were observed between healthy controls and patients in the levels and mRNA expressions of biomarkers related to arachidonic acid metabolism. Similar differences were also found between the severe and mild groups. Consequently, our findings strongly indicate that COVID-19 disease induces alterations in arachidonic acid metabolism, and these changes are associated with disease progression.

Our study revealed lower 5-LO levels and mRNA expression in both ICU and non-ICU patients compared to the healthy controls. Furthermore, within the patient groups, ICU patients exhibited even lower 5-LO levels and mRNA expression compared to non-ICU patients. Schwarz et al. found higher levels of lipid mediators linked to 5-lipoxygenase (5-LO) activity in severe patients compared to both control and moderate groups. They suggested a potential positive relationship between disease severity and increased 5-LO activity (13). This speculation was inconsistent with our findings. In another study higher 5-LO mRNA expression was observed in diabetic COVID-19 patients compared to non-diabetic patients (16). As there was no healthy control group in that study, our findings were not directly comparable with their results. To the best of our knowledge, our study is the first to evaluate changes in both 5-LO levels and mRNA expression patterns in healthy controls and COVID-19 patients with different severities. We propose that the decrease in 5-LO levels, rather than an increase, may have a significant impact on the molecular mechanisms and prognosis of the disease. 5-LO is responsible for the production of several immune modulator molecules, including lipoxins and leukotrienes. Therefore, we suggest that the decrease in both 5-LO levels and its mRNA expressions could lead to diminished downstream metabolites produced by the activity of 5-LO. Such changes may play key roles in the pathogenesis of COVID-19.

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In our study, we found that ALOX5AP levels were similar between COVID-19 patients and healthy controls, despite its known role in 5-LO activity and leukotriene synthesis (6). This discovery suggests two potential associations. Firstly, ALOX5AP might undergo changes during early infection, returning to baseline levels later, contributing to leukotriene production during specific disease stages. Alternatively, modifications, interactions with other molecules, or subcellular localization changes could influence ALOX5AP function without significantly affecting its expression.

In previous studies, lower monocyte and eosinophil levels were observed in COVID-19 patients compared to the control group (17-20). In line with the previous studies, we also identified a lower percentage of monocytes in both ICU and non-ICU patients compared to the control group. Our analysis revealed a weak positive correlation between monocyte (%) and 5-LO level. IL-4 and IL-13 levels are two main regulators of the 5-LO, suppressing 5-LO synthesis in monocytes (21). In previous studies, increased IL-4 and IL-13 levels were reported in COVID-19 patients, and these elevated levels were associated with the pathogenesis of the disease. (22, 23). Considering our findings and the evidence from previous studies, we hypothesized that the combined effects of monocytopenia and increased inflammation could potentially contribute to the observed decrease in 5-LO levels. Furthermore, we propose that this decrease in 5-LO may play a role in mitigating the heightened inflammatory state in COVID-19 patients.

5-Lipoxygenase is responsible for synthesizing mediators like LTC4, LTD4, and LTE4. These mediators promote the release of proinflammatory cytokines, stimulate chemotaxis, and trigger effects like airway smooth muscle contraction and mucus hypersecretion (6, 7, 24-26). In our study, decreased cysLT levels were detected in both non-ICU and ICU patients compared to the healthy controls. We hypothesize that the reduced level and expression of 5-LO may contribute to the observed decrease in cysLT levels in COVID-19. It has been reported that CYSLT is related with the signaling in T cells and T cell-driven immune responses (27-29). T cell-mediated immune response plays a role in COVID-19 disease and improper T cell activity related with severe outcome (30). In a study, elevated levels of CYSLT were associated with an increased survival rate in a mice influenza model (31). Thus, we think that decreasing CYSLT levels are related to the increased severity of the disease. However, in our previous study, we found upregulation in LTD4 in COVID-19. In the present

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study, decreased levels of CYSLT, reflecting the total levels of LTC4, LTD4, and LTE4, were determined in the patients. Therefore, measuring each leukotriene separately may provide a better understanding of the changes in leukotrienes in COVID-19.

CYSLTR1 and CYSLTR2 are associated with, increased vascular permeability, bronchoconstriction and increased mucus secretion (32). In a previous studies, it was suggested that montelukast, a CYSLTR1 receptor blocker, could be considered as a treatment option in COVID-19 patients due to the similarity between the pathological changes in the lung in COVID-19 and the physiological effects of CYSLTR1 (33, 34). In the present study, we found higher expressions of CYSLTR1 mRNA in COVID-19 patients compared to healthy controls. Additionally, we detected elevated expressions in ICU patients compared to non-ICU patients. Accordingly, we believe that increased CYSLTR1 expression in COVID-19 patients plays a role in the formation and progression of the disease, and the use of CYSLTR1 receptor blockers may be a therapeutic alternative to decrease the detrimental effects of the disease. In this study, we observed higher mRNA expression of CYSLTR1 in patients compared to controls, while no statistically significant changes were found in CYSLT levels. These findings might suggest potential mechanisms, including compensatory responses independent of CYSLT levels, sample heterogeneity, and differences in translational efficiency or protein turnover.

Various laboratory biomarkers have been associated with a higher risk of severe disease. However, the use of these biomarkers has some weaknesses, such as discrepancies in the methods used between studies and limitations in study design (35, 36). In this study, the sensitivity of CYSLTR1 mRNA expression was found to be 0.83, and the specificity of 5-LO was 0.84 for distinguishing between ICU and non-ICU patients. We propose that a CYSLTR1 mRNA expression threshold of ≤3.72 can be used as an additional marker to determine the need for intensive care in COVID-19 patients, while a 5-LO mRNA expression threshold of >9.95 can be used to rule out the ICU requirement. Moreover, considering the sensitivity value of the 5-LO level, a threshold of ≤867 can be an alternative indicator to determine the need for intensive care. The specific disease stage at which measurements were made could have influenced the expression of 5-LO. Moreover, genetic and environmental factors, could have influenced the results. Therefore, it is crucial to validate our

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findings in larger and more diverse cohorts to gain a better understanding of the role of 5-LO in COVID-19. Lastly, the low AUC values obtained in our study indicate the need for additional investigations with a larger number of patients to confirm and strengthen our findings.

Our findings highlight the importance of investigating key genes (COX1, COX2, LTA4H, LTC4S, GGT1, and DPEP2) involved in regulating the arachidonic acid leukotriene pathways. Studying genetic variations, mRNA expression, and potential interactions associated with these genes can greatly enhance our understanding of leukotriene metabolism in the context of COVID-19.

In conclusion, we found significant changes in leukotriene metabolism-related metabolite levels and mRNA expressions in COVID-19 patients compared to healthy controls. Additionally, variations in these parameters were also detected between ICU and non-ICU patients. These findings suggest that dysregulation in leukotriene metabolism may play a crucial role in the molecular mechanisms and progression of the disease. Consequently, selective leukotriene inhibitors like montelukast could be considered as potential treatment options. The decreased levels of 5-LO and mRNA expression in COVID-19 patients, particularly in ICU COVID-19 patients, could have important implications. 5-LO is an enzyme involved in the production of inflammatory mediators, and reduced levels might indicate a potential suppression of the inflammatory response. Additionally, it's important to explore whether this reduction is a cause or a consequence of the disease and whether modulating 5-LO could be a viable treatment strategy.

Acknowledgments: The study was supported by The Scientific and Technological Research Council of Turkey (TUBİTAK) with the grant number: 220S424 and Cumhuriyet University with the grant number: T-2021-914

Conflict of interest: None to declare.

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Figure legends:

Figure 1. Biosynthetic pathway of cysteinyl leukotrienes

Figure 2. Box plot of 5-LO, ALOX5AP and CYSLT concentrations (A) and box plot of 5-LO, ALOX5AP and CYSLTR1 CT values (B)

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Figure 3. ROC curves were generated for ALOX5AP, 5-LO and CYSLT levels and ALOX5AP, 5-LO and CYSLTR1 mRNA expressions in predicting the intensive care requirement in patients.

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Table 1. Demographic variables and laboratory findings of the study population

Variables	Groups			*p*
	Control (*n*=60)	Non-ICU (*n*=60)	ICU (*n*=60)	
Age (years)	58.45±12.74[a]	61.35±14.88[a]	69.33±12.41[b]	**<0.001**
Gender (Male/Female)	41(68.3)	29(48.3)	36(60.0)	0.082
Glucose (mg/dL)	90.0(85.0-96.75)[a]	108.50(95.0-140.0)[b]	134.50(102.75-184.0)[c]	**<0.001**
Creatinine (mg/dL)	0.71(0.63-0.81.0)[a]	0.80(0.60-1.03)[a]	1.14(0.75-2.54)[b]	**<0.001**
AST (U/L)	17.0(14.0-19.0)[a]	25.0(19.0-34.75)[b]	49.50(21.25-70.75)[c]	**<0.001**
ALT (U/L)	14.0(10.0-18.75)[a]	22.0(15.0-35.75)[b]	33.0(18.25-42.0)[b]	**<0.001**
LDH (U/L)	165.50(146.25-184.25)[a]	249.0(182.25-294.0)[b]	433.50(309.75-588.0)[c]	**<0.001**
Ferritin (µg/L)	33.55(13.08-99.38)[a]	169.90(63.38-339.25)[b]	492.50(349.0-601.25)[c]	**<0.001**
CRP (mg/L)	1.19(0.61-2.22)[a]	14.15(3.65-43.25)[b]	135.0(83.0-228.75)[c]	**<0.001**
D-Dimer (ng/mL)	-	437.0(240.0-617.50)	1117.50(423.50-2571.25)	**<0.001**
Troponin (ng/L)	-	3.20(2.20-12.0)	32.05(8.70-86.48)	**<0.001**
Albumin (g/L)	46.90(45.03-49.18)[a]	41.05(38.03-42.93)[b]	28.55(25.93-33.03)[c]	**<0.001**
Monocyte (%)	7.62±1.53[a]	7.65±2.34[a]	5.09±2.42[b]	**<0.001**
Lymphocyte (%)	30.65(23.88-35.08)[a]	24.50(16.48-35.70)[a]	6.55(4.23-10.28)[b]	**<0.001**
Neutrophile (%)	60.29±7.26[a]	65.81±13.74[b]	86.88±6.06[c]	**<0.001**
Hemoglobin (g/dL)	14.31±1.36[a]	13.53±1.54[b]	12.98±2.51[c]	**0.001**
Platelet (10^9/L)	263.50(223.25-293.50)	245.0(179.75-295.50)	228.50(170.25-304.0)	0.113
ELISA Results				
ALOX5AP (ng/mL)	0.30(0.27-0.67)	0.29(0.27-0.47)	0.29(0.28-0.35)	0.609
5-LO (pg/mL)	1560(977.5-2384)[a]	1029(418.4-1371)[b]	503(396.4-558.2)[c]	**<0.001**

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CYSLT (pg/mL)	745.5(388.7-868)[a]	591.3(448.7-637.7)[b]	596.7(293.7-631.4)[b]	**0.016**
mRNA Expressions				
ALOX5AP (CT value)	10.02±1.81	10.70±2.35	9.74±2.67	0.067
5-LO (CT value)	6.62±1.95[a]	8.26±1.85[b]	9.38±2.41[c]	**<0.001**
CYSLTR1 (CT value)	5.50±1.61[a]	3.37±1.72[b]	2.57±1.49[c]	**<0.001**

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C-reactive protein ICU: Intensive care unit, LDH: Lactate dehydrogenase, PLT: Platelet, ALOX5AP: 5-lipoxygenase activating protein, 5-lipoxygenase: 5-LO, CYSLT: Cysteinyl leukotriene. Data are summarized as *n*(%), mean±standard deviation or median(1st-3rd quartiles). Different lowercase letters (a,b,c) in the same row indicate a statistically significant difference among groups. Significant *p*-values are shown in bold.

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Table 2. Statistical diagnostic measurements of different biomarkers for ALOX5AP, 5-LO and CYSLT in predicting ICU in COVID-19 patients

Biomarker	ROC analysis results		Diagnostic Statistic			
	AUC (%95 CI)	*p*-value	SEN	SPE	PLR	NLR
ELISA Results						
ALOX5AP (ng/mL) ≤ 0.75	0.511(0.408-0.615)	0.829	0.87(0.75-0.94)	0.20(0.11-0.32)	1.08(0.92-1.27)	0.67(0.29-1.51)
5-LO (pg/mL) ≤ 868.55	0.678(0.571-0.784)	**<0.001**	0.92(0.82-0.97)	0.62(0.48-0.74)	2.39(1.72-3.33)	0.14(0.06-0.32)
CYSLT (pg/mL) > 594.97	0.509(0.403-0.615)	0.829	0.55(0.42-0.68)	0.65(0.52-0.77)	1.57(1.04-2.38)	0.69(0.50-0.97)
mRNA Expressions						
ALOX5AP (CT value) ≤ 9.45	0.619(0.518-0.720)	**0.021**	0.57(0.43-0.69)	0.70(0.57-0.81)	1.89(1.21-2.95)	0.62(0.44-0.86)
5-LO (CT value) > 9.97	0.639(0.540-0.738)	**0.006**	0.35(0.23-0.48)	0.88(0.77-0.95)	3.00(1.38-6.52)	0.74(0.60-0.91)
CYSLTR1 (CT value) ≤ 3.61	0.643(0.543-0.742)	**0.005**	0.82(0.70-0.90)	0.47(0.34-0.60)	1.53(1.17-2.00)	0.39(0.22-0.71)

ALOX5AP: 5-lipoxygenase activating protein, 5-lipoxygenase: 5-LO, CYSLT: Cysteinyl leukotriene. AUC: Area under the curve, ROC: Receiver operating characteristics, CI: Confidence interval, SEN: Sensitivity, SPE: Specificity, PLR: Positive Likelihood Ratio, NLR: Negative Likelihood Ratio

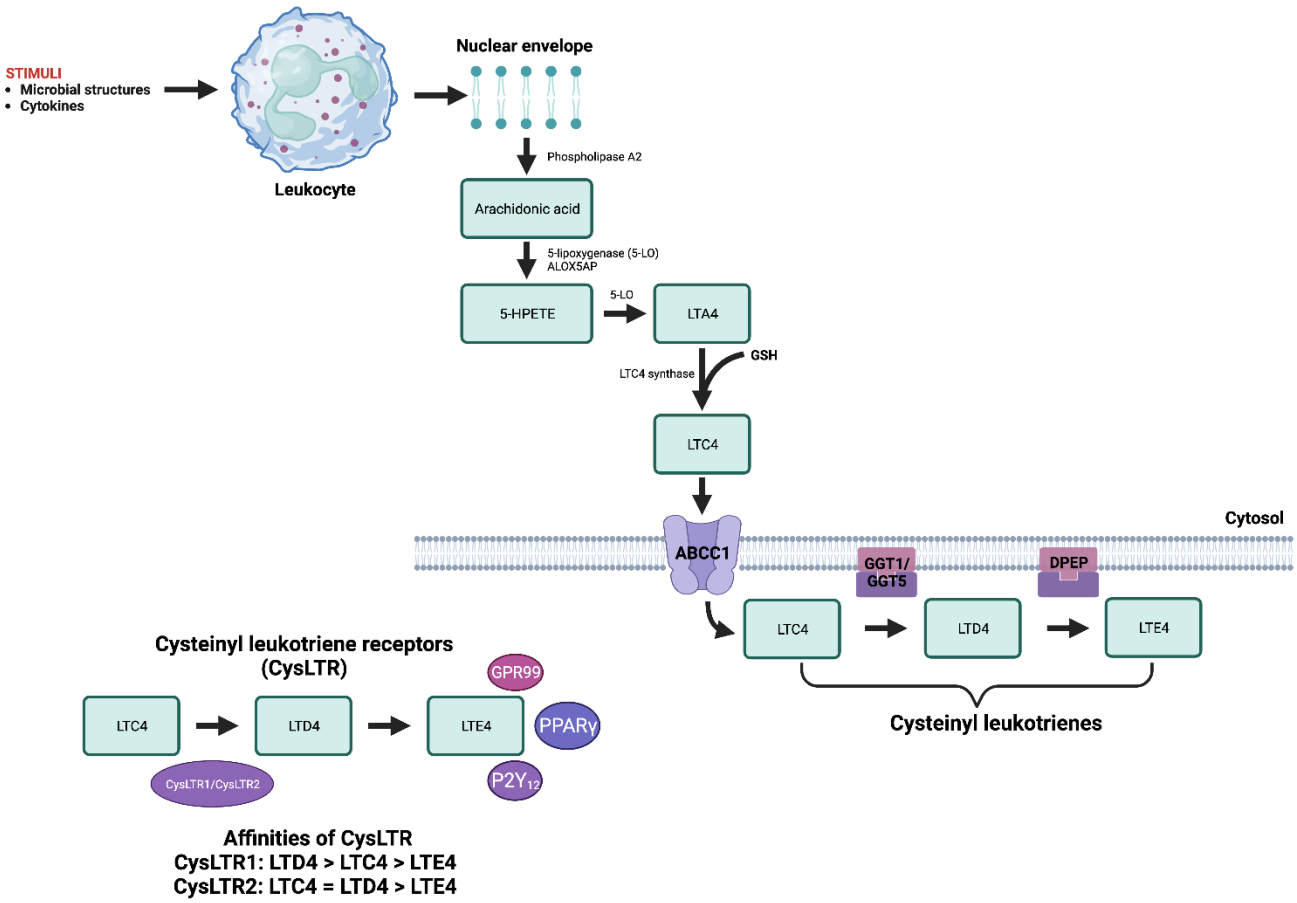
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Table 3. Binary logistic regression analyses computed for ALOX5AP, 5-LO and CYSLT in identifying ICU in COVID-19 patients

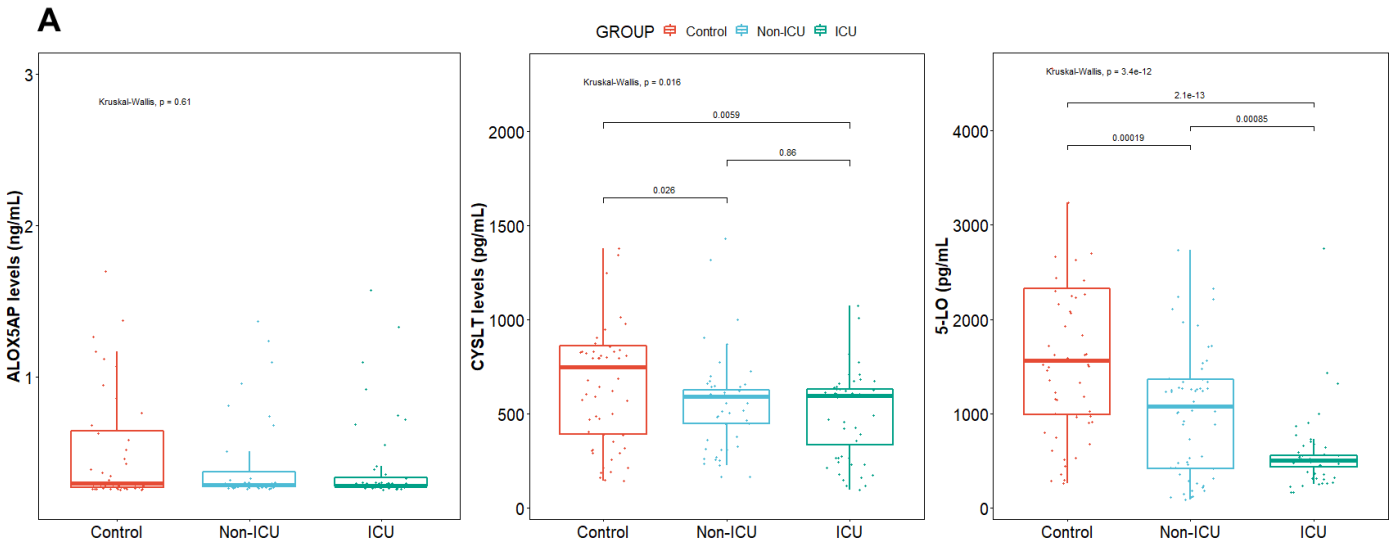
Variables	Crude Model		Adjusted Model*	
	OR(%95CI)	*p*	OR(%95CI)	*p*
ELISA Results				
ALOX5AP (pg/mL) (<0.75 / ≥0.75)	1.63(0.61-4.32)	0.330	1.76(0.63-4.91)	0.279
5-LO (pg/mL) (<868.55 / ≥868.55)	17.70(6.17-50.72)	**<0.001**	21.40(6.82-67.17)	**<0.001**
CYSLT (pg/mL) (> 594.97 / ≤ 594.97)	2.27(1.09-4.73)	**0.029**	1.98(0.92-4.26)	0.080
mRNA Expressions				
ALOX5AP (CT value) (<9.45 / ≥9.45)	3.05(1.44-6.47)	**0.004**	2.62(1.18-5.84)	**0.018**
5-LO (CT value) (> 9.97 / ≤9.97)	4.08(1.58-10.54)	**0.004**	6.88(2.29-20.64)	**0.001**
CYSLTR1 (CT value) (<3.61 / ≥3.61)	3.90(1.70-8.92)	**0.001**	3.77(1.59-8.98)	**0.003**

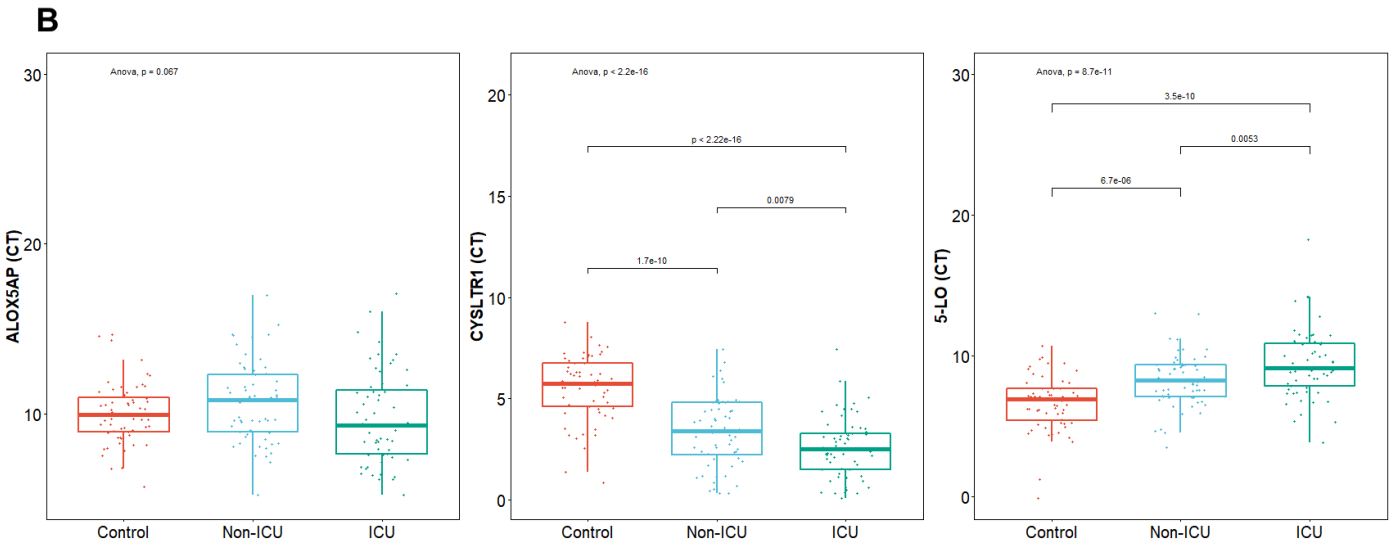
Odds ratios are adjusted for both age and gender. Significant *p*-values are shown in bold. ALOX5AP: 5-lipoxygenase activating protein, 5-LO: 5-lipoxygenase, CYSLT: Cysteinyl leukotriene. OR: odds ratio, CI: confidence interval, ICU: Intensive care unit.

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STIMULI
Microbial structures
Cytokines
Leukocyte
Nuclear envelope
Phospholipase A2
Arachidonic acid
5-lipoxygenase (5-LO)
ALOX5AP
5-HPETE
5-LO
LTA4
LTC4 synthase
GSH
LTC4
Cytosol
ABCC1
GGT1/
GGT5
DPEP
LTC4
LTD4
LTE4
Cysteinyl leukotrienes
Cysteinyl leukotriene receptors
(CysLTR)
LTC4
LTD4
LTE4
GPR99
PPARγ
CysLTR1/CysLTR2
P2Y12
Affinities of CysLTR
CysLTR1: LTD4 > LTC4 > LTE4
CysLTR2: LTC4 = LTD4 > LTE4

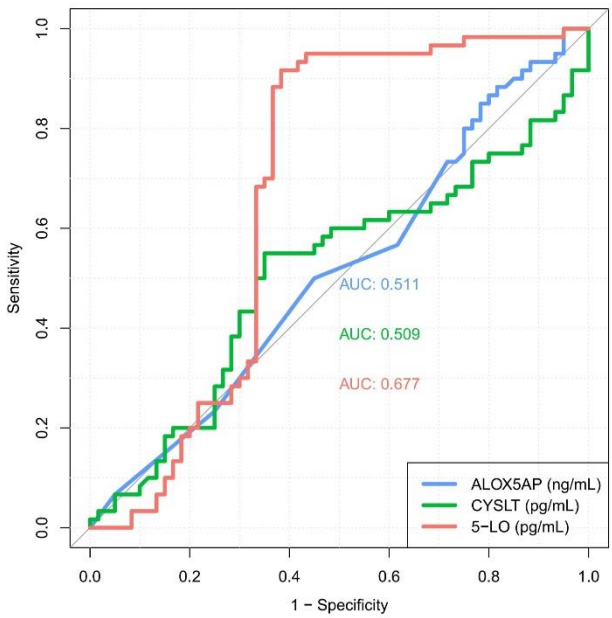
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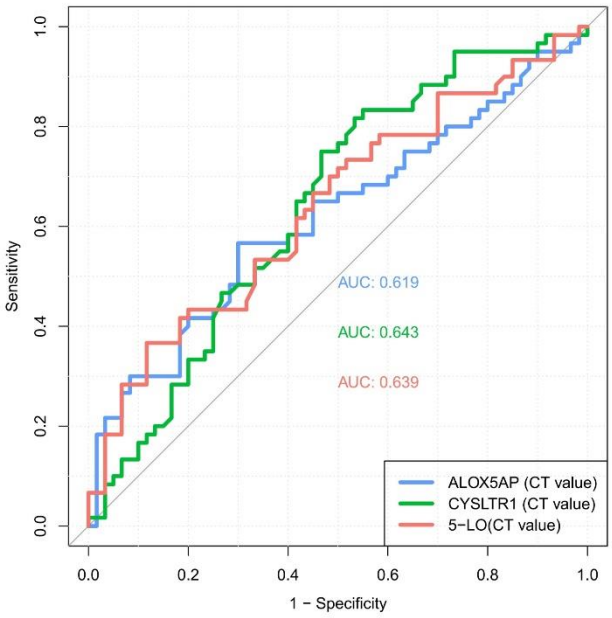

A
GROUP Control Non-ICU ICU
ALOX5AP levels (ng/mL)
Kruskal-Wallis, p = 0.61
Control
Non-ICU
ICU
CYSLT levels (pg/mL)
Kruskal-Wallis, p = 0.016
0.0059
0.86
0.026
5-LO (pg/mL
Kruskal-Wallis, p = 3.4e-12
2.1e-13
0.00085
0.00019


B
ALOX5AP (CT)
Anova, p = 0.067
Control
Non-ICU
ICU
CYSLTR1 (CT)
Anova, p < 2.2e-16
p < 2.22e-16
0.0079
1.7e-10
5-LO (CT)
Anova, p = 8.7e-11
3.5e-10
0.0053
6.7e-06

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ROC Curves for Biomarkers





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