LTB_4-driven inflammation and increased expression of *ALOX5/ACE2* during severe COVID-19 in individuals with diabetes.

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Diabetes Publish Ahead of Print, published online June 15, 2021

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Word count: 3,994

Abstract

Diabetes is a known risk factor for severe COVID-19, the disease caused by the new coronavirus SARS-CoV-2. However, there is a lack of knowledge about the mechanisms involved in the evolution of COVID-19 in individuals with diabetes. Therefore, we aimed to evaluate whether the chronic low-grade inflammation of diabetes could play a role in the development of severe COVID-19. We collected clinical data and blood samples of hospitalized patients for COVID-19, with diabetes and without diabetes. Plasma samples were used to measure inflammatory mediators and peripheral blood mononuclear cells, for gene expression analysis of SARS-CoV-2 main receptor system (*ACE2/TMPRSS2*) and main molecule of LTB_4 pathway (*ALOX5*). We found that diabetes activates LTB_4 pathway, and during COVID-19, it increases *ACE2/TMPRSS2* as well as *ALOX5* expression. Diabetes was also associated with COVID-19-related disorders, such as reduced SpO2/FiO2 and PaO2/FiO2 levels, and increased disease duration. In addition, the expression of *ACE2* and *ALOX5* are positively correlated, with increased expression in COVID-19 patients with diabetes requiring intensive care assistance. We confirmed these molecular results at the protein level, where plasma LTB_4 is significantly increased in individuals with diabetes. Besides, IL-6 serum levels are

increased only in individuals with diabetes requiring intensive care assistance. Together, these results indicate that LTB_4 and IL-6 systemic levels, as well as, *ACE2/ALOX5* blood expression could be early markers of severe COVID-19 in individuals with diabetes.

Keywords

COVID-19, Diabetes, Lipid mediators, LTB_4, ACE2, SARS-CoV-2

List of abbreviations

5-LO	5-lipoxygenase
ACE2	Angiotensin I Converting Enzyme 2
ACTB	β-actin
ALOX5	Arachidonate 5-Lipoxygenase
ALOX5AP	5-LO activating protein
ARDS	Acute Respiratory Distress Syndrome
CB	Clinical Beds
CCL2	C-C Motif Chemokine Ligand 2
CD147	Basigin
CO_2	Carbon dioxide
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus Sisease 19
CXCL10	C-X-C motif chemokine ligand 10
DEGs	Differentially Expressed Genes
DM	Individuals with diabetes
EHMN	Edinburgh Human Metabolic Network
ELISA	Enzyme-Linked Immunosorbent Assays

FDR	False Discovery Rate
FURIN	Furin, Paired Basic Amino Acid Cleaving Enzyme
GEO	Gene Expression Omnibus
ICU	Intensive Care Unit
IFN-γ	Interferon-gamma
IL-6	Interleukin 6
KEGG	Kyoto Encyclopedia of Genes and Genomes
LTB_4	Leukotriene B_4
LTB4R	LTB4 receptor
MCP1	Monocyte Chemoattractant Protein-1
MeSH	Medical Subject Headings
NDM	Individuals without diabetes
OGTT	Oral Glucose Tolerance Test
PBMC	Peripheral Blood Mononuclear Cells
RT-qPCR	Reverse Transcription followed by the quantitative Polymerase Chain Reaction.
SARS-CoV-2	Coronavirus of Severe Scute Respiratory Syndrome 2
TMPRSS2	Transmembrane Serine Protease 2
TNF-α	Alpha Tumor Necrosis Factors

Introduction

Coronavirus disease 19 (COVID-19) pandemic records more than 162 million confirmed cases and more than 3.3 million deaths worldwide, as of May 17, 2021 (1). The disease is caused by the new Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that emerged in China and rapidly spread around the world (2). Estimates

indicate that around 80% of infected individuals are asymptomatic or develop mild symptoms. The other 20% can develop moderate to severe disease, occasionally requiring medical assistance due to acute respiratory disease and pneumonia, burdening health care systems (3,4). Risk factors in developing severe COVID-19 include, among others, hypertension, age, obesity, and diabetes (5–9). Individuals with diabetes are at high risk of developing severe COVID-19 as accounted by their high rates of intensive care unit (ICU) admission and death (7).

Considering that 463 million people live with diabetes worldwide (10) and COVID-19 is a high transmissible disease, it is urgent to identify mechanisms that prevents infection of this population (6,7). As seen in multiple infectious diseases, including COVID-19, infection-induced inflammatory response can result in a cytokine storm, recruiting cells to infected tissues and establishing a pro-inflammatory feedback loop. This uncontrolled inflammation causes multi-organ damage, specially of the heart, liver and kidney, with high risk of death (11). Although several reports have described cytokines and chemokines involved in the inflammatory storm during COVID-19 (11,12), studies on lipid mediators of inflammation and their roles in this new disease are scarce.

Eicosanoids are potent lipid mediators produced by arachidonic acid's metabolism, found in cell surface, that signal many biological processes, including inflammation and immune responses (13). Some classes of eicosanoids, especially leukotrienes (LTs), have been associated with the pathogenesis of respiratory disease (14,15). We and others have already shown increased levels of leukotriene B_4 (LTB_4) in diabetes, which is associated with inflammation, compromised wound healing, insulin resistance, and susceptibility to infections (16–20). LTB_4 is a product from the action of 5-lipoxygenase (5-LO, encoded by *ALOX5* gene) and its activating protein (FLAP,

encoded by ALOX5AP gene), rapidly produced after several stimuli, mainly by neutrophils and monocytes/macrophages. After its release, LTB_4 can signal by an autocrine or paracrine manner by different cell types through the leukotriene receptor (encoded by the LTB4R gene), triggering an increase in chemotaxis and inflammatory exacerbation (18,21–23).

In the present study, we sought to evaluate whether LTB_4 plays a role in the severity of COVID-19 in individuals with diabetes.

Research design and methods

Ethics statement

This study followed the principles specified in the Declaration of Helsinki. The Institutional Board for Ethics in Human Research at the Gonçalo Moniz Institute (Oswaldo Cruz Foundation-IGM-FIOCRUZ, Salvador, Bahia-Brazil) and Irmã Dulce Social Works approved this study (protocol number: CAAE 36199820.6.0000.0040 and 33366020.5.0000.0047, respectively). Participants gave informed consent previous to any data and sample collection.

Acquisition of Microarray Dataset

Diabetes is considered a risk factor for complicated acute respiratory syndrome caused by SARS-CoV-2 infection (5,7). Given the lack of data on the mechanisms that drive these complications, we sought to analyze public transcriptome data of PBMCs from individuals with diabetes. Microarray analysis was performed from the NCBI Gene Expression Omnibus (GEO) database using the search terms "diabetes" and "human" which had a sample of peripheral blood mononuclear cells (PBMC) from healthy individuals with diabetes. Among the datasets found, we selected the dataset with GEO accession number GSE95849 that applied Phalanx Human lncRNA OneArray v1_mRNA

(GPL22448) platform (24). This dataset compared six samples of PBMCs from healthy controls (individuals with normal glucose tolerance and without a family history of diabetes or chronic diseases) and six samples from individuals with diabetes (DM). The criteria for including individuals in the DM group were: fasting plasma glucose ≥ 7 mmol/L, 2 h plasma glucose after oral glucose tolerance test (OGTT) ≥ 11.1 mmol/L, or use of glucose-lowering drugs or physician-diagnosed diabetes. Differentially expressed genes (DEGs) were considered when fold change ranged from −2.0 ≤ to ≥ 2.0 and FDR-adjusted *p-value* < 0.05.

Detection of metabolic network in diseases and pathway enrichment analysis

Metabolic networks (Compound-Reaction-Enzyme-Gene) were found based on the expression of significantly modulated genes comparing healthy controls with individuals with DM. We used MetDisease version 1.1.0 in the Cytoscape 3.7.2 software (USA) to build disease-based metabolite networks according to the Kyoto Encyclopedia of Genes and Genomes (KEEG). Next, data were further filtered to retain disease MeSH (Medical Subject Headings) terms relevant to reported clinical COVID-19 manifestations, such as pneumonia, respiratory distress syndrome (adult), acute lung injury, and inflammation. Matched metabolites found in these conditions were clustered using a Venn diagram to find common molecules.

The identification of enriched pathways was based on genes and compounds using integrated Kyoto Encyclopedia of Genes and Genomes (KEGG) and Edinburgh Human Metabolic Network (EHMN) databases stored at NCBI. Canonical pathways were detected by MetScape 3.1.3 in the Cytoscape 3.7.2 software (USA) using significantly modulated genes between healthy controls and individuals with DM.

Study design, cohort definition and clinical data

Patients were admitted with confirmed diagnosis of COVID-19 in Ernesto Simões Filho General Hospital (HGESF), Salvador, Bahia, Brazil. A convenience sample of fifty-three patients were enrolled in this study, 24 without diabetes (NDM) and 29 with diabetes (DM). This sample size considered a 95% confidence interval (two-sided), and the power estimated for each parameter measured in this study was above 80%, using Epi info TM software. All groups were matched for gender, age and hospitalization type [CB (clinical beds) or ICU (intensive care unit)]. According to the Brazilian Diabetes Society guidelines, 2019-2020 (25), the diagnosis of diabetes was confirmed by HbA1c levels measured during hospitalization. Patients with HbA1c ≥ 6.5% (48 mmol/mol) and medical history of insulin use were considered with diabetes. The NDM group includes individuals with HbA1c ≤ 6.4% (46 mmol/mol), considered without diabetes or with pre-diabetes (without the need for insulin during hospitalization). Comorbidity data were collected according to medical records. The study included patients diagnosed positive for COVID-19, based on the positivity of molecular test (RT-qPCR), serology, tomography, or clinical history for COVID-19. Patients who did not agree to sign the term of free and informed consent, pregnant women, time of symptoms ≥ 14 days, and those who had more than 48 hours after hospital admission were excluded. Clinical data from all patients, obtained from medical records, are shown in Table 1.

Sample collection

Blood samples from all patients were collected at the admission by venipuncture using tubes with Heparin. Plasma was separated (to quantify inflammatory mediators) and Peripheral Blood Mononuclear Cells (PBMC; to analyze gene expression) were purified using HISTOPAQUE® 1077 (Sigma Aldrich, USA).

Analysis of gene expression in PBMCs

Total RNA was extracted from PBMCs using miRNeasy Mini Kit (QIAGEN, GER) according to the manufacturer's guidelines. Relative expression of Arachidonate 5-Lipoxygenase (*ALOX5*; Assay ID Hs.PT.56a.28007202.g), Angiotensin I Converting Enzyme 2 (*ACE2*; Assay ID Hs.PT.58.27645939), Transmembrane Serine Protease 2 (*TMPRSS2*; Assay ID Hs.PT.58.4661363), Furin, Paired Basic Amino Acid Cleaving Enzyme *(FURIN*; Assay ID Hs.PT.58.1294962) and Basigin (*CD147*; Assay ID Hs.PT.56a.39293590.g) were analyzed. After RNA quantification and quality analysis by spectrophotometry, cDNA synthesis was performed using the SuperScript® III Reverse Transcriptase kit (Invitrogen, USA). Then, cDNA was amplified by quantitative real-time PCR (RT-qPCR) using the SYBR Green PCR Master Mix (Thermo Fisher Scientific, USA). Relative gene expression is showed as fold change between NDM and DM groups using the $2^{-\Delta\Delta CT}$ method [ΔΔCt = ΔCt (target DM) – mean ΔCt (target NDM)], where ΔCt = Ct (gene of interest) - Ct (housekeeping-gene)]. To identify the distribution within the control group (NDM), we applied ΔΔCt = ΔCt (target NDM) – mean ΔCt (target NDM), being ΔCt = Ct (gene of interest) - Ct (housekeeping-gene). β-actin the housekeeping gene (*ACTB*; Hs.PT.39a.22214847). All primers were purchased from IDT (Integrated DNA Technologies, USA).

Quantification of inflammatory mediators

Based on the inflammatory profile already described in the literature for DM COVID-19 (6,8,26), serum levels of TNF-α, IL-6 and IL-1β cytokines (Invitrogen, CA) were evaluated using sandwich enzyme-linked immunosorbent assays (ELISA). LTB_4

levels were determined by Competition ELISA Kit (Cayman Chemical, USA), considering the manufacturer's instructions.

Statistical analysis

Benjamini & Hochberg method was used to control false discovery rate (FDR) when evaluating Differentially Expressed Genes (DEGs) from the GEO transcriptome dataset. For variables with normal distribution, we used Student's t-test (two groups), one-way ANOVA test followed by Tukey (three or more groups). For non-normal distribution, we used Mann–Whitney test (two groups), Kruskal–Wallis with Dunn's post-test (three or more groups) and Spearman test we used for correlations analysis. Symptom and comorbidity analysis were performed using Chi-Square or Fisher's exact test. All tests were conducted using Prism 7 software (GraphPad, USA). Differences were considered statistically significant when $p < 0.05$, or adj. $p < 0.05$ for DEGs and multiple comparations.

Data and Resource Availability

The public data set analyzed during the current study is available in the GEO DataSets repository, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE95849.

The datasets generated during the current study are not publicly available but can be made available by the corresponding author upon request.

Results

LTB_4 signaling activated in individuals with diabetes is similar to that found in respiratory disorders

Initially, we found 3,585 genes were significantly modulated when comparing cells from individuals with or without diabetes. Of these, 3,405 were upregulated and 180 were downregulated in DM individuals (Fig. 1A).

Next, we searched for disorders associated with these differentially expressed genes (DEGs) by detecting molecule networks. We focused on conditions related to severe COVID-19, such as pneumonia, severe acute respiratory syndrome, acute lung injury; we also focused on inflammation. Interestingly, we found only two molecules in common between these conditions, carbon dioxide (CO_2) and the lipid mediator LTB_4 (Fig. 1B).

We further searched for signaling pathways associated with these DEGs and, among 61 routes found, the LTB_4 pathway was at a central position within the network (Fig. 1C). Next, we assessed the expression of molecules crucial for LTB_4 production, such as the *ALOX5* gene (it encodes the 5-LO enzyme that converts arachidonic acid into leukotrienes), *ALOX5AP* (the 5-LO activating protein), and *LTB4R* (the LTB_4 receptor), in this dataset. We found increased expression of all evaluated genes in the PBMCs from individuals with diabetes compared to healthy controls (Fig. 1D). Together, these findings indicate that LTB_4 is a potential target to study mechanisms under complicated COVID-19 in individuals with diabetes.

Increased expression of ALOX5 *and* ACE2/TMPRSS2 *in PBMCs from COVID-19 individuals with or without diabetes*

The expression of SARS-CoV-2 receptors (27), and the inflammatory response (11) are related to the complications found in COVID-19. We then assessed the expression of *ALOX5* (which encodes for the 5-LO enzyme), *ACE2/TMPRSS2*, *FURIN,* and *CD147* (surface molecules used by SARS-CoV-2 to invade human cells). The results

show a significant increase in the expression of *ALOX5* (Fig. 2A) and *ACE2/TMPRSS2* (Fig. 2B,C) in PBMCs from COVID-19 in DM compared to NDM. The increase in ALOX5, ACE2 and TMPRSS2 was also preliminarily assessed in the tracheal secretion of NDM and DM individuals with COVID-19 under mechanical ventilation, despite the small sample size, we observed a trend toward increased expression, indicating that blood cells mirror the immune response in the lungs ($p = 0.055$) (See supplementary figure 1). These findings confirm our previous result (from public transcriptome data), showing that *ALOX5* expression is increased in DM (Fig. 1D). Such findings support the possible role of 5-LO in the chronic low-grade inflammation observed in LTB_4 pathway-induced diabetes, rendering diabetic individuals more prone to infections (19,21). We also found increased expressions of SARS-CoV-2 main receptor system *ACE2 and TMPRSS2* in the PBMCs from DM individuals, suggesting that immune cells that will fight the infection are more prone to viral invasion.

Expression of* ALOX5 *correlates to* ACE2 *in PBMCs from COVID-19 patients with diabetes

ACE2 expression is crucial for cell invasion and progression in COVID-19 (11,27). Therefore, we sought to investigate whether the expression of *ALOX* could be correlated with *ACE2* expression. First, we correlated *ALOX5* with *ACE2/TMPRSS2* (summarized in the correlation matrix – Fig. 3A) separately between individuals DM or NDM. We found a positive correlation between *ACE2* and *TMPRSS2* in both groups (DM and NDM) since these molecules act together during viral invasion (11) (Fig. 3B,C). However, the correlation between *ALOX5* and *ACE2* is only present in the DM group (Fig. 3D) (See supplementary Fig. 2), suggesting that cells that have high levels of ALOX5 also have increased ACE2 expression in the DM group.

Next, we evaluated whether *ALOX5* and *ACE2* expressions are correlated with clinical evolution of COVID-19. To assess that, we first compared the need for intensive care unit (ICU) between DM and NDM individuals stratified by the expression levels of *ALOX5* and *ACE2*. The results show that DM individuals with higher levels of *ACE2* (Fig. E) and *ALOX5* (Fig. 3F) required ICU more frequently compared to individuals with low expression of these genes, but no difference was found with the gene expression of *TMPRSS2* (Fig. 3G). Together, these findings indicate that the increased expressions of *ALOX5* and *ACE2* in blood cells from DM individuals are associated with more severe conditions of COVID-19, requiring ICU.

Increased systemic levels of LTB_4 in COVID-19 patients with diabetes

The cytokine storm described in COVID-19 is characterized by several inflammatory mediators. However, the role of lipid mediators in this context is still unknown (11). Then, we measured the levels of inflammatory cytokines (IL-6, TNF-α and IL-1β) and a lipid mediator of inflammation (LTB_4) in the plasma of NDM or DM individuals with COVID-19. The results show a significant increase of LTB_4 levels in the sera from DM individuals (Fig. 4A). No statistical differences in the levels of IL-6 (Fig. 4B), TNF-α (Fig. 4C) or IL-1β (Supplementary Fig. 3) were found when comparing DM and NDM individuals. The supplementary Fig 4 shows the production of these inflammatory mediators individually for each patient between NDM and DM groups.

We further detailed the productions of LTB_4, IL-6 and TNF-α among NDM and DM based on the hospitalization type. No differences were found for LTB_4 and TNF- α production (Fig. 4D, F). Regarding IL-6 production, there is a significant increase in the ICU group compared with CB for DM individuals (Fig. 4E). Together, these findings indicate the predominance of LTB_4 production in DM group compared to NDM.

Moreover, IL-6 production seems to be an indicative for COVID-19 severity (hospitalization type) in DM group.

ALOX5 *expression, involved in LTB$_4$ synthesis*, is correlated with clinical outcomes of COVID-19 in individuals with DM

Despite studies reporting diabetes as a risk factor for COVID-19, few studies explored the mechanisms related to these patients' worse prognosis (7,28,29). We compared LTB_4 signaling, in patients with different clinical outcomes associated with COVID-19. Analyzing days spent at the hospital (Fig. 5A) and death rate (Fig. 5B), we found no difference between NDM and DM individuals. However, there is a significantly longer disease duration (the period between symptoms onset and disease outcome - death or hospital discharge) in the DM group (Fig. 5C). These data suggest that DM individuals develop COVID-19 symptoms for prolonged periods, possibly due to the low-grade inflammation already present in DM individuals, even in the absence of an infectious agent. Furthermore, the pulmonary condition in DM group is more severe than in NDM group, measured by Sp02 / Fio02 (Fig. 5D), PaO_2/FiO_2 ratio (Fig. 5E) and O_2 saturation (See supplementary figure 5) at the moment of admission to the hospital. For both parameters, DM individuals arrived at the hospital in a more critical condition.

Finally, we correlated these clinical aspects with LTB_4 production, *ALOX5* and *ACE2* expressions in all individuals (Fig. 6A). The results show a positive correlation between LTB_4 and *ALOX5*, as expected since the 5-LO enzyme produces LTB_4 ($r = 0.5$) (See supplementary figure 6A). Then, we found that *ALOX5* negatively correlates with the worse pulmonary condition, such as $Sp0_2/Fi0_2$ ($r = -0.6$) and $Pa0_2/Fi0_2$ ($r = -0.9$) (Fig. 6B, C). In addition, we found that patients with a low Sp02/Fio02 ratio and increased production of IL-6 have a longer hospital stay for COVID-19 (Fig. 6D).

Taken together, these results show that COVID-19 patients with DM develop a more pronounced systemic inflammatory response, with the predominance of LTB_4 and increased expression of SARS-CoV-2 receptor system *ACE2/TMPRSS2*. These individuals require more frequently critical care assistance due to lung injury, suggesting that LTB_4 signaling could be a mediator produced by DM individuals that increases the risk for severe COVID-19.

Discussion

As SARS-CoV-2 emerged and spread globally, identifying mechanisms involved in severe COVID-19 and its risk factors is crucial for improving disease management. Diabetes is considered a risk factor for severe COVID-19 (5,7,28), but the mechanisms under these complications remain unknown. Inflammation associates with severe COVID-19 (18,21,22), and LTB_4 drives the chronic low-grade inflammation observed in experimental models of diabetes, while its role is not fully elucidated in humans with diabetes (17–19,21,31–33). The present study shows that DM individuals with COVID-19 have increased expression of genes from the LTB_4 pathway in blood cells. During COVID-19, the expression of *ACE2* and *TMPRSS2*, that encode the main receptor system for SARS-CoV-2 cell invasion, are also increased in PBMCs of DM individuals. Moreover, the increased expression of *ALOX5* correlates with *ACE2*, which was present in patients with critical conditions requiring intensive care.

As revealed by pathway analysis, LTB_4 is critical in several physiological disorders (observed in severe COVID-19), including inflammation and respiratory complications, such as pneumonia, respiratory distress syndrome, acute lung injury (11,28). LTB_4 is also an essential molecule in diabetes pathogenesis. Several studies with experimental models indicate that LTB_4 dictates the chronic low-grade inflammation in

diabetes, rendering mice more prone to infections (17,19,34). Our group previously showed that increased production of LTB_4 induced by diabetes alters the outcome of Cutaneous Leishmaniasis (17). Another study showed that LTB_4 is associated with pulmonary complications, such as pneumonia, acute lung injury, acute respiratory distress syndrome (ARDS), and respiratory failure (15,35,36).

The interaction between SARS-CoV-2 and host cells involves several molecules, such as ACE2 and TMPRSS2 that interact with the viral Spike (S) protein (11,37,38). High glucose concentrations increase the expression of *ACE2* and SARS-CoV-2 viral load in human monocytes (27). A meta-analysis revealed an increase of *ACE2* expression in the lung patients with comorbidities, including diabetes (5), and another study showed an increase in the ACE2 protein in the lungs of diabetic individuals (39). Besides the expression of ACE2 in the lung, monocytes and lymphocytes are crucial for the COVID-19 immunopathogenesis (5,11,12,27,37). Our data show that *ACE2* and *TMPRSS2* expression are increased in PBMCs of DM individuals with COVID-19, which can be related to a greater susceptibility to SARS-CoV-2 infection (27,39).

Additionally, *ALOX5* expression positively correlates with *ACE2,* and ICU admission is associated with increased *ALOX5/ACE2* expression in DM patients with COVID-19. The interaction between LTB_4 and ACE2 pathways is still unknown, but the positive independent regulation of these genes in monocytes can influence the process of inflammation and infection, respectively (21,27). During SARS-CoV-2 infection, mononuclear cells are recruited to the lung tissue, where they probably contribute to the control of infection and the healing process, but also causing tissue damage (11).

In the present study, DM individuals with COVID-19, age- and gender-matched to NDM individuals also with COVID-19, had a higher frequency of dyspnea, agreeing with data from Wuhan, China (7). Hypertension is more frequent in diabetic COVID-19

patients and a known risk factor for severe COVID-19 (7,39). According to previous studies, diabetes and hypertension are frequent in COVID-19 patients and may have a role in increased death rates (6,7,40). In our study, mortality rates are similar between COVID-19 patients with or without diabetes, but the disease severity is more pronounced in DM individuals. Although our cohort show no difference in obese individuals between NDM and DM groups, the influence of weight differences among the groups should not be excluded, since obesity condition was determined only by medical observation.

The cytokine storm contributes to mortality in about 28% of fatal COVID-19 cases (11). This condition encompasses several cytokines and chemokines, such as IL-1β, IL-6, IFN-γ, MCP1, CCL2, CXCL10, and TNF-α (11,28). The IL-6 cytokine is one of the most related to the severity of COVID-19, as well as previous studies, our findings also demonstrate this association in the context of COVID-19 in individuals with diabetes (6,8,26). However, there is a lack of knowledge about lipid mediators' implication in the inflammatory response during COVID-19. LTB_4 is a potent inducer of inflammatory cytokines, including those of the cytokine storm, which may drive COVID-19 severity (16,21). Bronchoalveolar lavage fluid exhibits high levels of LTB_4 in an experimental model of acute lung injury (35). LTB_4 plays a significant role in the Chronic Obstructive Pulmonary Disease (COPD), and individuals with severe COPD have high levels of LTB_4 in exhaled air, and such levels correlate with disease severity (14). LTB_4 levels better correlate with lung injury severity and clinical outcomes in Acute Respiratory Distress Syndrome (ARDS) than several other eicosanoids (36).

The number of patients with severe COVID-19 that requires intensive care is a challenge for healthcare systems worldwide. Individuals with ARDS exhibit three to five times more LTB_4 levels than controls (41). The role of LTB_4 in the outcome of lung diseases is associated with neutrophils tissue infiltration, a condition present in COVID-

19 (12). Our group has recently shown that LTB_4 is involved in the activation of pathogen-induced inflammasome (18). A recent preliminary study associated the activation of inflammasome in the lung of COVID-19 patients with a worse disease prognosis (30).

The RECOVERY study showed that dexamethasone slightly reduced death rates among patients with COVID-19 requiring invasive mechanical ventilation or oxygen therapy (42). Additionally, Montelukast, a leukotriene antagonist, is proposed for the prophylaxis of COVID-19 symptoms (43). Together, these studies suggested strategies to treat COVID-19 that, directly or indirectly, act through eicosanoids. Our results confirm that LTB_4 signaling is a crucial branch of the inflammatory response observed in COVID-19 and reinforces the possibility of its inhibition in clinical practice.

Several studies reported the association of diabetes and increased COVID-19 death rates (4,5,19,22), whereas others did not find such an association with disease severity (4,5,34). We have not found a direct association between diabetes and mortality rates in our cohort. The participants with diabetes enrolled in this study developed severe forms of COVID-19, requiring ICU hospitalization, but their evolution seemed similar to patients without diabetes. On the other hand, we found a significant longer disease duration in COVID-19 patients with diabetes. The disease duration refers to the period between the onset of symptoms until the patient's discharge or death, indicating that DM patients develop COVID-19 symptoms for prolonged periods.

Although we have not found a direct association between systemic levels of LTB_4 and a worse COVID-19 prognosis in DM individuals, our findings show that COVID-19 patients with diabetes more frequently present reduced Pa-SpO_2/FiO_2 levels that correlates with ALOX5 expression in the blood. The dissociation between the expression of gene *ALOX5* and its metabolic product may be due to different sources of LTB_4

detected in the bloodstream. Different immune cell types are able to produce LTB_4, such as neutrophils (14), a cell type not represented in our sample of mononuclear cells. LTB_4 is also locally produced at the site of infection caused by different agents (17–19,44) and has been associated with increased lung injury in experimental models (35). Our results add a new player at the inflammation panorama of COVID-19, suggesting that circulating mononuclear cells already present a pro-inflammatory profile that, once recruited to the lung, may amplify local inflammation and tissue injury. Further studies are necessary to confirm pulmonary production of LTB_4 and its role in COVID-19 outcomes.

In summary, our findings show that diabetes induces a pro-inflammatory profile on circulating immune cells with increased expression of *ACE2* and *ALOX5* genes, rendering these cells more prone to SARS-CoV-2 invasion. Together, our data reveal a potential role of LTB_4 in COVID-19, which is poorly explored, and open new ways to study implications and applications of this mediator in SARS-CoV-2 infection. Furthermore, we found that IL-6, a known cytokine for COVID-19 severity, is also a potential indicative for DM individuals in need of intensive care assistance.

Acknowledgments

We thank the developers of the MetScape and MetDisease software for making it possible to analyze the data in a more integrated way. Dra. Manuela da Silva Solcà for her help in the construction of the Table 1. To Health professionals who participated directly and indirectly in the care of patients.

Author Contributions

I.B.S., T.C.S., S.N., R.L.S., R.K., P.R.S.O., A.B., C.B., M.B.N., V.B., and N.M.T. contributed to the writing of the article or substantial involvement in its revision before

submission. M.R.S.C., A.F.A.M., and J.R.C. conducted the medical care of the research participants. I.B.S., J.S., and S.N. conducted the processing of biological samples in the laboratory. I.B.S., A.F.A.M., T.C.S., H.C.S and S.N. contributed to the acquisition of the data or the analysis and interpretation of information. I.B.S., N.M.T., and V.B.B. were involved in the conception, hypotheses delineation, and design of the study. N.M.T. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Foundation for Scientific and Technological Development in Health (FIOTEC), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) under Finance Code 001 and Conselho Nacional de Desenvolvimento Científico e Tecnológico – BRAZIL (CNPq).

References

1. WHO Coronavirus (COVID-19) Dashboard [updated 17 May 2021]. Available from: https://covid19.who.int

2. Zhou P, Yang X Lou, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature [Internet]. 2020;579(7798):270–3. Available from: http://dx.doi.org/10.1038/s41586-020-2012-7

3. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical Characteristics of

Coronavirus Disease 2019 in China. N Engl J Med. 2020;382(18):1708–20.

4. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA - J Am Med Assoc. 2020;323(11):1061–9.

5. Pinto BGG, Oliveira AER, Singh Y, Jimenez L, Gonçalves ANA, Ogava RLT, et al. ACE2 Expression is Increased in the Lungs of Patients with Comorbidities Associated with Severe COVID-19. J Infect Dis. 2020;

6. Sardu C, D'Onofrio N, Balestrieri ML, Barbieri M, Rizzo MR, Messina V, et al. Outcomes in Patients with Hyperglycemia Affected by COVID-19: Can We Do More on Glycemic Control? Diabetes Care. 2020;43(7):1408–15.

7. Shi Q, Zhang X, Jiang F, Zhang X, Hu N, Bimu C, et al. Clinical Characteristics and Risk Factors for Mortality of COVID-19 Patients with Diabetes in Wuhan, China: A Two-Center, Retrospective Study. Vol. 43, Diabetes Care. 2020. p. 1382–91.

8. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol [Internet]. 2020;146(1):110–8. Available from: https://doi.org/10.1016/j.jaci.2020.04.006

9. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting Characteristics, Comorbidities, and Outcomes among 5700 Patients Hospitalized with COVID-19 in the New York City Area. JAMA - J Am Med Assoc. 2020;323(20):2052–9.

10. Federation ID. IDF Diabetes Atlas - 2019. International Diabetes Federation.

2019. 144 p.

11. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol [Internet]. 2020;20(6):363–74. Available from: http://dx.doi.org/10.1038/s41577-020-0311-8

12. Zhang B, Zhou X, Qiu Y, Feng F, Feng J, Jia Y, et al. Clinical characteristics of 82 death cases with COVID-19. medRxiv [Internet]. 2020;2020.02.26.20028191. Available from: http://medrxiv.org/content/early/2020/02/27/2020.02.26.20028191.abstract

13. Peters-golden M, Henderson WR. Leukotrienes. new Engl J o f Med. 2007;357:1841–54.

14. Biernacki WA, Kharitonov SA, Barnes PJ. Increased leukotriene B4 and 8-isoprostane in exhaled breath condensate of patients with exacerbations of COPD. Thorax. 2003;58(4):294–8.

15. Auner B, Geiger E V., Henrich D, Lehnert M, Marzi I, Relja B. Circulating leukotriene B4 identifies respiratory complications after trauma. Mediators Inflamm. 2012;2012.

16. Serezani CH, Lewis C, Jancar S, Peters-golden M. Leukotriene B 4 amplifies NF- κ B activation in mouse macrophages by reducing SOCS1 inhibition of MyD88 expression. 2011;121(2):671–82.

17. Bonyek-Silva I, Nunes S, Santos RLS, Lima FR, Lago A, Silva J, et al. Unbalanced Production of LTB 4/PGE 2 Driven by Diabetes Increases Susceptibility to Cutaneous Leishmaniasis. Emerg Microbes Infect. 2020;

18. Salina ACG, Brandt SL, Klopfenstein N, Blackman A, Bazzano JMR, Sá-Nunes A, et al. Leukotriene B4 licenses inflammasome activation to enhance skin host defense. Proc Natl Acad Sci [Internet]. 2020; Available from: https://www.pnas.org/content/early/2020/11/11/2002732117

19. Brandt SL, Wang S, Dejani NN, Klopfenstein N, Winfree S, Filgueiras L, et al. Excessive localized leukotriene B4 levels dictate poor skin host defense in diabetic mice. JCI insight. 2018;3(17).

20. Li P, Oh DY, Bandyopadhyay G, Lagakos WS, Talukdar S, Osborn O, et al. LTB4 promotes insulin resistance in obese mice by acting on macrophages, hepatocytes and myocytes. Nat Med [Internet]. 2015;21(3):239–47. Available from: http://www.nature.com/doifinder/10.1038/nm.3800

21. Brandt SL, Serezani CH. Too much of a good thing: How modulating LTB 4 actions restore host defense in homeostasis or disease. Semin Immunol [Internet]. 2017;33(August 2016):37–43. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1044532316300793

22. Afonso P V., Janka-Junttila M, Lee YJ, McCann CP, Oliver CM, Aamer KA, et al. LTB4 Is a Signal-Relay Molecule during Neutrophil Chemotaxis. Dev Cell [Internet]. 2012;22(5):1079–91. Available from: http://dx.doi.org/10.1016/j.devcel.2012.02.003

23. Morato CI, da Silva IA, Borges AF, Dorta ML, Oliveira MAP, Jancar S, et al. Essential role of leukotriene B4 on Leishmania (Viannia) braziliensis killing by human macrophages. Microbes Infect. 2014;

24. Luo L, Zhou WH, Cai JJ, Feng M, Zhou M, Hu SP, et al. Gene Expression

Profiling Identifies Downregulation of the Neurotrophin-MAPK Signaling Pathway in Female Diabetic Peripheral Neuropathy Patients. J Diabetes Res. 2017;2017.

25. Lyra R, Oliveira M, Lins D, Cavalcanti N, Gross JL, Maia FFR, et al. Sociedade Brasileira de Diabetes. Vol. 5, Diabetes Mellitus Tipo 1 e Tipo2. 2020. 709–717 p.

26. Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Emerg Microbes Infect. 2020;9(1):1123–30.

27. Codo AC, Davanzo GG, Monteiro LDB, Souza GF De, Muraro SP, Carregari VC, et al. Elevated glucose levels favor SARS-CoV-2 infection and monocyte response through a HIF-1α/glycolysis dependent axis. Cell Metab. 2020;

28. Muniyappa R, Gubbi S. COVID-19 pandemic, coronaviruses, and diabetes mellitus. Am J Physiol - Endocrinol Metab. 2020;318(5):E736–41.

29. Rao S, Lau A, So HC. Exploring Diseases/Traits and Blood Proteins Causally Related to Expression of ACE2, the Putative Receptor of SARS-CoV-2: A Mendelian Randomization Analysis Highlights Tentative Relevance of Diabetes-Related Traits. Diabetes Care. 2020;43(7):1416–26.

30. Rodrigues TS, Sa KSG, Ishimoto AY, Becerra A, Oliveira S, Almeida L, et al. Inflammasome activation in COVID-19 patients. medRxiv [Internet]. 2020 Jan 1;2020.08.05.20168872. Available from: http://medrxiv.org/content/early/2020/08/06/2020.08.05.20168872.abstract

31. Zhang Y, Olson RM, Brown CR. Macrophage LTB4 drives efficient

phagocytosis of Borrelia burgdorferi via BLT1 or BLT2. J Lipid Res [Internet]. 2017 Jan 4 [cited 2017 Jan 12];jlr.M068882. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28053185

32. Filgueiras LR, Henrique Serezani C, Jancar S. Leukotriene B4 as a potential therapeutic target for the treatment of metabolic disorders. Front Immunol. 2015;6(OCT):13–6.

33. Das UN. Is There a Role for Bioactive Lipids in the Pathobiology of Diabetes Mellitus? 2017;8(August).

34. Filgueiras LR, Brandt SL, Wang S, Wang Z, Morris DL, Evans-Molina C, et al. Leukotriene B 4 -mediated sterile inflammation favors susceptibility to sepsis in murine type 1 diabetes HHS Public Access. Sci Signal. 2015;8(361).

35. Eun JC, Moore EE, Mauchley DC, Johnson CA, Meng X, Banerjee A, et al. The 5-lipoxygenase pathway is required for acute lung injury following hemorrhagic shock. Shock. 2012;37(6):599–604.

36. Masclans JR, Sabater J, Sacanell J, Chacon P, Sabin P, Roca O, et al. Possible prognostic value of leukotriene B4 in acute respiratory distress syndrome. Respir Care. 2007;52(12):1695–700.

37. Radzikowska U, Ding M, Tan G, Zhakparov D, Peng Y, Wawrzyniak P, et al. Distribution of ACE2, CD147, CD26 and other SARS-CoV-2 associated molecules in tissues and immune cells in health and in asthma, COPD, obesity, hypertension, and COVID-19 risk factors. Allergy. 2020. 0–3 p.

38. Ing P, Bello I, Areiza M, Oliver J. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein Ke. J Chem Inf Model. 2020;53(9):45–50.

39. Wijnant SR, Jacobs M, Van Eeckhoutte HP, Lapauw B, Joos GF, Bracke KR, et al. Expression of ACE2, the SARS-CoV-2 Receptor, in Lung Tissue of Patients With Type 2 Diabetes. Diabetes. 2020;(September):db200669.

40. Mudatsir M, Fajar JK, Wulandari L, Soegiarto G, Ilmawan M, Purnamasari Y, et al. Predictors of COVID-19 severity : a systematic review and meta-analysis [version 1 ; peer review : 2 approved]. 2020;2019:1–24.

41. Davis JM, Yurt RW, Barie PS, Verma M, Dineen P, Shires GT, et al. Leukotriene B4 Generation in Patients With Established Pulmonary Failure. Arch Surg. 1989;124.

42. Building RD, Campus OR, Drive R. Effect of Dexamethasone in Hospitalized Patients with COVID-19 – Preliminary Report. 2020;

43. Sanghai N, Tranmer GK. Taming the cytokine storm: repurposing montelukast for the attenuation and prophylaxis of severe COVID-19 symptoms. Drug Discov Today [Internet]. 2020;00(00):1–4. Available from: https://doi.org/10.1016/j.drudis.2020.09.013

44. Serezani CH, Perrela JH, Russo M, Peters-Golden M, Jancar S. Leukotrienes Are Essential for the Control of Leishmania amazonensis Infection and Contribute to Strain Variation in Susceptibility. J Immunol [Internet]. 2006;177(5):3201–8. Available from: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.177.5.3201

Table 1 Characteristics of individuals hospitalized due to complications of COVID-19, Salvador, Brazil, 2020, (n = 53).

	COVID-19		
Parameter	Without diabetes	With diabetes	*p valor*
Patients number, *n*	24	29	
Male sex, *n* (%)	15 (62.5)	16 (55)	0.59
Age Years, median (min-max)	59 (27-88)	59 (43-93)	0.12
HbA1c % (mmol/mol), median [min-max]	5.6 (38) [4.5 (26)-6.3 (45)]	7.9 (63) [6.5 (48)-12.9 (117)]	<0.0001
Comorbidities, *n/N* (%)			
Obesity	3/18 (16.6)	7/21 (33.3)	0.23
Dyslipidemia	3/13 (23.0)	3/11 (27.2)	0.99
Liver disease	1/22 (4.5)	0/24 (0.0)	0.47
Kidney disease	8/24 (33.3)	5/27 (18.5)	0.22
COPD	3/16 (18.7)	3/14 (21.4)	0.99
HAS	9/24 (37.5)	22/26 (84.6)	0.001
Symptoms, *n/N* (%)			
Fever	12/19 (63.1.0)	14/21 (66.6)	0.99
Cough	16/23 (69.5.5)	16/22 (72.7)	0.81
Dyspnea	13/22 (59.0)	22/24 (91.6)	0.01
Expectoration	1/17 (5.8)	3/16 (18.7)	0.33
COVID-19 confirmed, *n/N* (%)	18/21 (85.7)	26/27 (96.3)	0.30

HAS *Systemic Arterial Hypertension;* ***COPD*** *Chronic Obstructive Pulmonary disease;* ***n*** *Positive number;* ***N*** *Valid numbers.*

Figure legends

Figure 1. Upregulation of LTB_4 signaling in individuals with diabetes (DM). Volcano plot with differentially expressed genes (DEGs) (blue, upregulated genes; yellow, down-regulated genes) in PBMCs from DM individuals compared to non-DM (NDM) (A). Workflow to identify molecules associated with inflammation and respiratory disorders based on gene expression showed in A and the resulting Venn diagram showing molecules in common between pneumonia, respiratory syndrome, acute lung injury and inflammation (B). Enriched pathways raised from DEGs analyses of PBMCs from DM individuals compared to NDM, highlighting in red the central position of leukotriene metabolism among pathways (C). Fold change of genes involved with LTB_4 production (*ALOX5AP* and *ALOX5*) and signaling (*LTB4R*) in PBMCs of DM individuals compared to NDM (D). Dotted line = Cutoff point for a DEG; Complete line = Average of the control group. Data shown as median. ** $p<0.01$.

Figure 2. Increased expression of *ALOX5* and *ACE2/TMPRSS2* receptor system for SARS-CoV-2 infection in diabetic (DM) individuals with COVID-19. Expressions of *ALOX5* (A), *ACE2* (B), *TMPRSS2* (C), *FURIN* (D) and *CD147* (E) in PBMCs from DM and non-DM (NDM) individuals. Data shown as mean. * $p<0.05$; ** $p<0.01$.

Figure 3. *ALOX5* expression positively correlates with ACE2 expression in diabetic (DM) individuals with COVID-19 and this is associated with increased rate in intensive care unit (ICU) admission. Correlation matrix between *ALOX5* and *ACE2/TMPRSS2* expressions in PBMCs from DM (red) and non-DM (NDM; gray) individuals (A). Correlation analysis between *ACE2* and *TMPRSS2* expressions in PBMCs from all individuals with COVID-19 (B). Correlation analysis between *ALOX5*

and *ACE2* expressions in PBMCs from DM patients (C). Types of hospitalization among DM or NDM individuals with COVID-19: based on the expression of *ACE2* (D) or *ALOX5* (E). CB: Clinical Beds. Data shown as median. Spearman r correlation. * $p<0.05$; ** $p<0.01$

Figure 4. Increased systemic levels of LTB_4 in diabetic (DM) individuals with COVID-19. Levels of LTB4 (A), IL-6 (B) and TNF-α (C) in plasma samples from DM and non-DM (NDM) individuals affected by COVID-19. Global and individualized view of LTB4 (red), IL-6 (green) and TNF-α (blue) production in NDM (D) and DM (E) individuals with COVID-19 through Cubic spline analysis. Data shown as mean. * $p<0.05$.

Figure 5. Diabetes induces greater severity of COVID-19. Number of days that non-DM (NDM) and DM individuals remained hospitalized in Clinical Beds (CB) or Intensive Care Unit (ICU) due to COVID-19 (A). Percentage of survival between NDM and DM individuals hospitalized with COVID-19 (B). Disease duration measured from the onset of symptoms to hospital discharge for NDM and DM individuals with COVID-19 (C). O_2 saturation of NDM and DM individuals with COVID-19 (D). Degree of lung injury in NDM and DM individuals with COVID-19 (E). Data shown as median in A, B, D and mean in C. * $p<0.05$.

Figure 6. *ALOX5* plays a role in the severity of COVID-19 diabetic (DM) individuals with COVID-19. Correlation matrix between genes, inflammatory parameters and clinical outcome changes found in all patients with COVID-19 (A). Dispersion of values with all patients between the correlation of ALOX5 with $Sp0_2/Fi0_2$ (B), $Pa0_2/Fi0_2$ (C),

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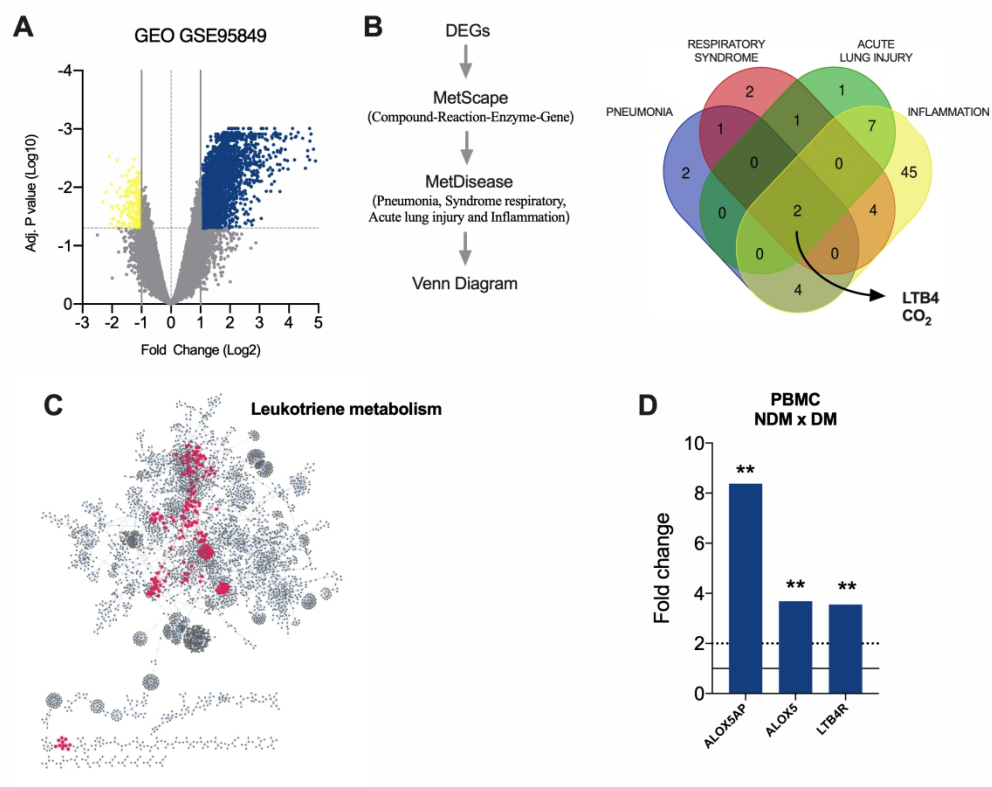

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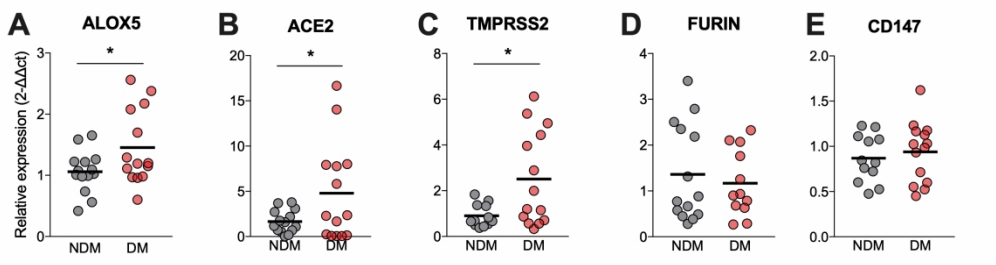

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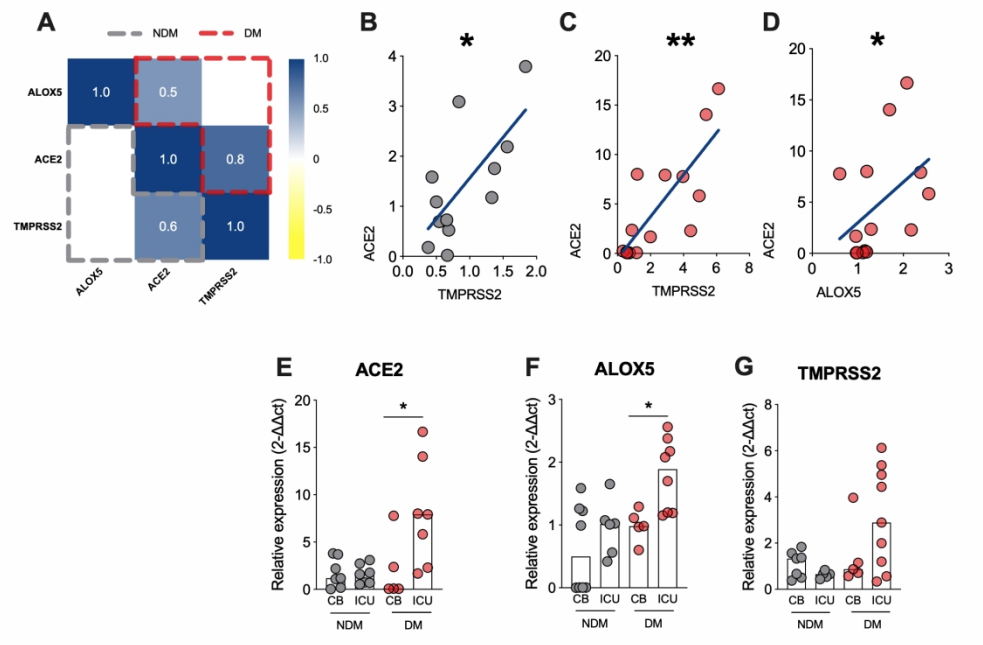

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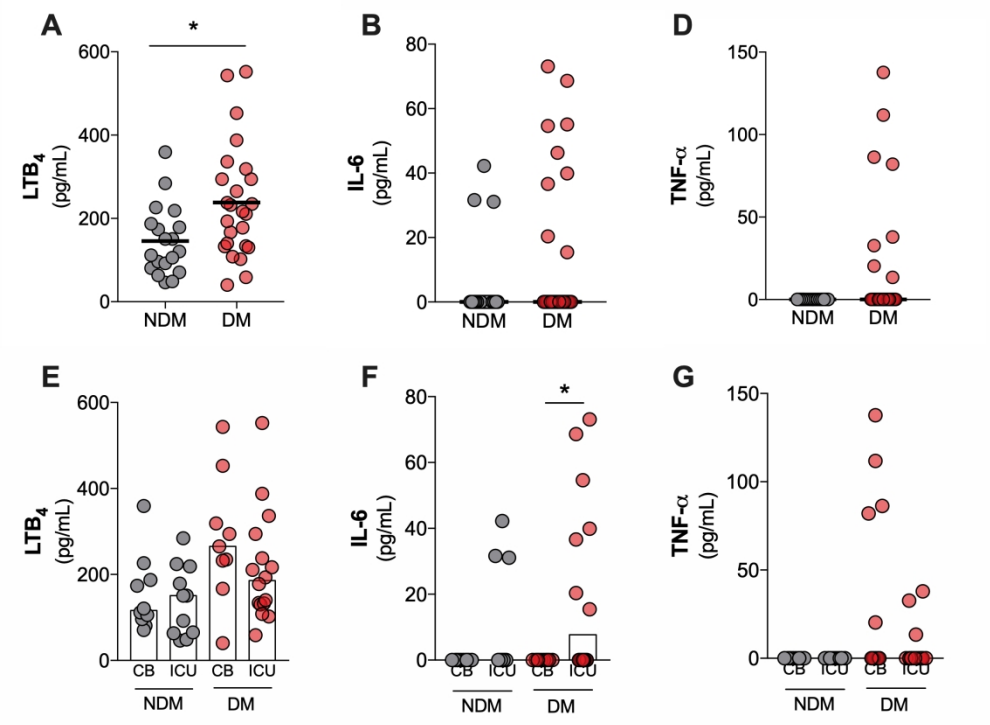

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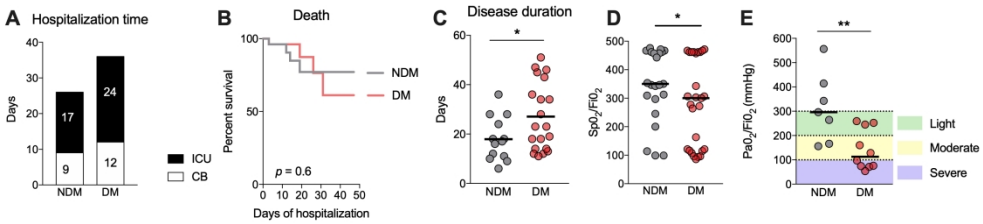

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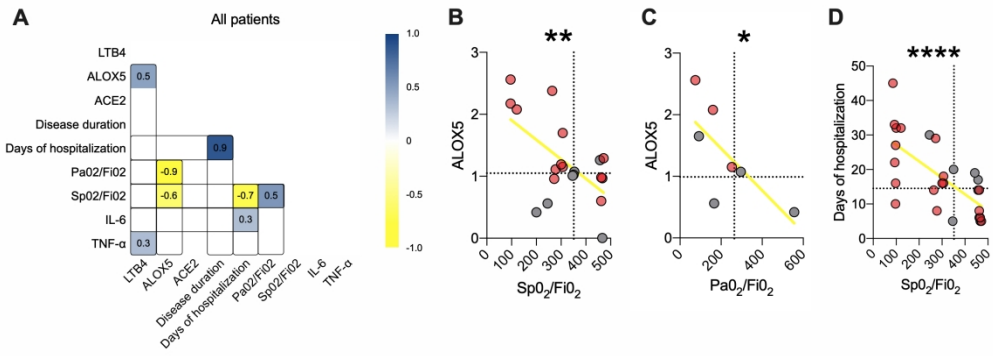

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Supplementary Figure 1. Tracheal secretion from COVID-19 patients with DM trends toward increased expression of ALOX5 and ACE2/TMPRSS2 viral receptors. Relative expression of ACE2 (A), TMPRSS2 (B) and ALOX5 (C) in tracheal secretion of NDM and DM patients with COVID-19. Dotted line = control group relarive expression. Data shown as median.

Supplementary Figure 2. No correlation between ACE2 and ALOX5 is observed in NDM COVID-19 patients. Correlation between the expression of ALOX5 and ACE2 in PBMCs of NDM individuals with COVID-19 (A). Spearman r correlation.

Supplementary Figure 3. No difference in IL-1β production between NDM and DM groups of patients with COVID-19. IL-β serum levels in NDM and DM individuals with COVID-19. Dotted line = cut-off for detection limit. Data shown as median.

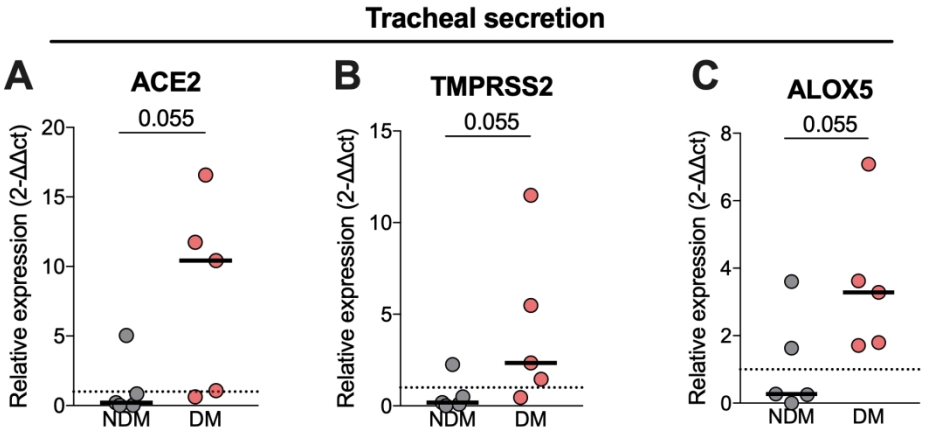
Supplementary Figure 4. LTB_4 is the most prevalent inflammatory mediator at systemic levels. Global and individualized view of LTB4 (red), IL-6 (green) and TNF-α (blue) production in NDM (A) and DM (B) individuals with COVID-19 through Cubic spline analysis.

Supplementary Figure 5. O_2 saturation levels are reduced in DM patients with COVID-19 compared with NDM individuals. SpO2 in NDM and DM patients with COVID-19. Data shown as median. * $p<0.05$.

Supplementary Figure 6. Correlation between inflammatory mediators and clinical parameters of NDM and DM individuals with COVID-19. Correlation between ALOX5 and LTB_4 (A), PaO_2/FiO_2 and SpO_2/FiO_2 (B), disease duration and hospitalization days (C), IL-6 and hospitalization days (D), TNF-α and LTB_4 (E) in NDM and DM patients with COVID-19. Dotted lines = median of NDM group. Spearman r correlation. * $p<0.05$. **** $p<0.0001$.

Supplementary Figure 7. Liver enzymes are altered in DM patients with COVID-19 compared to NDM group. Quantification of Leucogram (A), platelets (B), C-reactive protein (C), Lactate dehydrogenase (D), Glutamic-oxalacetic Transaminase (E), Glutamic

Pyruvic Transaminase (F), Gamma-glutamyl transferase (G), Alkaline phosphatase (H), Lactate (I), Urea (J) and Creatinine (K) in NDM and DM patients with COVID-19. Data shown in median. * $p<0.05$.



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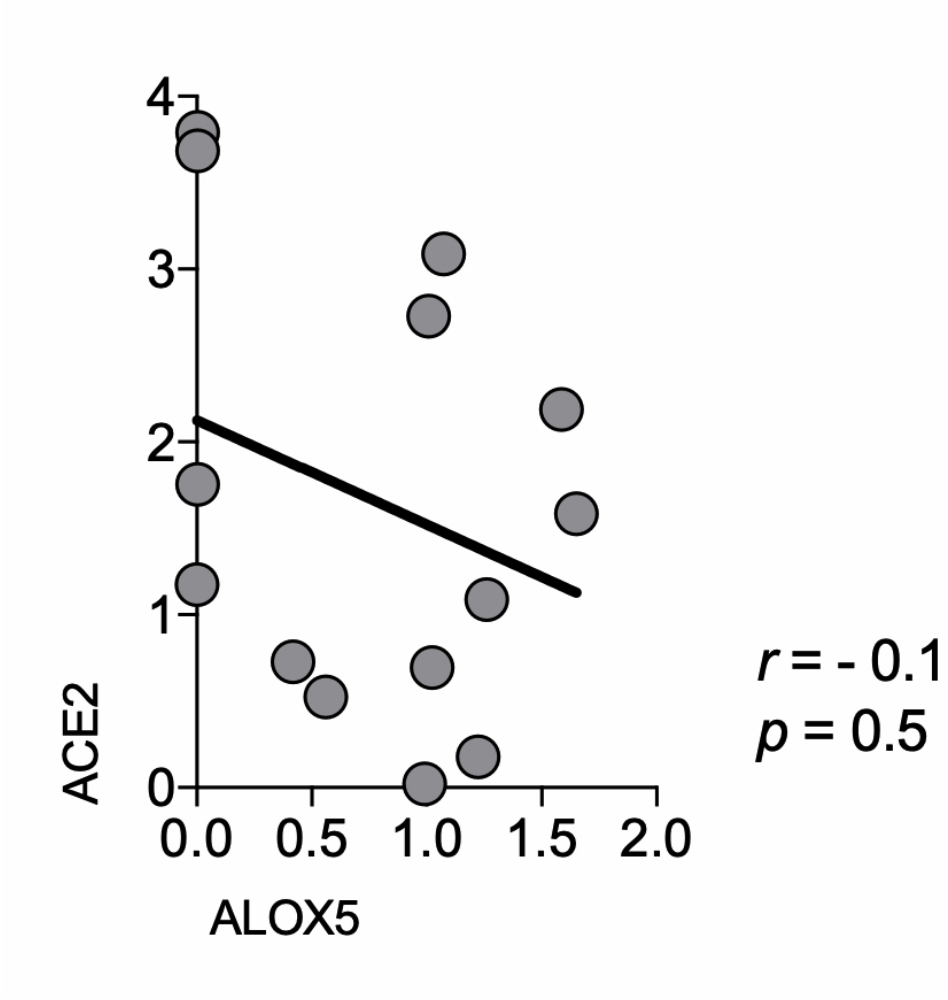

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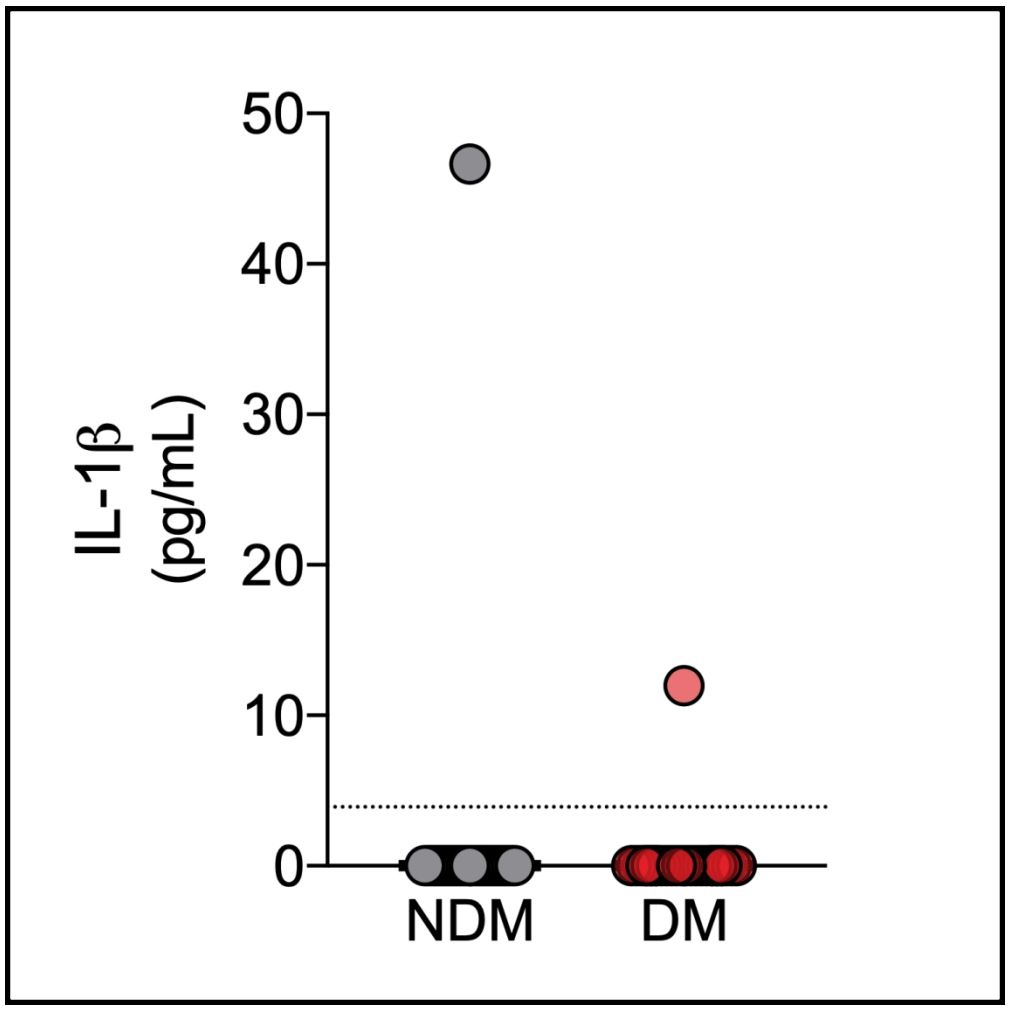

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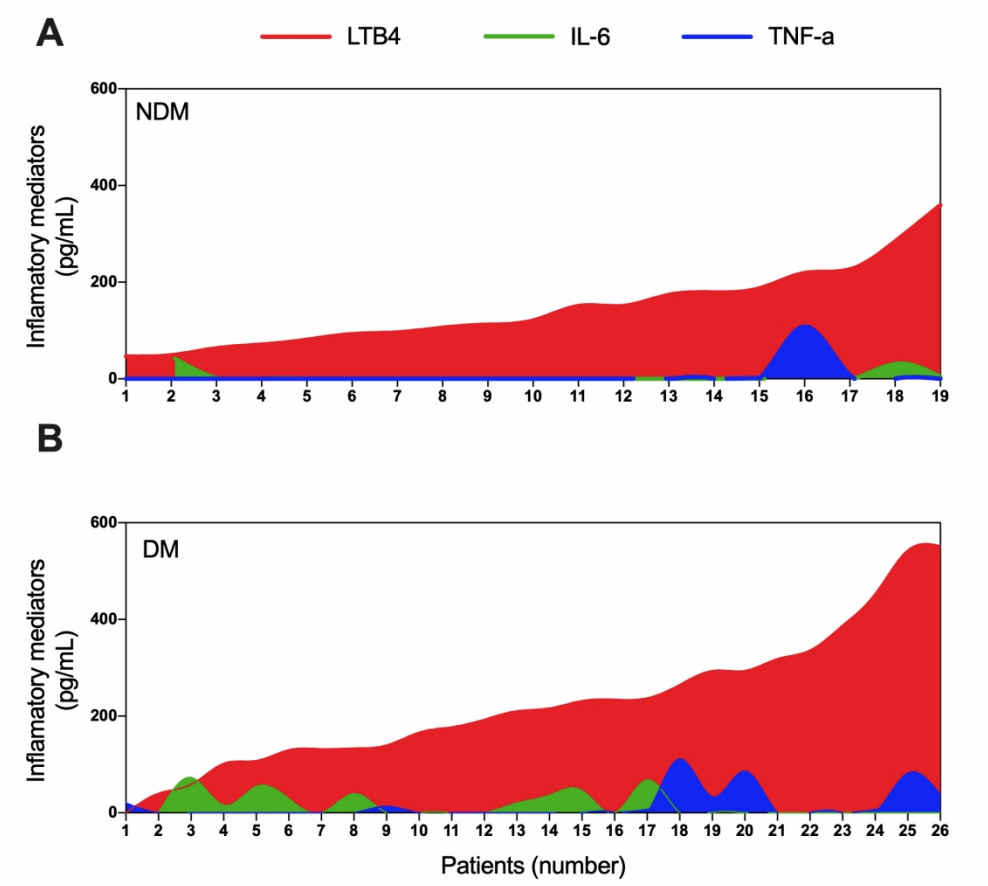

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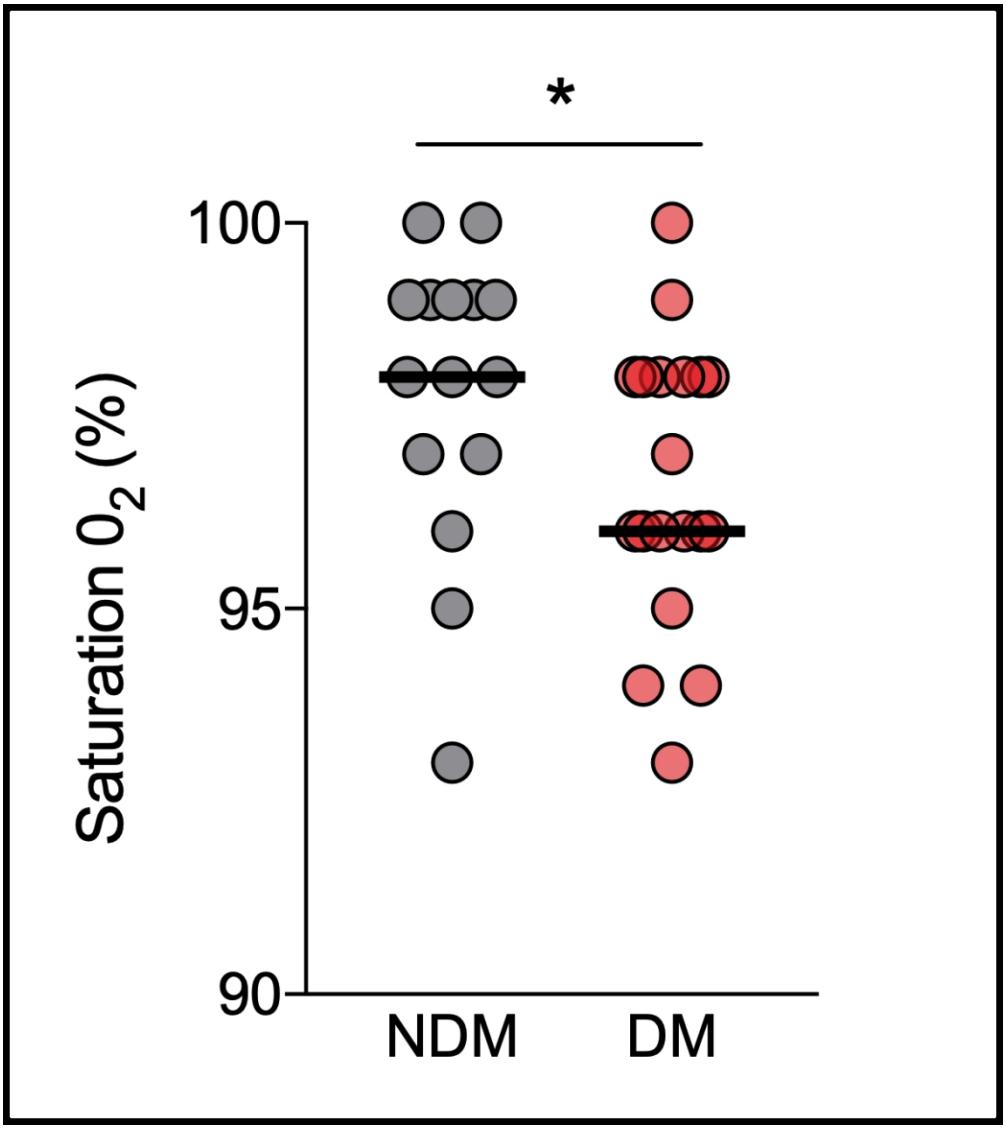

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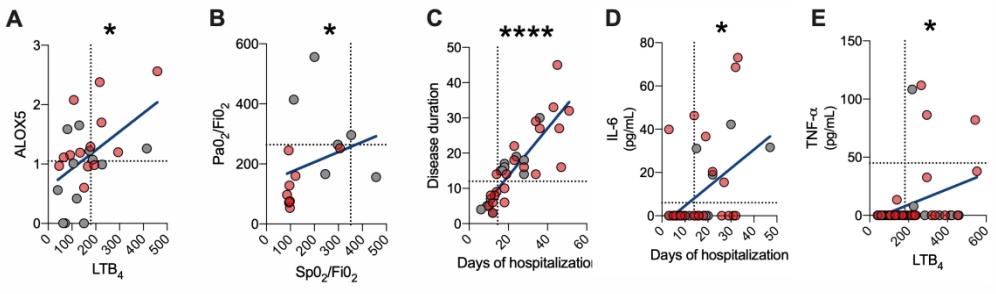

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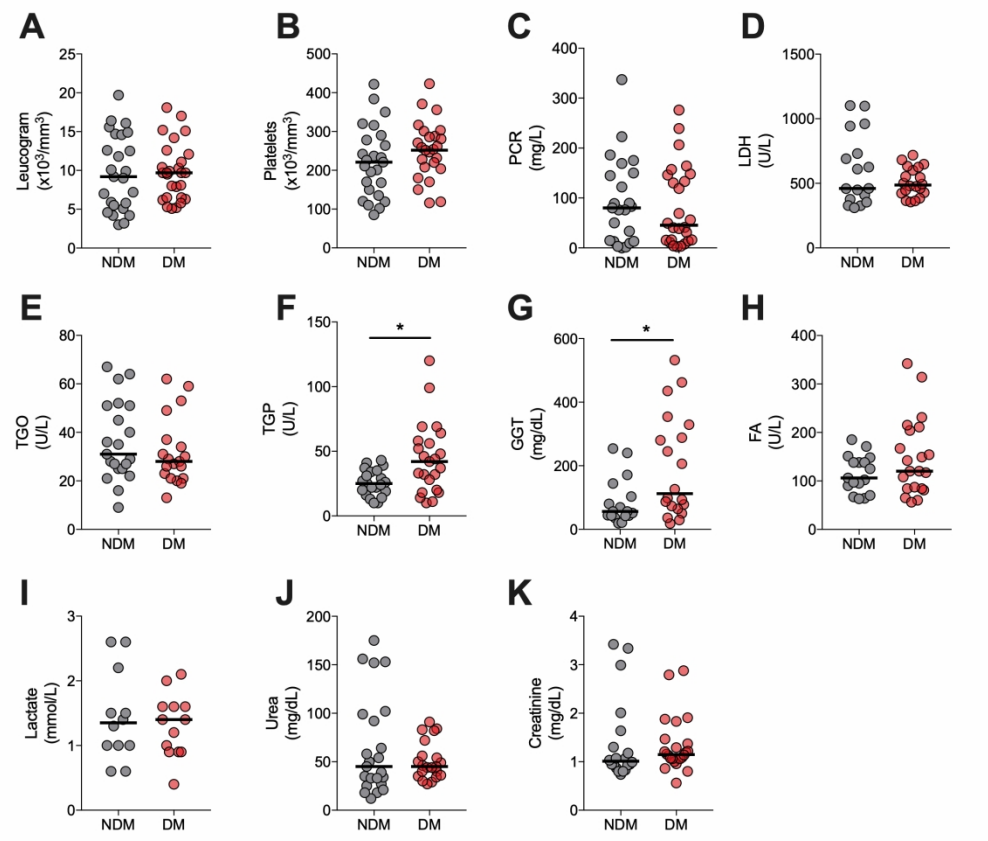

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Supplementary Table 1. Pathways found on the basis of DEGs between NDM and DM
3-oxo-10R-octadecatrienoate beta-oxidation
Aminosugars metabolism
Androgen and estrogen biosynthesis and metabolism
Arachidonic acid metabolism
Bile acid biosynthesis
Biopterin metabolism
Butanoate metabolism
C21-steroid hormone biosynthesis and metabolism
De novo fatty acid biosynthesis
Dimethyl-branched-chain fatty acid mitochondrial beta-oxidation
Di-unsaturated fatty acid beta-oxidation
Endohydrolysis of 1,4-alpha-D-glucosidic linkages in polysaccharides by alpha-amylase
Fructose and mannose metabolism
Galactose metabolism
Glycerophospholipid metabolism
Glycine, serine, alanine and threonine metabolism
Glycolysis and Gluconeogenesis
Glycosphingolipid biosynthesis - ganglioseries
Glycosphingolipid biosynthesis - globoseries
Glycosphingolipid biosynthesis - neolactoseries
Glycosphingolipid metabolism
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis
Histidine metabolism
Leukotriene metabolism
Linoleate metabolism
Lysine metabolism
Methionine and cysteine metabolism
Mono-unsaturated fatty acid beta-oxidation
N-Glycan biosynthesis
O-Glycan biosynthesis
Omega-3 fatty acid metabolism
Omega-6 fatty acid metabolism
Pentose phosphate pathway
Phosphatidylinositol phosphate metabolism

Phytanic acid peroxisomal oxidation
Porphyrin metabolism
Propanoate metabolism
Prostaglandin formation from arachidonate
Prostaglandin formation from dihomo gama-linoleic acid
Proteoglycan biosynthesis
Purine metabolism
Putative anti-Inflammatory metabolites formation from EPA
Pyrimidine metabolism
Saturated fatty acids beta-oxidation
Selenoamino acid metabolism
Squalene and cholesterol biosynthesis
TCA cycle
Trihydroxycoprostanoyl-CoA beta-oxidation
Tryptophan metabolism
Tyrosine metabolism
Urea cycle and metabolism of arginine, proline, glutamate, aspartate and asparagine
Valine, leucine and isoleucine degradation
Vitamin A (retinol) metabolism
Vitamin B2 (riboflavin) metabolism
Vitamin B3 (nicotinate and nicotinamide) metabolism
Vitamin B5 - CoA biosynthesis from pantothenate
Vitamin B6 (pyridoxine) metabolism
Vitamin B9 (folate) metabolism
Vitamin E metabolism
Vitamin K metabolism
Xenobiotics metabolism