# Circulating Dickkopf1 parallels metabolic adaptations and predicts disease trajectories in patients with Covid-19

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None of the authors has any conflict of interest to declare.

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**Abstract**

*Background and aims*

Coronavirus disease 19 (Covid-19) trajectories show high interindividual variability, ranging from asymptomatic manifestations to fatal outcomes, the latter of which may be fueled by immunometabolic maladaptation of the host. Reliable identification of patients, who are at risk of severe disease remains challenging. We hypothesized that serum concentrations of Dickkopf1 (DKK1) indicate disease outcomes in SARS-CoV-2 infected individuals.

*Methods*

We recruited hospitalized patients with PCR-confirmed SARS-CoV-2 infection and included 80 individuals, for whom blood samples from two independent time points were available. DKK1 serum concentrations were measured by ELISA in paired samples. Clinical data was extracted from patient charts and correlated with DKK1 levels. Publicly available datasets were screened for changes in cellular DKK1 expression upon SARS-CoV-2 infection. Plasma metabolites were profiled by NMR spectroscopy in an unbiased fashion and correlated with DKK1 data. Kaplan Meier and Cox regression analysis were used to investigate the prognostic value of DKK1 levels in the context of Covid-19.

*Results*

We report that serum levels of DKK1 predict disease outcomes in patients with Covid-19. Circulating DKK1 concentrations are characterized by high interindividual variability and change as a function of time during SARS-CoV-2 infection, which is linked to platelet counts. We further find that the metabolic signature associated with SARS-CoV-2 infection resembles fasting metabolism and is mirrored by circulating DKK1 abundance. Patients with low DKK1 levels are twice as likely to die from Covid-19 than those with high levels and DKK1 predicts mortality independent of markers of inflammation, renal function and platelet numbers

*Conclusion*

Our study suggests a potential clinical use of circulating DKK1 as a predictor of disease outcomes in patients with Covid-19. These results require validation in additional cohorts.

## Introduction

The coronavirus disease 2019 (Covid-19) pandemic emerged in 2019 as a cluster of pneumonia of unknown cause as reported by the Chinese Center for Disease Control and Prevention. Following successful isolation and genome identification, the Covid-19-inducing virus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), corresponding to its close phylogenetic relationship with other members of the *Betacoronavirus* genus such as severe acute respiratory syndrome coronavirus (SARS-CoV) or middle east respiratory syndrome coronavirus (MERS-CoV) (1,2). Since its initial discovery, SARS-CoV-2 has spread rapidly across the globe and imposed considerable socioeconomic and medical costs on societies (3). This spreading has been paralleled by the evolution of novel SARS-CoV-2 variants, which differ in their molecular characteristics and virulence (4). Covid-19 is characterized by highly variable clinical manifestations; with symptoms ranging from fully asymptomatic to severe forms that result in multiorgan failure and death(2-5). Reliable tools to identify patients at risk of developing severe Covid-19 remains challenging. In this study, we explored if circulating levels of Dickkopf1 (DKK1), a multifunctional protein produced by platelets and the skeleton with immunomodulatory properties (6-9), change in response to SARS-CoV-2 infection and if variation in DKK1 serum concentration indicates fatal disease trajectories.

## Methods

### *Study participants*

Hospitalized patients with PCR-confirmed SARS-CoV-2 infection were recruited as part of BioBank Dresden (BBD) project, which was approved by the Ethics Committee of the TU Dresden (EK378092017 and EK9012022). Written informed consent was obtained from all study participants prior to inclusion and blood samples were collected at various time points. Collection and processing of blood samples was performed according to standard operating

procedures. Serum was obtained by centrifugation of whole blood for 10 min, 2000xG at room temperature. Samples were processed in aliquots, which were immediately cooled down, cryopreserved using liquid nitrogen and stored at minus 80 degrees Celsius until further analysis. From this cohort of patients, individuals were included in the current study if serum samples from two independent time points were available, allowing for a paired design. Patients with only one sample available were excluded from the study. No other in- or exclusion criteria were prespecified. In total, 80 subjects (2 samples/patient) met the criteria and were included in the study. Clinical data and routine laboratory testing data was extracted from patient charts, which were incomplete or unavailable from 4 individuals. Detailed characteristics of the study population are summarized in **Table 1.** Of note, the relatively long sampling period of the study (April 2020 – June 2021) together with the rapidly evolving treatment recommendations for patients with Covid-19 may have introduced some bias into our study (e.g. glucocorticoids became standard of care during the pandemic but were rarely administered in the beginnings).

Following approval by the Ethics Committee of the TU Dresden (EK273062016), serum samples from healthy individuals were obtained at the Department of Transfusion Medicine, Technische Universität Dresden, between June and July 2022. Samples were collected during routine visits for blood donation. All study participants provided written informed consent. Subjects were randomly selected. Chronic illnesses and ongoing pharmacotherapy were considered exclusion criteria as defined by blood donor standards.

*Enzyme linked sorbent assay (ELISA)*

Circulating DKK1 levels were measured in serum samples using a commercially available ELISA kit (Biomedica, Vienna, Austria, cat# BI-20413, RRID: AB_2922680**)** according to the manufacturer's instructions. The sensitivity of the assay was 1.7 pmol/l and intra-assay precision was <3% CV. Samples were processed in a blinded fashion and measurements were performed on a conventional plate-reader (Omega, BMG Labtech, Vienna, Austria). Data were analyzed using 4-parameter logistic regression and expressed as pmol/l.

*NMR spectroscopy and bioinformatics*

$^{1}$H-nuclear magnetic resonance (NMR) spectroscopy measurements were performed according to established protocols(10). The frozen serum samples were thawed at room temperature for 30 minutes before being mixed with phosphate buffer (1:1) to a volume of 600µl. The phosphate buffer also contained the internal reference TMSP. The resulting suspension was pipetted into the NMR sample tube and immediately prepared for measurement. All NMR measurements were run on a Bruker 600 Mhz Avance III Neo equipped with a BBI Probe and a Bruker SampleJet robot with a cooling system for sample storage at 4°C. The samples were measured at 310 K and a full quantitative calibration was completed before the measurement. All measurements followed the Bruker in-vitro diagnostics (IVDr) standard operating procedures and methods. All data was processed in automation using Bruker TopSpin 4.1.1 and ICON NMR. Automatic metabolite and lipoprotein reports were obtained using Bruker IVDr B.I Methods Plasma (B.I.Quant-PS, v2.0.0) and Bruker IVDr Lipoprotein Sublcass Analysis (B.I.LISA, v1.0.0). The analysis was performed on a 1D Nuclear Overhauser Spectroscopy experiment. Metabolites that were undetectable in serum samples were excluded from statistical analysis. All other metabolites were ranked according to their median fold-change over time (sample2/sample1) and clustered via the HMDB KEGG database.

*Publicly available datasets*

Changes in DKK1 transcript expression in human lung epithelial cells as well as human lung tissue in response to SARS-CoV-2 and influenza infection were evaluated using publicly available datasets (https://www.immgen.org/Databrowser19/COVID19Databrowser.html). Lung specimens were obtained post-mortem from two Covid-19-positive males (age>60), while uninfected lung control tissues were collected post-surgery at Icahn School of Medicine (11). Raw data were downloaded, visualized and analyzed using Prism (Graphpad, LaJolla, CA).

*Statistics*

No sample size calculations were performed prior to conducting the study. Data were collected and analyzed using IBM SPSS version 28 (SPSS Inc., Chicago, IL). Additional statistical analysis as well as graphical illustration were performed using Prism (Graphpad Inc., LaJolla, CA). All *P*-values shown were calculated using two-tailed tests and significance was assumed at $P \leq 0.05$. Paired groups were analyzed by Wilcoxon rank sum test, while two independent groups were compared by Mann-Whitney-U test. Parametrically distributed data from lung epithelial cells was analyzed by student's t-test. Frequencies between categorical variables were compared by chi-square test. Associations between different variables were explored using Spearman's correlation and/or linear regression analysis. If dependent variables were dichotomous, binary logistic regression was applied. For survival analysis, Kaplan Meier plots and log-rank testing were used. Multivariate analysis was performed by Cox regression (backward selection method).

## Results

*Circulating DKK1 levels change as a function of interindividual variability and time during SARS-CoV-2 infection*

We recruited hospitalized patients with PCR-confirmed SARS-CoV-2 infection between April 2020 and June 2021 as part of the Biobank Dresden Project (BBD) and included individuals, for whom 2 independent blood samples across the course of the disease were available. In total, 80 patients met the selection criteria and were included in the study. Blood samples were obtained approximately one week (sample 1, median=6 days, IQR=9) and two weeks (sample 2, median=15 days, IQR=15) following hospitalization (Figure 1 A). Clinical data was unavailable or incomplete from four individuals, who were excluded from survival analysis. Patient characteristics, comorbidities, therapeutic interventions and disease characteristics

are summarized in **Table 1**. None of the patients were vaccinated and the majority (68%) received glucocorticoids during hospitalization. In contrast, few patients were treated with remdesivir (16%), IL6 blocking agents (4%) or convalescent plasma (20%). Of note, many patients were admitted at an early time-point of the pandemic, where these agents were not yet standard of care. Patients were hospitalized for a median of 24 days. In hospital death occurred in 23 cases. Diabetes was common among study participants but did not confer elevated mortality (*B*=0.065, *P*=0.902, binary logistic regression analysis).

Pulmonary embolism was more frequent among patients who died than those who did not (60 vs. 30%) and this was associated with lower platelet counts in the later (sample 2) but not early phase (sample 1) of hospitalization (Fig. S1 A)(12). Most patients suffered from severe disease manifestations as reflected by the high proportion of individuals requiring intubation and extra-corporal membrane oxygenation (ECMO) (27.3 and 31.2%) (Figure 1 B).

To study DKK1 biology in the context of SARS-CoV-2 infection, we measured DKK1 serum levels in the two samples available from the respective patients (= paired design) (Figure 1 A). We found that DKK1 serum concentration increased across the disease trajectory (31.04 vs. 35.87 pmol/L, *P*=0.06). Further data analysis revealed that DKK1 levels showed high interindividual (absolute differences between patients) and modest intraindividual variability (differences between the two samples from the same patient), i.e. the trajectory of DKK1 concentration occurred within an individual spectrum (Figure 1 C). Consistently, DKK1 levels during follow-up (sample 2) could be predicted by the baseline measure (sample 1) of the individual as demonstrated in linear regression analysis (Fig. S1 B)(12). Neither inflammatory markers (c-reactive protein, IL6, leukocyte counts, ferritin, procalcitonin, soluble IL2 receptor), nor surrogates for kidney function (creatinine), cell damage (LDH) and coagulation activity (D-Dimer), glucose, age or sex were correlated with circulating DKK1 abundance (Figure S1 C,D)(12). In contrast, DKK1 levels at both time points as well as their changes over time could be predicted by platelet counts (Fig. 1 D,E). Of note, platelets are known as rich sources for DKK1 (9).

As samples from hospitalized, SARS-CoV-2-negative patients were not collected during the recruiting phase of our study, we were unable to compare DKK1 levels between our cohort and a matched control group. Instead, we obtained blood samples from healthy blood donors (n=71, median age: 40 years, IQR=24; 45% females, 55% males), none of whom received ongoing pharmacotherapy. Interindividual variability in DKK1 serum concentration was less pronounced in healthy controls than in Covid-19 patients. Yet, DKK1 levels did not differ significantly between the two groups at either time point investigated (sample 1 and 2, respectively) (Fig. S1 E, F)(12). DKK1 levels in healthy controls were neither correlated with age (Spearman's r=0.068, p=0.573), nor shaped by sex (p=0.93, Mann-Whitney-U-test). We conclude that DKK1 serum abundance is a variable trait, which is only mildly responsive to viral infection.

Finally, because SARS-CoV-2 first infects the lungs, we screened publicly available experimental datasets and found that DKK1 expression was suppressed in three human lung epithelial cell lines by SARS-CoV-2 but not influenza infection (Fig. 1 F-G). Likewise, DKK1 expression was reduced in lung tissue from patients with Covid-19 compared to non-infected controls (Fig. S1 G)(12).

Taken together, these data suggest that circulating DKK1 levels are variable in humans and change as a function of time during SARS-CoV-2 infection, which is linked to platelet counts.

*The metabolic signature associated with SARS-CoV-2 infection resembles fasting*

Metabolic adaptations are a hallmark of infection and DKK1 has been linked to lipid homeostasis(13-16). Thus, we next profiled the plasma metabolome of patients in an unbiased fashion using nuclear magnetic resonance (NMR) spectroscopy. We first ranked and filtered signals according to their fold change (sample 2 vs. sample 1). Subsequent clustering showed that metabolites annotated to lipid metabolism and specifically ketones, were most differentially regulated in abundance over time (Fig. 2 A), reminiscent of fasting metabolism. In response to fasting, organismal insulin secretion is suppressed, and

triacylglycerides (TAGs) are hydrolyzed by lipases (e.g. hormone sensitive lipase, HSL) to release free fatty acids (FFAs), which are then converted to ketone bodies by hepatocytes as a fuel for energy production(17,18). In addition, ketone bodies elicit signaling events, modulate the immune response and exert tissue protective effects(19). The same metabolic adaptations are engaged in response to infection and infection-induced anorexia (14) (Figure 2 B). Consistent with a fasting metabolism signature, levels of ketone bodies such as 3-hydroxybutyrate (=beta hydroxybutyrate), acetoacetate and acetone were high, whereas triglycerides were low during the early phase of the disease (sample 1). Later, triglyceride levels recovered, while the abundance of ketone bodies dropped to barely detectable limits (Fig. 2 C-E and Fig. S2 A)(12). These changes were mirrored by circulating DKK1 (Fig. 2 F). Concentrations of certain amino acids (isoleucine, leucine, valine, threonine) and associated breakdown products (2-hydroxybutyrate) followed a similar pattern (Fig. S2 B)(12), suggestive of concomitant protein catabolism. Of note, oxidation of isoleucine, leucine or threonine yields acetyl CoA, which can be fed into ketogenesis ("ketogenic" amino acids)(20). The presence of diabetes did not significantly affect any of these changes ($P$>0.05 for all metabolite comparisons; Mann-Whitney-U test)

Because fasting metabolism is considered as an adaptive response during infection(14), we next stratified patients into those who maintained lower triglyceride levels throughout the disease (TAG levels below median in sample 2) and those who did not (above median). We observed that the loss of a fasting metabolism signature (as reflected by high TAG levels) was associated with higher markers of inflammation, coagulation activity and cell damage (Fig. S2 C, D)(12). Moreover, these patients tended to be more likely affected by pulmonary artery embolism (41% vs. 21%, $P$=0.059, chi-square test). Vice versa, higher DKK1 levels during the same phase of the disease were linked to reduced signs of inflammation (CRP, IL6, ferritin), cell damage (LDH) and protein turnover (creatine, ornithine, phenylalanine) (Fig. 2G). Moreover, DKK1 was positively associated with markers of cholesterol shuttling (LDL, HDL, Apo A1), while correlations with ketone levels were modest (Fig. 2 G).

As most individuals received glucocorticoids during hospitalization and cortisol triggers lipolysis and proteolysis (21), we asked whether the fasting metabolism signature (beta hydroxybutyrate, acetoacetate, TAG) is affected by glucocorticoid treatment. However, we did not find evidence for this notion ($P$>0.6, two-tailed student's t-test for the three metabolite comparisons between groups). Of note, we cannot rule out that lipids (and other metabolites) were affected by parenteral nutrition and/or lipid-rich drugs (e.g. propofol), both of which are preferentially administered to critically-ill individuals.

We conclude that SARS-CoV-2 infection promotes metabolic adaptations resembling fasting, which are mirrored by circulating DKK1 levels.

*DKK1 predicts disease trajectories in patients with Covid-19*

Our results prompted us to explore if circulating DKK1 levels predict disease outcomes in SARS-CoV-2-infected individuals. We found no association between basal DKK1 serum concentration and the use of glucocorticoids during hospitalization, nor did DKK1 levels differ between patients who required intubation or ECMO and those who did not (Fig. 3 A, B). We then stratified individuals as $DKK1_{high}$ or $DKK1_{low}$ according to their respective serum levels at baseline (sample 1) relative to the median of the study population (Fig. 3 C). $DKK1_{low}$ patients were twice as likely to die from Covid-19 than $DKK1_{high}$ patients (HR=2.28, 95%CI 0.99-5.3; p=0.05) (Fig 3 D). Accordingly, DKK1 levels in quartiles 1 and 2 (Q1, Q2) associated with worse outcomes than those in quartiles 3 and 4 (Q3, Q4) (Fig. S3 A)(12). We found somewhat similar trends using our DKK1 measurements from sample 2 (Fig. S3 B)(12), although these results were less prominent. Neither relative changes in DKK1 levels, nor platelet counts over time (ΔDKK1 and Δplatelets, respectively) predicted mortality in our cohort (Fig. 3 E, F).

To further explore this finding, we used Cox regression analysis. Applying the backward selection method (likelihood ratio), we computed a model using DKK1 (sample 1), IL6, CRP and creatinine levels as well as age, leukocyte and platelet counts as covariates and

identified DKK1 and age as the only significant, independent predictors of Covid-19-related mortality (Fig. 3 G). In this model, the regression coefficient *B* for DKK1 serum levels was -0.027, corresponding to a 2.7% decrease in risk for death for every 1 pmol/l increase in DKK1 serum levels.

## Discussion

Our observations that a.) DKK1 expression is suppressed in epithelial cell lines as well as human lung tissue in response to SARS-CoV-2 infection and b.) low DKK1 serum levels predict mortality in individuals suffering from Covid-19 raise the question if reduced DKK1 levels indicate excessive viral replication and/or inflammatory sequalae. Vice versa, genetic variation in DKK1 expression could actively impinge on disease trajectories in the context of SARS-CoV-2 infection. As individual DKK1 levels only changed modestly over time in our study cohort (intraindividual variability), irrespective of whether patients recovered or not, but absolute DKK1 abundance markedly differed between subjects (high interindividual variability), we suggest that genetic variation in DKK1 expression, rather than differences in the "DKK1 response" upon viral infection, accounts for the association between low DKK1 levels and mortality. This notion is supported by our finding that relative changes in circulating DKK1 (ΔDKK1) do not predict disease trajectories, whereas absolute levels do as well as our observations in healthy individuals.

Our correlation analysis showed that platelet numbers were the only significant predictors of DKK1 levels. Experimental studies have already demonstrated important contributions of platelet-derived DKK1 to pulmonary inflammation in response to influenza infection and platelet numbers show considerable changes upon microbial inflammation (e.g. resulting from activation of the coagulation cascade)(22,23). Yet, we found no association between platelet counts and disease outcomes in our patients. As such, platelets may explain a considerable proportion of variation in DKK1 serum levels but potential contributions of DKK1 to SARS-CoV-2 pathology may at least in part be platelet-independent. Alternatively,

circulating DKK1 abundance could reflect platelet activity more accurately than circulating platelet themselves, particularly in the context of diseases associated with thromboembolic events such as Covid-19.

Given the reciprocal regulation of circulating DKK1 and metabolic adaptations of the host (TAG hydrolysis, ketogenesis, protein catabolism), experimental studies investigating immunometabolic functions of DKK1 in viral infection are warranted. Moreover, our results raise the question if a ketogenic state (either induced endo- or exogenously) is beneficial in patients suffering from Covid-19, which is supported by experimental studies in mice (24).

In summary, our results suggest a potential clinical use of measuring circulating DKK1 as an indicator of disease severity in Covid-19. These results require validation in a larger cohort of patients.

**Data availability statement**

All datasets included in this study are available from the corresponding author (NPJ) upon reasonable request.

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## Figure Legends

**Figure 1.** *Circulating DKK1 levels change as a function of interindividual variability and time during SARS-CoV-2 infection.* (A) Study design. Days shown correspond to the median of the study population. (B) Maximum therapy expressed as proportion of total. (C) Circulating DKK1 levels in samples 1 and 2 from study participants (paired) measured by ELISA (n=80/time point). Data were analyzed by Wilcoxon rank sum test. (D) Association between platelet counts and circulating DKK1 levels at the two time points analyzed. Results were calculated by linear regression analysis (least square fit). (E) Association between change in platelet count (Δplatelets) and circulating DKK1 (ΔDKK1) over time. Data were analyzed by linear regression analysis (F) DKK1 transcript expression in human lung epithelial cell lines (A549-ACE2, Calu3 and NHBE) 24h following infection with SARS-CoV-2 or mock treatment. Data were extracted from publicly available platforms and visualized by Prism. Data is expressed as mean± s.e.m. ***<0.001 according to two-tailed, student's t-test (G) DKK1 mRNA expression in NHBE cells in response to Influenza A infection.

**Figure 2.** *The metabolic signature associated with SARS-CoV-2 infection resembles fasting.* (A) Heatmap visualization of changes in metabolite signatures over time (median sample 2 vs. 1). AA= amino acid metabolism, Glc=glucose metabolism, lip= lipid metabolism, ket= ketone bodies (B) Schematic illustration of host metabolic adaptations in response to fasting, infection and infection-induced anorexia (FFAs= free fatty acids, TG=triglycerides, HSL= hormone-sensitive lipase). (C-E) Absolute change in circulating beta hydroxybutyrate, acetoacetate and triacylglyceride (TAG) levels over time (samples 1 vs 2) assessed by Wilcoxon rank sum test (n=80/time point). (F) Relative change in DKK1 and beta hydroxybutyrate serum abundance over time (n=80/time point) (G) Heatmap visualization of correlations between DKK1 serum concentration in sample 2 (S2) and different variables. The color mapping corresponds to the respective Spearman correlation coefficient.

**Figure 3.** *DKK1 predicts disease trajectories in patients with Covid-19.* (A) DKK1 serum levels (sample 1) in patients treated with (+) or without (-) glucocorticoids (GC) during hospitalization. Data were analyzed by Mann-Whitney-U test (B) as in (A) but stratified according to the initiation of intubation (+intub) vs. less invasive ventilation procedures (-intub) (also see Table 1). (C) Stratification scheme of patients according to their basal DKK1 levels (sample 1) relative to the median DKK1 serum concentration of the study population ($DKK1_{high}$ and $DKK1_{low}$, respectively). (D) Probability of survival in $DKK1_{high}$ and $DKK1_{low}$ patients (n=76) analyzed by log-rank test. Censored cases (= no event during follow-up) are visualized by black ticks. (E, F) Survival according to changes in DKK1 (ΔDKK1) and platelets (Δplatelets) over time. (G) Cox regression analysis. The first and last step of the analysis are shown, the former including all covariates analyzed, the latter only comprising variables selected by the backward selection method (likelihood ratio) as independent predictors of Covid-19-related survival.

| Table 1: Characteristics of the study population | |
|---|---|
| | **Number/total (frequency)** |
| **Sex** | |
| Male | 46/77 (59%) |
| Female | 32/77 (41%) |
| **Comorbidities** | |
| Coronary artery disease | 13/77 (16.9%) |
| Malignant disease | 10/77 (13%) |
| Diabetes | 26/77 (33.8%) |
| **Vaccination status** | |
| Vaccinated | 0 (0%) |
| **Disease characteristics** | |
| Pulmonary embolism | 24/77 (31.2%) |
| Deaths | 23/78 (29.5%) |
| **Covid-19 directed therapy** | |
| Glucocorticoids (Dexamethason/Prednisolone) | 52/76 (68.4%) |
| Remdesivir | 12/76 (15.8%) |
| IL6 neutralization | 3/76 (3.9%) |
| Convalescent plasma | 15/76 (19.7%) |
| **Maximum therapy** | |
| No oxygen | 3/77 (3.9%) |
| Oxygen mask | 13/77 (16.9%) |
| High flow oxygen | 13/77 (16.9%) |
| Non-invasive ventilation | 3/77 (3.9%) |
| Intubation | 21/77 (27.3%) |
| ECMO (Extracorporeal membrane oxygenation) | 24/77 (31.2%) |
| | **mean ± SD (median; IQR)** |
| **Metrics** | |
| Age | 64.95 ± 13.12 (65; 21) |
| Days of hospitalization | 23.68 ± 14.22 (21; 15.5) |
| Days from hospitalization to collection of sample 1 | 7.32 ± 8.22 (6; 8.75) |
| Days from hospitalization to collection of sample 2 | 19.38 ± 24.49 (15; 14.75) |
| Days between sample 1 and 2 | 12.06 ± 22.47 (7; 8) |

**Table 1**: *Summary of patient characteristics. (SD= standard deviation, IQR= interquartile range)*

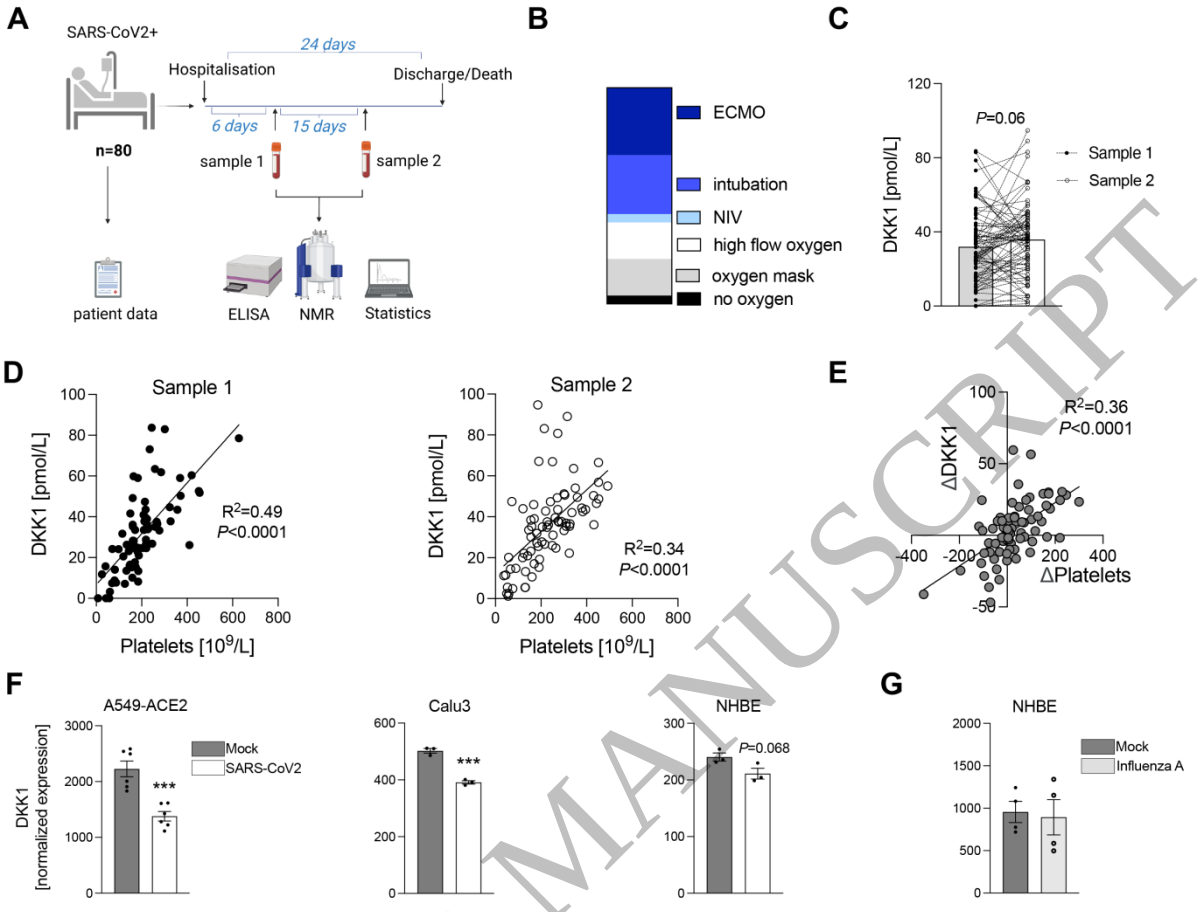



**Figure 1**
**254x190 mm (.00 x DPI)**

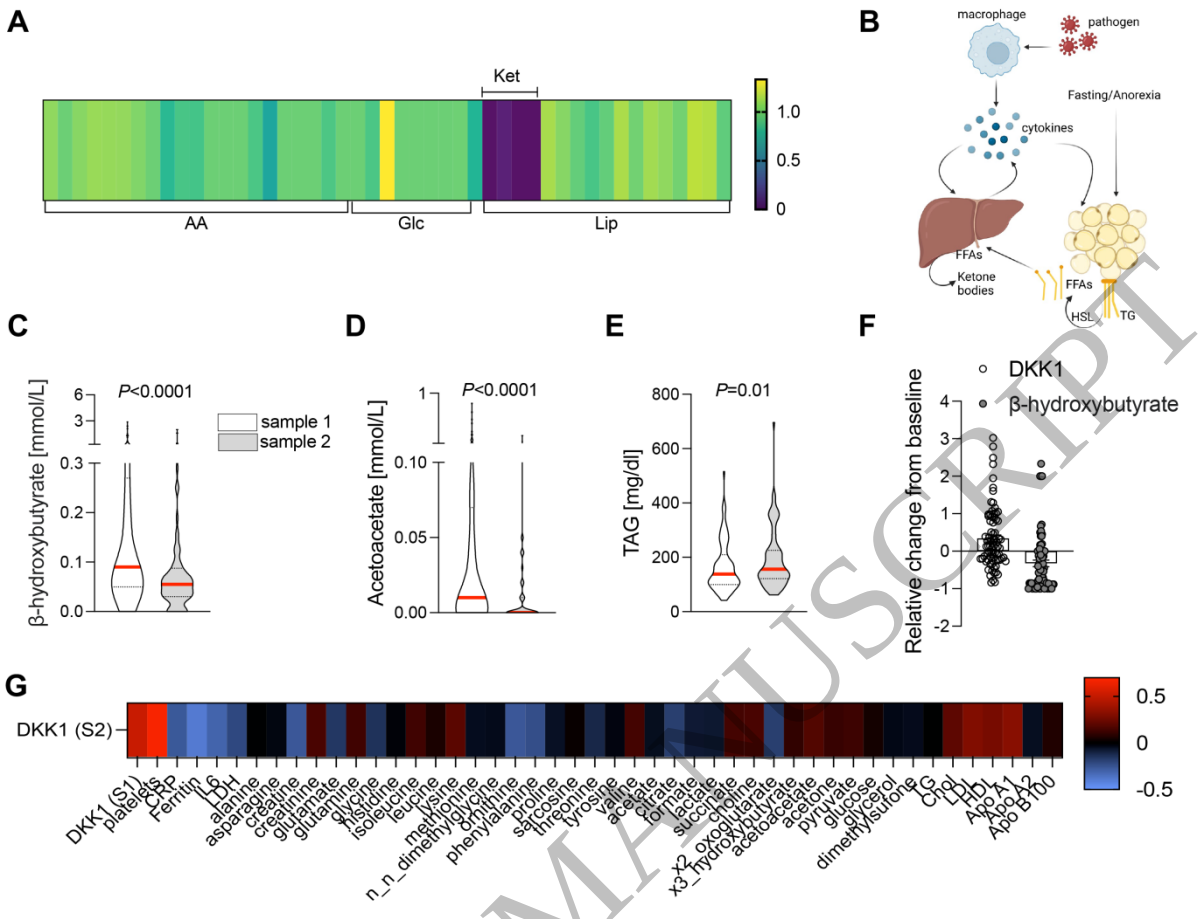
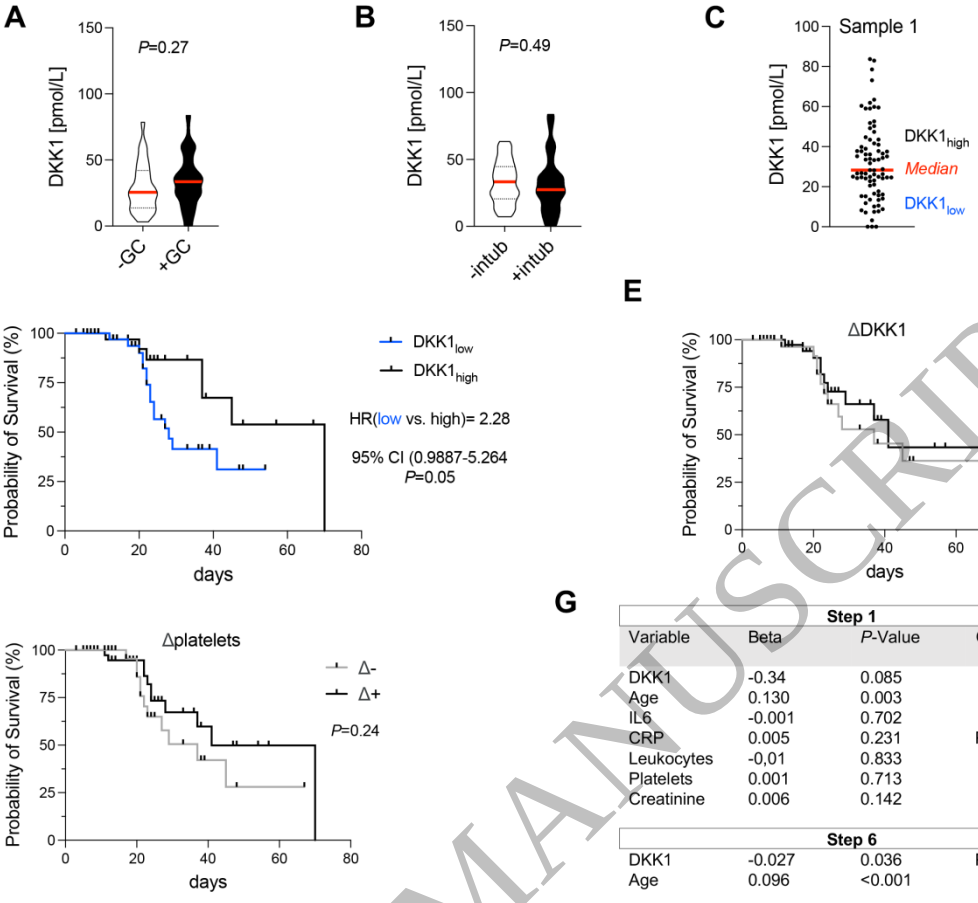



**Figure 2**
**254x190 mm (.00 x DPI)**

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| Step 1 | | | |
|---|---|---|---|
| Variable | Beta | *P*-Value | Omnibus test |
| DKK1 | -0.34 | 0.085 | |
| Age | 0.130 | 0.003 | |
| IL6 | -0.001 | 0.702 | |
| CRP | 0.005 | 0.231 | P=0.004 |
| Leukocytes | -0,01 | 0.833 | |
| Platelets | 0.001 | 0.713 | |
| Creatinine | 0.006 | 0.142 | |
| **Step 6** | | | |
| DKK1 | -0.027 | 0.036 | P<0.001 |
| Age | 0.096 | <0.001 | |

**Figure 3**
**254x190 mm (.00 x DPI)**