

Early and accurate prediction of COVID-19 hospitalization risk - methodological framework for pandemic preparedness

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Abstract

As demonstrated by the public health crisis caused by the COVID-19 pandemic, pandemic preparedness requires rapid identification of early biomarkers predicting need of hospitalization. Furthermore, COVID-19 treatment eligibility is currently determined by patient demographics and pre-existing clinical conditions, but these factors do not provide accurate severity predictions. However, previous research on COVID-19 severity biomarkers has focused on already hospitalized patients. Here, we investigated point-of-care biomarkers capable of accurately predicting COVID-19-related hospitalization risk and disease progression shortly after a positive diagnosis. Previously uninfected non-vaccinated individuals (N=201) were recruited at, or shortly after, SARS-CoV-2 diagnosis, and biochemistry and serum cytokine levels were longitudinally measured. Biomarker combinations were tested by multi-dimensional clustering followed by dimension reduction. The combination of high levels of LDH or serum IL-6 showed the most accurate prediction (sensitivity=100%, specificity=94.5%) for hospitalization risk. The combination of four cytokines (IFN- α , IFN- γ , IL-6, IL-8) was the most accurate predictor for moderate versus mild disease. Age and BMI showed significantly lower predictive values for hospitalization risk and were not predictive of mild versus moderate symptoms. Thus, early accurate prediction of COVID-19 hospitalization and severity is possible using biomarkers feasibly measurable at point-of-care, thus allowing optimizing personalized treatment and public health management. The methodology used here sets out a paradigm for severity prediction in the framework of pandemic preparedness.

Introduction

The COVID-19 pandemic, sparked by the spread of the SARS-CoV-2 virus after December 2019, resulted in approximately 6 million deaths by 2021¹. Subsequently, SARS-CoV-2 became endemic, but COVID-19 mortality remains high (around 200,000 deaths in 2023), primarily among those with outdated vaccinations, despite the less severe Omicron variant². At the onset of the COVID-19 pandemic, a major public health challenge was the lack of biomarkers to effectively triage patients requiring hospitalization, thus leading to a critical overload of hospital systems globally.

Currently, several anti-viral drugs are available^{3,4,5} or under investigation^{6,7}, also for patients with mild-moderate disease⁸. However, these must be initiated early, within 5–7 days post symptom onset (DPSO), and targeted at patients that are at risk for hospitalization or developing considerable symptomatic disease. Immunomodulatory drugs that inhibit inflammatory cytokines (e.g., IL-6 inhibitors) are also available^{9,10}. However, currently, anti-viral therapy is underused¹¹ and cytokine inhibitors are only given to hospitalized severe patients, largely because of absence of biomarkers that allow early patient stratification¹². Thus, it is important to identify as early as possible which individuals are at risk for hospitalization and/or suffering a moderate disease course, in order to optimize early personalized treatment.

COVID-19 shows a versatile range of severity, from asymptomatic¹³ to mild or moderate symptomatic disease and up to severe disease requiring oxygen support and hospitalization, potentially leading to

multi-organ failure and death¹⁴. Symptoms such as chest pain, confusion, dizziness, fatigue, fever, and shortness of breath are linked to dyspnea and a moderate to severe course of infection¹⁵. Severe disease is defined by a low oxygen saturation ($\text{SpO}_2 < 94\%$) requiring oxygen support and hospitalization¹⁶.

Patients' characteristics, such as age and BMI, and underlying comorbidities, are known factors associated with risk for severe COVID-19¹⁶ and are currently used as guidelines for early treatment with anti-viral therapy¹⁷. Some biochemistry and hematological blood biomarkers, such as elevated levels of C-reactive protein (CRP)¹⁸ and lactate dehydrogenase (LDH)¹⁹, and low lymphocyte counts, were reported to be predictive of more severe COVID-19 in hospitalized patients²⁰. SARS-CoV-2 viral load was also reported to be associated with COVID-19 severity, but a recent meta-analysis has shown that lack of viral load standardization prevents it from being a good biomarker²¹.

Additionally, as severe COVID-19 patients suffer from a "cytokine storm"^{22,23}, an excessive release of pro-inflammatory cytokines, various cytokines were identified as biomarkers predictive of more severe COVID-19 in hospitalized patients, such as elevated serum levels of IL-6^{23–25}, IL-10^{26,27}, IL-17A²⁸, IFN- γ ²⁹ and tumor-necrosis-factor-alpha (TNF- α)^{25,30}. Contrarily, hospitalized patients with increased IFN- α levels showed more favorable COVID-19 disease course³¹. We previously reported that the ratio between type-I interferon response and inflammatory cytokines, at the day of hospitalization, gives the highest accuracy for predicting COVID-19 severity and mortality³².

However, while previous studies assessed biomarkers in already hospitalized patients, identifying early biomarkers measurable before hospitalization at the point-of-care, ideally within 5–7 DPSO, is crucial. Therefore, in this study, we analyzed the predictive value of biochemical, hematological, and cytokine markers measured at or shortly after SARS-CoV-2 diagnosis for prediction of hospitalization risk and moderate versus mild disease severity.

Results

Hospitalization risk prediction

While both age and BMI were significantly ($p < 0.001$) higher in hospitalized versus non-hospitalized patients (Supplementary Figures 2,5), they showed only low predictive value for hospitalization risk (age: J-index=55.2%, sensitivity=84.5% and specificity=70.7%; BMI: J-index=39.9%, sensitivity=81.3% and specificity=58.6%; Figure 1F, Table 2). Also, the combination of age and BMI did not improve prediction of hospitalization risk (Supplementary Figure 2).

Serum SARS-CoV-2 N-antigen levels were significantly higher in hospitalized patients (Supplementary Figures 2,5), but showed only low predictive value for hospitalization risk (J-index=60.9%, sensitivity=79.3% and specificity=80.7%; Supplementary Figure 6). Standardized SARS-CoV-2 RNA load was unfortunately not available for most hospitalized patients.

Of all biochemistry and hematology biomarkers measured at the first sample per patient (median 2, IQR 0-3, days after diagnosis; median 5, IQR 3-7, DPSO), LDH and CRP showed the most significant ($p<0.001$) elevation in hospitalized versus non-hospitalized patients (Figures 1A-B, Supplementary Figures 3,5), and were the only ones to show accurate prediction of hospitalization risk with a J-index larger than 80% (LDH: J-index=89.8%, sensitivity=95.3% and specificity=94.5%; CRP: J-index=89.2%, sensitivity=94.7% and specificity=94.5%; Figure 1F, Table 2). D-dimers, ferritin, creatinine kinase, and neutrophil percentage were also significantly elevated, while lymphocyte percentage was significantly lower, in hospitalized patients, but none of those showed a good prediction (Supplementary Figures 3,5,6).

Among the 8 cytokines measured at the first available sample, serum IL-6 levels were the most significantly ($p<0.001$) elevated in hospitalized patients (Figure 1C), and the only cytokine that alone accurately predicted hospitalization risk (J-index=89.4%, sensitivity=94.9% and specificity=94.6%; Figure 1F and Table 2). IL-8, IL-10, IL-17A, IL-22, TNF- α , and IFN- γ were also significantly elevated in hospitalized versus non-hospitalized patients, but none of those showed a good prediction of hospitalization risk (Supplementary Figures 4,5,6).

Next, we used principal component analysis (PCA) for a multidimensional analysis of all the cytokine normalized (Z-scaled) serum levels. Starting with a PCA including all 8 cytokines, a clear separation was observed between hospitalized and non-hospitalized patients (Supplementary Figure 7A, Supplementary Table 3). Dimension reduction showed a good separation using only 4 cytokines (IL-6, IL-10, TNF- α , and IFN- α), which was also validated for the cytokine raw values (Supplementary Figure 7B). Using the separatrix line between hospitalized and non-hospitalized patients, this was translated to a prediction function of the ratio between the inflammatory cytokines and IFN- α , termed here IFN-INF function,

$$IFN-INF = \log (2 * IL-6 * IL-10 * TNF-\alpha / IFN-\alpha)$$

which was significantly ($p<0.001$) higher in hospitalized patients (Figure 1D) and resulted in accurately predicting hospitalization risk with J-index=92.6% (sensitivity=96.7% and specificity=95.9%; Figure 1F and Table 2), showing slightly higher sensitivity and specificity than IL-6, LDH, or CRP each alone.

Lastly, we investigated whether combining the biochemistry and cytokine biomarkers increases the prediction accuracy. LDH, CRP, IL-6, and the IFN-INF function were all significantly ($p<0.001$) correlated with each other (Supplementary Figure 8). Nevertheless, combining (with an OR function) LDH with either IL-6, the IFN-INF function, or CRP allowed the increase of sensitivity to 100% with only a minor reduction of specificity (Table 2). The IL-6-OR-LDH-function (Figure 1E) showed the highest predictive value with a J-Index of 94.5% (Sensitivity=100%, Specificity=94.5%) (Figure 1F, Table 2). Other combinations, in particular with CRP (Supplementary Figure 8), did not improve predictive value.

ROC analysis showed that IL-6-OR-LDH function, IFN-INF function, and LDH have the largest areas under the curve, although not statistically significantly better than CRP or IL-6 alone (Table 2, Figure 1F, Supplementary Figure 6).

While LDH, CRP, IL-6, IFN-INF function, and IL-6-OR-LDH function are all strongly correlated with age and BMI, but not with the time the sample was taken, their predictive value was independent of these factors or of gender and diabetes status (Supplementary Figures 9-11). Also, the prediction of hospitalization risk of these biomarkers held for all SARS-CoV-2 variants in the initial analysis, and the n=12 patients in the Omicron validation cohort (Supplementary Figures 9-11).

Symptom severity prediction in non-hospitalized patients

Next, we focused on only non-hospitalized patients to discover a biomarker predicting mild versus moderate disease. Only n=59 patients with the first sample available at DPSO≤6 (Supplementary Table 1) were included in this analysis because some relevant biomarkers significantly declined after day 6 (Supplementary Figure 12). Age and BMI were not good predictors for moderate versus mild symptoms (age: J-index=11.6%; BMI: J-index=26.7%; Figure 2F, Table 2, Supplementary Figures 2,5,13). Serum SARS-CoV-2 N-antigen levels, but not SARS-CoV-2 oropharyngeal RNA loads, were significantly higher in moderate versus mild patients (Supplementary Figures 2,5C), but showed only low predictive value (J-index<45.0%; Supplementary Figure 13A).

None of the biochemistry biomarkers, including CRP and LDH, were significantly different between moderate and mild patients (Supplementary Figures 3,5C) and none of them predicted moderate versus mild symptoms (Supplementary Figure 13B).

Among the cytokines, serum levels of IFN-α, IFN-γ, IL-6 and IL-10 were significantly elevated in moderate versus mild patients (Figure 2A-D, Supplementary Figures 4,5C), but only IFN-α and IL-6 exhibited a modest predictive value (J-index=56.3% and 52.9%) for moderate disease course (Figure 2F, Table 2, Supplementary Figure 13C). PCA performed for only non-hospitalized patients with all eight cytokines showed some separation between mild and moderate disease, and dimension reduction showed that IFN-α, IFN-γ, IL-6, and IL-8 are the most important cytokines for that separation (Supplementary Figure 7C-D). Using the raw values of IFN-α and IFN-γ combined by the OR function with the raw values of the inflammatory cytokines IL-6 and IL-8 (Figure 2E) we obtain the IMM-INF function:

$$IMM-INF = [\log(IL-6) + \log(IL-8)] > 1.75 \text{ OR } [\log(IFN-\alpha) + \log(IFN-\gamma)] > -0.25$$

being significantly ($p < 0.001$) predictive for moderate versus mild symptom severity with J-index=71.7% (sensitivity=95.2% and specificity=76.5%; Figure 2F, Table 2). Combination of IMM-INF function (or any single cytokine) with the biochemistry biomarkers did not improve the predictive value for moderate symptoms.

IMM-INF is not correlated with age and BMI and its predictive value is independent of these factors or of gender and diabetes status (Supplementary Figure 14). IMM-INF was stable within the range of 0-6 DPSO, and its predictive value was independent of DPSO in that range (Supplementary Figure 14).

Decision tree for combined prediction of hospitalization risk and symptomatic status

In summary, by measuring only LDH and 4 cytokines (IL-6, IL-8, IFN- α and IFN- γ) it was possible to obtain a decision tree (Figure 3), for a combined prediction of both hospitalization risk with sensitivity of 100% (using the IL-6-OR-LDH function) and, among those predicted not to be hospitalized, of developing moderate symptoms with sensitivity of 93.1% (using IMM-INF).

Longitudinal profiles of cytokine and biochemistry biomarkers

Longitudinal measurements starting as early as the day of diagnosis (median 2, IQR 0-3, days after) and up to 5 weeks later, reveal differences in the kinetic profiles of different biomarkers between hospitalized versus non-hospitalized and between mild versus moderate patients (Figure 4). LDH, CRP, IL-6, IL-8, IL-10, and TNF- α are significantly elevated in hospitalized patients already at 0-7 DPSO, stay significantly elevated up to 28 DPSO in hospitalized patients, and are not higher in mild patients versus negative controls. On the other hand, IFN- α and IFN- γ levels show no significant difference between hospitalized and non-hospitalized patients, but are higher in moderate versus mild patients, and in mild patients versus controls, and decline faster to negative control levels.

Discussion

Our findings indicate that it is possible to predict COVID-19 disease progression with biomarkers measurable at point-of-care already as early as 0-7 days post symptom onset and even at the time of the first positive diagnosis of SARS-CoV-2 infection. Most importantly, we show that highly accurate (Balanced Accuracy >94.5%) early prediction of COVID-19 hospitalization risk is possible either by high LDH levels, or by high IL-6 levels, or by high ratio between inflammatory cytokines (IL-6, IL-10 and TNF- α) and IFN- α ³², termed here IFN-INF predictor function, or by their combinations. It should be noted that all above predictors are not statistically significantly different in their predictive values. However, the combination of LDH with IL-6 biomarkers enables predicting hospitalization with sensitivity of 100% and specificity of 94.5%, giving the best predictive value per number of biomarkers used. While some of above biomarkers have been previously shown by us³² and others^{18,19,23-31} to be associated with COVID-19 severity in hospitalized patients, our results now show that they are also good early predictors for hospitalization risk. Of note, the predictive value of these biomarkers is significantly better than that obtained by age and/or BMI, as observed here similarly to other studies³⁴, which, together with clinical pre-conditions, are currently used to determine eligibility for early anti-viral therapy.

Furthermore, we show that the IMM-INF function, combining serum IL-6, IL-8, IFN- α and IFN- γ levels, is an early predictor (accuracy=89.8%) for moderate versus mild disease. Interestingly, none of the single cytokines, nor the IFN-INF function, nor any of the biochemistry or hematological parameters, age, or BMI were good predictors for moderate symptom severity. While the prediction of moderate disease course for non-hospitalized patients is clinically less important, still it could be used to guide personalized treatment with SARS-CoV-2 anti-viral therapy, as proven in clinical trials to preserve quality of life in moderate patients and decrease public health burden^{3,7}.

For a large fraction (22%) of the patients (29% of non-hospitalized) the above predictions are based on samples taken already on the day of SARS-CoV-2 positive diagnosis, and for the other patients the sample was taken within 7 days of the first positive diagnosis. Furthermore, we show that the prediction of hospitalization risk (or of moderate disease) is independent of the time of sampling within 0-11 (or 0-6) DPSO.

Similar to previous studies^{16,17,34}, we also observed that the risk of hospitalization is associated with older age and higher BMI. However, prediction by the biomarkers we have identified is independent of such factors as age, gender, BMI or diabetes, probably since these are already factored into the early levels of the biomarkers in response to SARS-CoV-2 infection.

Our results also shed further light on the role of the different cytokines in response to SARS-CoV-2 infection. While high levels of the inflammatory cytokines (e.g. IL-6, IL-8 and TNF- α), and also interestingly IL-10, are associated with both the risk for hospitalization and higher risk for moderate course of infection, conversely IFN- α , IFN- γ and IL-17A are less associated with hospitalization risk, but rather with moderate versus mild symptoms.

Our longitudinal results show that the kinetics of the cytokines IL-6, IL-8, TNF- α and IL-10 differ in hospitalized patients, showing higher expression early after symptoms onset and staying consistently high even 4 weeks after. While it was shown that inflammatory cytokines are high in severe COVID-19 patients²³⁻²⁷, we show here that in some patients they are already high very early on, thus indicating that if immune modulatory treatment is to be successful it should be initiated early, personalized according to the cytokine profile¹². As expected, we also see an early rise in IFN- α and IFN- γ but these are not as significantly different between hospitalized and moderate disease patients and they decline within 2-3 weeks also in hospitalized patients.

The patients analyzed here were all non-vaccinated, which is the group most susceptible to hospitalization and mortality, and most important in framework of pandemic preparedness. While most of the hospitalized patients in this study were infected with the wild-type virus and the non-hospitalized patients infected with other variants (mostly Alpha but also Beta, Eta, Iota and Delta), we also have some hospitalized patients infected with other variants. In general, our predictors were not affected by the different variants, including a small cohort of Omicron patients. Due to our study design, the hospitalized group was overrepresented; thus, for hospitalization risk we report sensitivity, specificity, Youden J-index, and balanced accuracy, rather than accuracy. Larger sequential recruitment prospective studies, especially including more Omicron cases, are needed for validation.

Based on our findings, we propose an early predictive decision tree to classify patients as mild, moderate, or severe. Soon after SARS-CoV-2 diagnosis, LDH, IL-6, IL-8, IFN- α , and IFN- γ should be measured, ideally at the point-of-care. LDH and IL-6 can be used to estimate hospitalization risk, while the IMM-INF function can predict moderate disease severity, thus supporting early personalized treatment with antivirals or cytokine inhibitors.

Last but not least, we suggest that the methodology we used here should be considered as the basis for early severity prediction in the framework of pandemic preparedness. As shown by the public health mayhem during the early stages of the COVID-19 pandemic, early prediction of disease severity due to a pandemic pathogen is as important as the identification of the new pathogen. The methods we used in this study: focusing on early severity prediction immediately after symptoms onset, rather than in hospitalized patients^{18-19,23-32}, considering non-linear combinations of biomarkers, rather than only single biomarkers or their linear contribution, and aiming at biomarkers that are easy to measure at point-of-care, would serve as an advantageous methodological paradigm for pandemic preparedness.

Methods (more details in supplementary methods)

Patients and study cohorts

The Early-Opt-COVID-19 longitudinal observational cohort study was performed at the University Hospital Augsburg and approved by the local ethics committee of the Technical University of Munich (internal code 799/20 S). Informed consent was obtained from all study participants before enrollment. All clinical research and experiments were performed in accordance with the Declaration of Helsinki and all relevant guidelines and regulations. Adult study participants (n=201) were enrolled, either actively at the test center on the day of SARS-CoV-2 positive diagnosis, or within 1-7 days through recruitment via flyers. The main inclusion criteria were either the presence of COVID-19-related symptoms or contact with a SARS-CoV-2 infected person within the last five days. Exclusion criteria encompassed individuals with chronic virus infections, immunosuppressant treatment, immune deficiency or pregnancy. Only non-vaccinated and previously uninfected patients were included in the current analysis, in order to avoid the effect of a boosted immune response. Visit 1 was at the day of diagnosis, for patients recruited at the test center, while the other patients started at visit 2 (1-7 days after diagnosis), with following visit 3 (2-3 weeks after) and visit 4 (4-5 weeks after). The patients completed a symptoms questionnaire, under physician guidance, at each visit to classify the disease course.

Demographic and clinical patient characteristics for the n=189 non-omicron patients used for the initial analysis are summarized in Table 1. Only patients with the first visit within 11 DPSO were included, since at later time points there was a significant decline in many biomarker levels. For the analysis of mild versus moderate disease in non-hospitalized patients, only patients with first visit at $\text{DPSO} \leq 6$ were included (Supplementary Table 1), since the levels of the relevant biomarkers started to decline after day 6.

Patients were divided into two main groups, non-hospitalized (n=92) and hospitalized (n=97) patients. Non-hospitalized patients were divided into 3 groups (Table 1): asymptomatic, (n=5, combined with mild for analysis), mild (n=21), and moderate symptom severity (n=66), where moderate was defined (Supplementary Figure 1) as having at least 1 of 6 symptoms (chest pain, confusion, dizziness, fatigue, fever and shortness of breath) that are associated with more severe disease¹⁵.

Biochemistry, hematology and immunoassays

Biochemistry, hematology, and differential blood count measurements for: CRP, LDH, creatine kinase (CK), platelets (PLT), ferritin, percent of lymphocytes count, and neutrophils count, were performed at the University Hospital Augsburg by standard clinical procedures.

Serum IFN- γ , IL-6, IL-8, IL-10, IL-22, and TNF- α were measured using multiplex planar array immunoassay (CorPlex panel, SP-X platform, Quanterix). IFN- α and IL-17A were measured by ultra-sensitive single-plex Simoa assay (HD-1/X Analyser, Quanterix).

SARS-CoV-2 load and variant

SARS-CoV-2 RNA load was measured by standardized RT-qPCR using two different specific oligonucleotide targets: N-Gene and NSP10.

Serum SARS-CoV-2 N-antigen levels were measured by ELISA using the COV-QUANTO (AAZ) kit.

SARS-CoV-2 variant was determined by RT-PCR amplification followed by sequencing on the Illumina NextSeq 1000 (Illumina) instrument.

Bioinformatics and statistical analysis

Principal component analysis (PCA) was used to cluster patients and compute principal component weights for each parameter. For the hospitalization risk prediction, predictive value of the various biomarkers was evaluated by the Youden J-index (Informedness=Specificity+Sensitivity-1), since accuracy could be taken only indicatively as the ratio between numbers of hospitalized and non-hospitalized patients does not represent real world ratio. The thresholds used for prediction were selected as the point with the highest J-index on the ROC curve for each biomarker. Longitudinal data was analyzed using piecewise linear regression³³. Statistical significance between groups was evaluated using the non-parametric Mann-Whitney-U test for continuous variables and the Fisher-Exact test for discrete variables. Correlations were evaluated using the non-parametric Spearman test. A two-sided p-value lower than 0.05 was considered statistically significant. Since 18 biomarkers were tested, p-value lower than 0.0027 was considered significant after multiple testing correction using the Bonferroni rule.

Abbreviations

AUC	Area under the curve
BMI	Body Mass Index
CI	Confidence Interval
CK	Creatine Kinase
CoVaKo	Corona-Vakzin-Konsortium
COVID	Coronavirus Disease
CRP	C-reactive protein
DPSO	Days Post Symptom Onset
ECMO	Extracorporeal membrane oxygenation
EDTA	ethylene diamine tetra-acetic acid
EOC	Early-Opt-COVID19 Study
IFN	Interferon
IL	Interleukin
IQR	Interquartile Range
J-Index	Youden Index
LDH	Lactate Dehydrogenase
MVS	Mechanical Ventilation
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PLT	Platelet
ROC	Receiving Operator Curve
SARS-CoV	Severe-acute-respiratory-syndrome-related coronavirus
TNF	Tumor necrosis factor
WBC	White Blood Cell

Declarations

Competing Interests

UP received personal fees from Abbott, Abbvie, Arbutus, Gilead, GSK, Leukocare, J&J, Roche, MSD, Sanofi, Sobi and Vaccitech. UP is a co-founder and shareholder of SCG Cell Therapy. All other authors declare no conflict of interest. The study was independently designed, run, analyzed, and summarized by the authors with no involvement from the funding agencies. The manuscript was written by the authors with no involvement of the funding agencies. The funding agencies did not pay for or were involved in any way of writing the manuscript.

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Role of the Funder/Sponsor

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Conflict of Interest Disclosure

UP received personal fees from Abbott, Abbvie, Arbutus, Gilead, GSK, Leukocare, J&J, Roche, MSD, Sanofi, Sobi and Vaccitech. UP is a co-founder and shareholder of SCG Cell Therapy. All other authors declare no conflict of interest. The study was independently designed, run, analyzed, and summarized by the authors with no involvement from the funding agencies. The manuscript was written by the authors with no involvement of the funding agencies. The funding agencies did not pay for or were involved in any way of writing the manuscript.

Author Contribution

C.H., M.G., K.D., C.P., M.R., S.G., C.R., G.G., C.T.-H., and A.N. conceived and designed the study. C.H., M.G., K.D., C.P., M.T., C.R., L.B., A.D-T., K.Z., G.G., and A.N. developed the methodology. C.H., M.G., and A.N. validated the experimental data and results. C.H., M.G., and A.N. performed formal data analysis. C.H., M.G., K.D., P.E., D.L., O.d.M.-J., C.P., M.T., A.S., D.R., G.H., M.A., A.B., B.B., A.D-T., A.F., C.G., C.K., L.K., E.K., S.K., A.M., F.N., N.P., C.R., and M.W. conducted investigations and experiments. P.S., U.P., C.R., C.D., M.F., A.F., H.H., A.M., M.W., G.G., and C.T.-H. provided study materials and resources. C.H., C.G., and M.S. curated and maintained the data. C.H., M.G., and A.N. wrote the original draft. M.G., K.D., O.d.M.-J., C.P., M.T., A.S., M.R., P.S., S.G., U.P., C.R., G.G., C.T.-H., and A.N. reviewed and edited the manuscript. C.H., M.G., M.R., G.G., and A.N. created visualizations and figures. M.G., U.P., C.R., H.M., G.G., C.T.-H., and A.N. supervised the research. C.H., M.G., D.R., G.H., C.R., V.F., and A.N. managed project administration. G.G., C.T.-H., and A.N. acquired funding. All authors read and approved the final manuscript.

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Data Availability

The SARS-CoV-2 RNA sequencing datasets generated and analyzed during the current study are available in the GenBank repository [accession numbers QB065616–QB065686]. All other demographic, clinical and laboratory data described in the manuscript is freely available to any researcher wishing to use them for non-commercial purposes, in compliance with the ethical approval and informed consent, by request to the corresponding author.

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Tables

Table 1 and 2 are available in the Supplementary Files section.

Figures

Figure 1

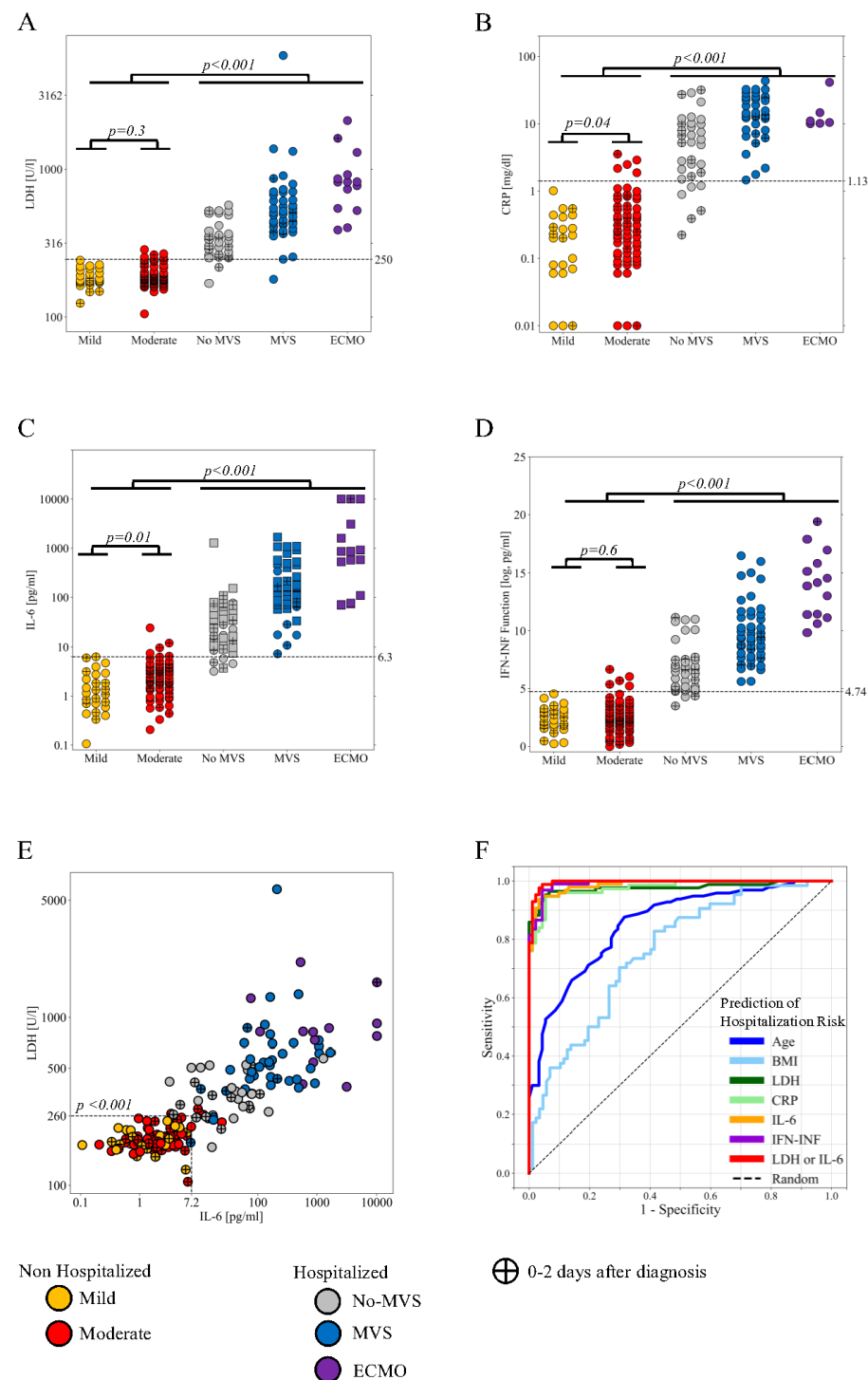


Figure 1

Biochemistry and cytokine biomarkers predictive of COVID-19 hospitalization risk. Early levels of LDH (A), CRP (B) and IL-6 (C), depicted as function of severity groups, show significant difference between hospitalized and non-hospitalized patients. Also, the ratio between inflammatory cytokine (IL-6, IL-10 and TNF- α) levels compared to IFN- α (IFN-INF function) is significantly higher in hospitalized patients (D). Using an OR function between LDH and IL-6, allows significant separation of the hospitalized (out of the

square) and non-hospitalized (in the square) patients (E). Receiver Operating Characteristic (ROC) curves for LDH-OR-IL-6, IFN-INF, LDH, IL-6 and CRP show significantly larger AUC for the prediction of hospitalization risk as compared to age and BMI (F). Dotted horizontal black lines (A, B, C, D, E) represent the threshold for each biomarker for which the hospitalization risk prediction has the largest J-index (see Table 2). All other biochemistry and cytokine biomarkers show lower prediction values for hospitalization risk (Supplementary Figures 2-6). Crossed circles depict patients measured already at the day of SARS-CoV-2 positive diagnosis or within 2 days after. The IFN-INF function is defined by: $\log(2 * IL-6 * IL-10 * TNF-\alpha / IFN-\alpha)$.

Figure 2

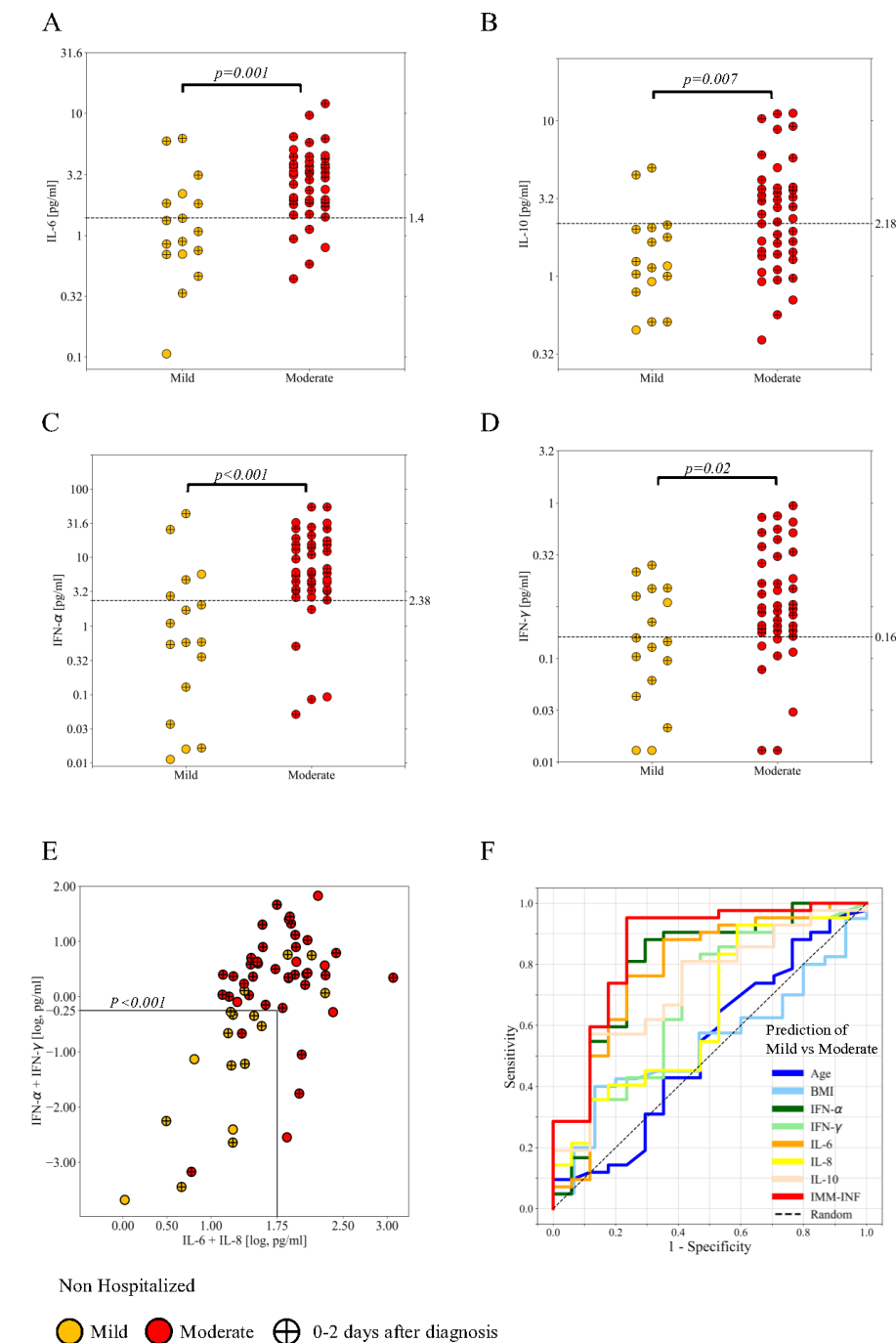


Figure 2

Cytokine biomarker combination predictive of COVID-19 moderate versus mild disease. Early levels of IL-6 (A), IL-10 (B), IFN-α (C), and IFN-γ (D) depicted as function of symptom severity, show significant difference between mild versus moderate non-hospitalized patients. Using an OR function between the combined levels of IL-6 and IL-8 as compared to the combined levels of IFN-α and IFN-γ, allows significant separation of the moderate (out of the square) and mild (in the square) patients (E), thus

leading to the IMM-INF function. Receiver Operating Characteristic (ROC) curves of the prediction for moderate severity show largest AUC for the IMM-INF function as compared to IFN- α , IFN- γ , IL-6, IL-8, IL-10, age and BMI (F). Dotted horizontal black lines (A, B, C, D, E) represent the threshold for each biomarker for which the moderate disease prediction has the largest J-index (see Table 2). All other biochemistry and cytokine biomarkers show no prediction of moderate severity (Supplementary Figures 2-5,13). Crossed circles depict patients measured already at the day of the SARS-CoV-2 positive diagnosis or within 2 days after. The IMM-INF function is defined by: $[(\log(\text{IL-6})+\log(\text{IL-8}) > 1.75)]$ OR $[\log(\text{IFN-}\alpha)+\log(\text{IFN-}\gamma) > -0.25]$.

Figure 3

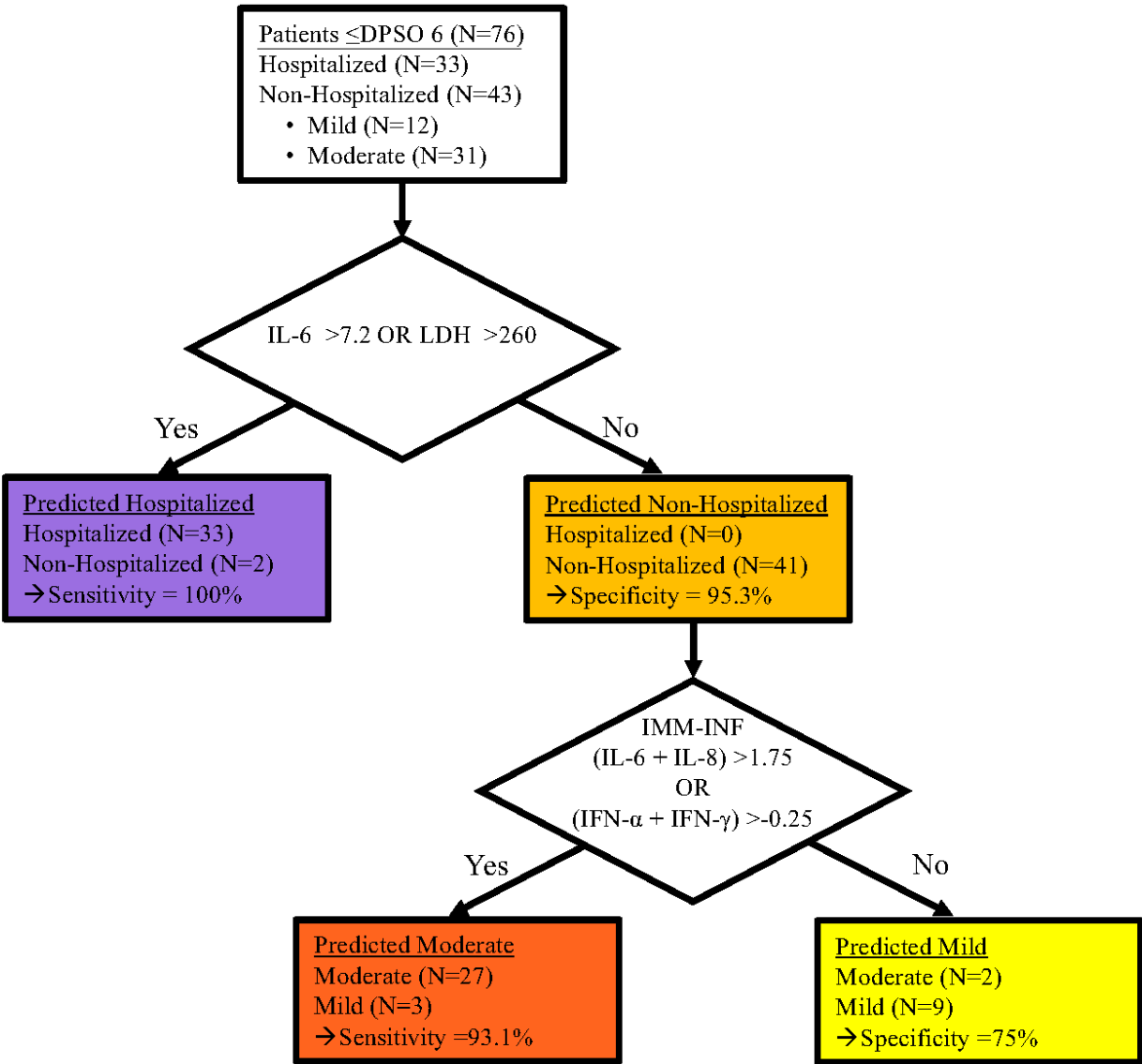


Figure 3

Schematic flowchart of a combined decision tree for the prediction of hospitalization risk and moderate versus mild disease. A flowchart showing the stratification of patients in the study to those predicted (using the LDH-OR-IL-6 combination) to be hospitalized (with 100% sensitivity) or non-hospitalized, and the latter further stratified to those predicted (using the IMM-INF function) to have moderate (sensitivity=93.1%) versus mild symptom severity. Only 76 patients are stratified since only patients with $DPSO \leq 6$ are used here (Supplementary Table 1), and 23 of those were missing LDH values (Supplementary Table 2).

Figure 4

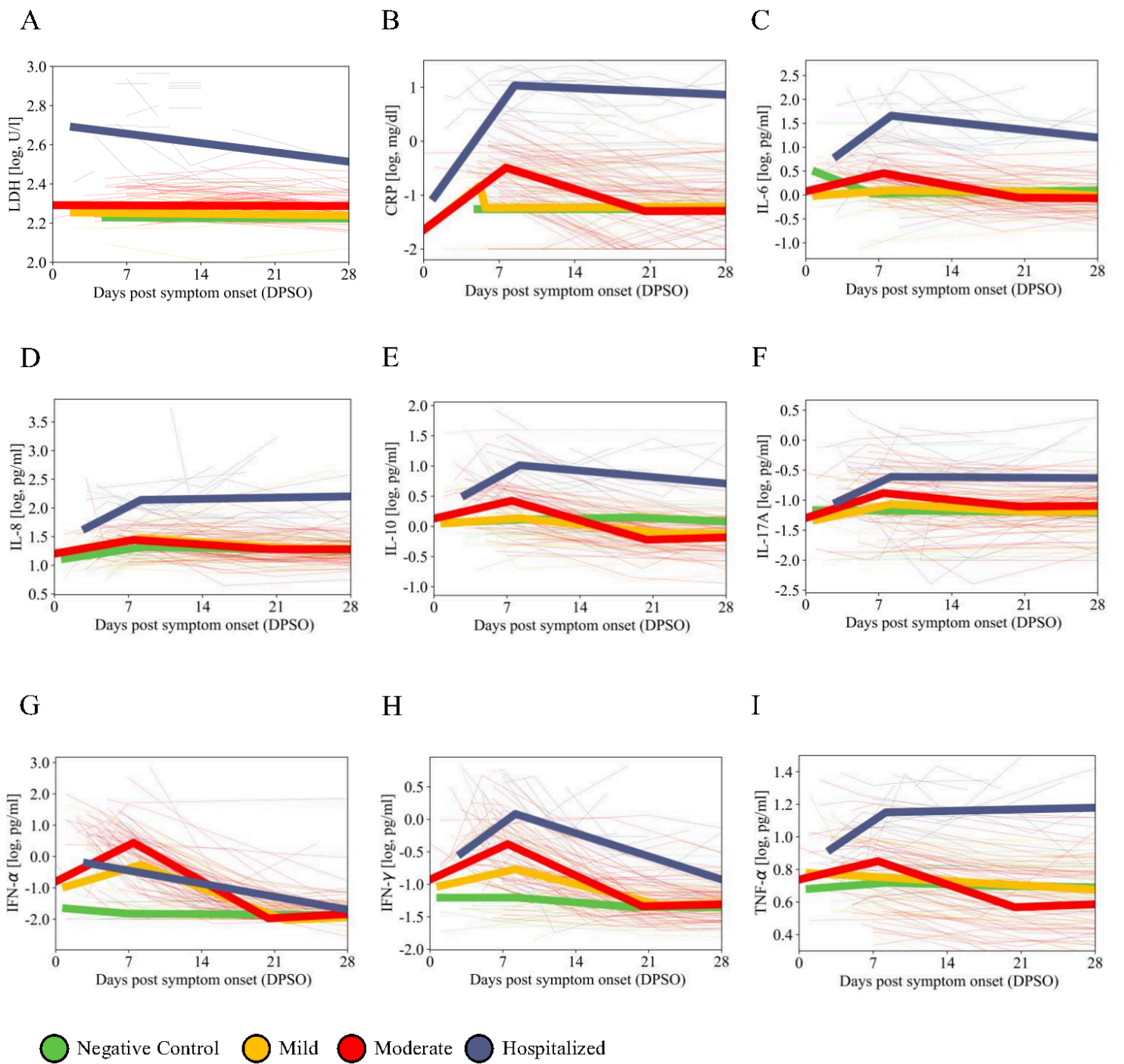


Figure 4

Kinetics of cytokine and biochemistry biomarkers stratified by the different severity groups. Serum cytokine and biochemistry levels in SARS-CoV-2 infected patients, as well as the negative control group, were measured 3-4 times over the span of 28 days post symptoms onset (DPSO). Different kinetic profiles for LDH (A), CRP (B), IL-6 (C), IL-8 (D), IL-10 (E), IL-17A (F), IFN- α (G), IFN- γ (H) and TNF- α (I) are observed in the hospitalized patients versus non-hospitalized mild and moderate symptom severity groups, using the average piecewise linear regression of the patients in each group. The dotted lines in the background represent the kinetics of the individual patients, while the thick lines represent average kinetic profile per group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [EOCPredictionSupplementarymaterial20260829.pdf](#)
- [Table1AND2.docx](#)