

## Absolute Lymphocytes, Ferritin, C-Reactive Protein, and Lactate Dehydrogenase Predict Early Invasive Ventilation in Patients With COVID-19

Salvador Payán-Pernía, MD,<sup>1,\*</sup> Lucía Gómez Pérez, MD,<sup>1</sup> Ángel F. Remacha Sevilla, MD, PhD,<sup>1</sup> Jordi Sierra Gil, MD, PhD,<sup>1</sup> Silvana Novelli Canales, MD, PhD<sup>1</sup>

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### ABSTRACT

**Objective:** Early detection of patients with COVID-19 who will need mechanical invasive ventilation (MIV) may aid in delivering proper care and optimizing the use of limited resources.

**Methods:** In this single-center retrospective observational study, we aimed to identify simple laboratory parameters that in combination with ferritin (a surrogate marker of severe inflammation) may help predict early (first 48 hours) MIV. A total of 160 patients with COVID-19 in whom serum ferritin, absolute lymphocyte count (ALC), platelet count, C-reactive protein (CRP), and lactate dehydrogenase (LDH) had been analyzed at admission were included.

**Results:** We found that ferritin, LDH, ALC, and CRP predicted with 88% accuracy the probability of early MIV. Results indicated that LDH showed the greater area under the curve (AUC), with a value of 89.1%. Using the AUC, we established cutoff values for clinical application. Finally, we developed a classification tree based on LDH for its clinical use.

**Conclusion:** Ferritin, LDH, ALC, and CRP predict with 88% accuracy the probability of early MIV.

**Keywords:** ferritin, COVID-19, hematology, biomarkers, mechanical ventilation, lactate dehydrogenase

As of October 4, 2020, the World Health Organization reported a total of 34,804,348 confirmed COVID-19 cases of infection globally, including 1,030,738 deaths.<sup>1</sup> The most common clinical features at the onset of the illness caused by SARS-CoV-2 are fever, fatigue, and dry cough. Patients with severe illness may develop dyspnea and hypoxemia within 1 week after onset of the disease, which may quickly progress to acute respiratory distress syndrome (ARDS) or end-organ failure.<sup>2</sup>

### Abbreviations:

MIV, mechanical invasive ventilation; ALC, absolute lymphocyte count; CRP, C-reactive protein; LDH, lactate dehydrogenase; AUC, area under the curve; ARDS, acute respiratory distress syndrome; ICU, intensive care unit; IL-6, interleukin-6; MAS, macrophage activation syndrome; RT-PCR, reverse-transcriptase polymerase chain reaction; ROC, receiver operating characteristic

<sup>1</sup>Haematology Department, Hospital de la Santa Creu i Sant Pau, Sant Pau Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain

\*To whom correspondence should be addressed.

[spayan@gmail.com](mailto:spayan@gmail.com)

Since the outbreak in December 2019, the sudden increase in COVID-19 cases of infection is putting high pressure on healthcare services worldwide, with particular significance in intensive care units (ICU). Reported rates of ICU admission represent up to one-quarter of hospitalized patients, but rates vary among countries.<sup>3</sup> These differences may relate to the availability of ICU beds, variations in practice and admission criteria, and differences in predisposing factors and testing availability.

Researchers have learned that ARDS is the most common complication for ICU admission; in a series of 1300 patients admitted to the ICU in the Lombardy region of Italy, 88% required endotracheal intubation and mechanical ventilation.<sup>3</sup> Similarly, two-thirds of patients with COVID-19 who required critical care in the United Kingdom had mechanical ventilation within 24 hours of admission.<sup>4</sup> Therefore, early detection of patients who will need mechanical invasive ventilation (MIV) may aid in delivering proper care and optimizing the use of limited resources, and this is of particular interest in lower- and middle-income countries.

Several laboratory parameters have been associated with worse outcomes in patients with COVID-19: elevated liver enzymes, ferritin, IL-6, lactate dehydrogenase (LDH), C-reactive protein (CRP), D-dimer, prothrombin time, troponin, and creatine phosphokinase, along with lymphopenia and acute kidney injury.<sup>5-7</sup>

Hyperferritinemia has been linked to macrophage activation syndrome (MAS), which is present in serious inflammatory disease<sup>8</sup>; MAS is quite possibly the origin of the severest clinical manifestations of SARS-CoV-2 infection.<sup>9</sup>

In this setting, we aimed to identify simple laboratory parameters that in combination with ferritin may help to predict early (first 48 hours) MIV by orotracheal intubation.

## Methods

This retrospective observational study was performed at Hospital de la Santa Creu i Sant Pau, a first-level hospital in Barcelona, Spain. The study was conducted according to the Declaration of Helsinki and approved by the institutional ethics committee. For patient enrollment, all consecutive blood tests that included ferritin between March 15, 2020 and April 6, 2020 were reviewed. In this way, patients diagnosed with COVID-19 in whom serum ferritin, ALC, platelet count, CRP, and LDH had been analyzed at admission were selected. In all patients, these parameters were determined in the same sample or with a maximum time difference of 24 hours. We assessed ALC on a Sysmex XN-10 (Sysmex Corporation, Kobe, Japan) analyzer. Serum ferritin was measured on an Architect c16000 System (Abbott Laboratories, IL) using a 2-step chemiluminescent microparticle immunoassay. We determined CRP and LDH in serum using the Alinity c system (Abbott Laboratories, IL), the former using a particle-enhanced immunoturbidimetric assay, the latter using spectrophotometry.

One hundred sixty patients aged 23 to 75 years were recruited. Informed consent was obtained from all participants. One hundred fifty-eight patients had a laboratory-confirmed SARS-CoV-2 infection according to World Health Organization guidance<sup>10</sup>: a positive result of real-time reverse-transcriptase polymerase chain reaction (RT-PCR) assay of a nasopharyngeal swab. In 2 patients, RT-PCR was negative and the diagnosis of COVID-19 was made

presumptively based on a compatible clinical presentation and an exposure risk. Patients who received tocilizumab, a monoclonal antibody against IL-6, at any time during their hospital stay were excluded for the study because this agent has been associated with a decrease in CRP and ferritin levels.<sup>11</sup> Patients with active neoplasia were also excluded because of iron overload.

Demographic, clinical, laboratory, and outcome data were extracted from electronic medical records. An image on chest radiograph was considered typical of COVID-19 in the presence of consolidation and ground-glass opacities, with bilateral, peripheral, and lower lung zone distributions. All patients were evaluated until death or hospital discharge.

Descriptive analyses of the variables were expressed as mean (range) or the number of patients (%). We chose MIV as the dependent variable, and the independent variables were LDH, ALC, platelet count, ferritin, and CRP. To analyze the association between the independent variables and MIV, the Pearson  $\chi^2$  test was used, considering a type I error < 5%. A binomial logistic regression analysis was used for the joint evaluation of variables associated with MIV. The significant variables with  $P < .05$  in the univariate analysis were selected for the regression analysis performed by the stepwise backward method (likelihood ratio). The variables that kept  $P \leq .05$  after adjustments remained in the multiple regression model. A receiver operating characteristic (ROC) analysis was performed to measure the diagnostic/predictive accuracy of each significant variable.

Moreover, we developed a classification tree analysis using the chi-squared automated interaction detection (CHAID) growing method. This nonparametric analysis examines interactions among variables to create a decision tree without assuming that independent and dependent variables are linked by linear relationships. All statistical analyses were carried out using SPSS, version 21.0. Because the sample size was small ( $n = 160$ ), we could not conduct an internal validation analysis.

## Results

**Table 1** shows the demographic and clinical characteristics of the 160 patients included in the study. Overall, 58.10% were men. The median age was 57 years (minimum: 23;

Table 1. Patient Characteristics

	Frequency (n)	Percentage (%)
Age, y, mean (minimum–maximum)		
57 (23–75)		
Sex		
Female	67	41.90
Male	93	58.10
Total	160	100.00
COVID-19 diagnosis		
Possible	2	1.30
Confirmed	158	98.80
Total	160	100.0
Typical X-ray pattern		
Yes	158	98.80
No	2	1.30
Total	160	100.00
Lactate dehydrogenase, U/L, mean (minimum–maximum)	405 (147–5250)	
Ferritin, µg/L, mean (minimum–maximum)	993 (107–5127)	
CRP, mg/L, mean (minimum–maximum)	125.3 (5.6–404.3)	
Platelets, × 10 <sup>9</sup> /L, mean (minimum–maximum)	223.2 (59–495)	
Lymphocytes, × 10 <sup>9</sup> /L, mean (minimum–maximum)	1.11 (0.11–2.85)	
Thrombotic event		
Yes	5	3.10
No	155	96.90
Total	160	100.00
MIV		
Yes	32	20.00
No	128	80.00
Total	160	100.00

Abbreviations: CRP, C-reactive protein; MIV, mechanical invasive ventilation.

Table 2. Variables Included in the Logistic Regression Model

Step 1	B	SE	Wald	df	Sig.	Exp (B)	95% CI for Exp (B)	
							Lower	Upper
Absolute lymphocytes (× 10 <sup>9</sup> /L)	−2.750	0.960	8.201	1	0.004	0.064	0.010	0.420
Lactate dehydrogenase (U/L)	0.007	0.002	10.941	1	0.001	1.007	1.003	1.011
C-reactive protein (mg/L)	0.006	0.003	3.970	1	0.046	1.007	1.000	1.013
Ferritin (µg/L)	0.001	0.000	4.026	1	0.045	1.001	1.000	1.001
Constant	−3.615	1.220	8.777	1	0.003	0.027	...	...

In the logistic regression model, all predictors were included in the first step. B is the coefficient in the model; SE is the standard error corresponding to B; Wald is the  $\chi^2$  distributed with 1 degree of freedom (df); Sig. is the corresponding P value; Exp (B) is the exponentiation of the B coefficient, which is an odds ratio (OR); and the 95% confidence intervals (CIs) around the OR are also presented.

maximum: 75). A total of 32 patients (20%) required endotracheal intubation and mechanical ventilation, which happened in 96.9% of these patients within 48 hours after the emergency room admission.

Four independent variables were selected for logistic regression model fitting (Table 2): LDH, ALC, ferritin, and CRP. The platelet count was excluded ( $P > .05$ ). Although each variable in the equation remained statistically significant, CRP

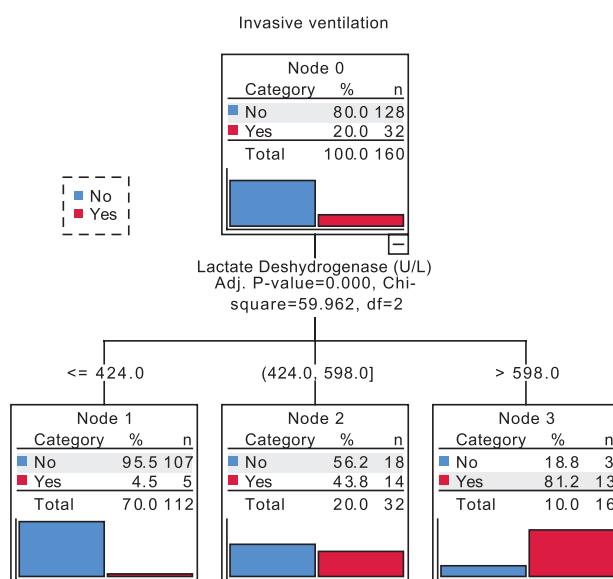
and ferritin were close to the limit of significance ( $P = .046$  and  $P = .045$ , respectively). Without including independent variables in the model, the probability of not being intubated was 80%. After the inclusion of the independent variables, the model's capacity to predict no intubation improved to 88%.

In the ROC analysis, LDH showed the greater AUC, with a value of 89.1%, followed by CRP (80.5%), ALC (77.6%), and ferritin (77.5%). Using the AUC, we established cutoff

**Table 3. AUC Scores for Significant Variables and Cutoff Values Predictive of MIV**

Variables	AUC Score (%)	Cutoff Value	Sensitivity (%)	Specificity (%)	MIV (yes/no)
LDH (U/L)	89.1	<219	100.0	11.7	No
		<310	93.8	52	
		>1102	6.3	100	Yes
		>506	69.0	94	
CRP (mg/L)	80.5	<65.65	100.0	39	No
		>76.45	96.9	44	Yes
Absolute lymphocytes ( $\times 10^9/L$ )	77.6	<0.37	100	19	Yes
		<0.53	95	28	Yes
		>1.28	36.7	93.7	No
		>1.56	21.9	100	No
Ferritin ( $\mu g/l$ )	77.5	<300	93.8	18.0	No
		>3496	18.8	99.2	Yes

Abbreviations: AUC, area under the curve; CRP, C-reactive protein; LDH, lactate dehydrogenase; MIV, mechanical invasive ventilation.

**Figure 1**

Classification tree. The root node (node 0) shows the binary (Yes/No) distribution of the outcome of the dependent variable, MIV. The  $\chi^2$  test is then applied to ensure that the branch is associated with a statistically significant predictor of MIV—in this case, LDH. As seen in node 1, LDH  $\leq 424.0$  U/L could predict that 95.5% of those patients did not need MIV; in addition, 70% of all patients had LDH  $\leq 424.0$  U/L. On the other hand, as shown in node 3, LDH  $> 598.0$  U/L could anticipate that 81.2% of patients needed MIV; only a minority of all patients (10%) reached those LDH levels. MIV, mechanical invasive ventilation; LDH, lactate dehydrogenase.

values for clinical application, as shown in **Table 3**. We found that LDH might represent the most useful discrimination variable for clinical application: when it was  $< 219$  U/L, patients did not need MIV, with a sensitivity of 100% and a

specificity of 11.7%. No patients with CRP  $< 65.65$  mg/L or an ALC  $> 1.56 \times 10^9/L$  needed MIV. Ferritin levels  $< 300 \mu g/L$  predicted no MIV with a sensitivity of 93.8%.

On the classification tree analysis (**Figure 1**), LDH  $> 598$  U/L increased the likelihood of MIV, and an  $< 424$  U/L defined a category with the lowest probability of MIV.

## Discussion

Our work provides an approximation for the prediction of early (first 48 hours) MIV in patients with COVID-19 by means of simple laboratory tests that can be easily collected in any hospital. Although our results are concordant with those published by other authors about risk factors for severe disease and death, they refer specifically to the risk of MIV, which is of particular interest in the context of the high pressure on ICUs. Therefore, our data can help prioritize patients quickly when healthcare resources are limited.

We found that LDH is the biomarker that better can predict early MIV; with a sensitivity of 100%, patients will not be intubated if LDH on admission is  $< 219$  U/L. Based on LDH, we developed a classification tree to estimate the risk of MIV quickly. Research has shown that LDH is an intracellular enzyme found in nearly all organ systems that catalyzes the interconversion of pyruvate and lactate, with concomitant interconversion of Reduced nicotinamide adenine dinucleotide and Nicotinamide adenine dinucleotide. Abnormal values can not only result from cardiac damage or hemolysis but also from multiple organ injury and decreased oxygenation with upregulation of the glycolytic pathway.

Because LDH is present in lung tissue, elevated levels seen in COVID-19 and other viral respiratory infections, such as Middle East Respiratory Syndrome, may represent the extent of lung injury that influences clinical outcomes.<sup>12</sup>

In severe COVID-19 infection, a deviation of the protective immune response into a dysfunctional program occurs, leading to cytokine release syndrome with severe inflammation and, eventually, multisystemic failure. A better understanding of the mechanisms lying at the root of immune response failure is needed; serum levels of inflammatory markers, such as CRP, ferritin, IL-6, and other cytokines, are increased in COVID-19.<sup>13</sup> In the setting of ongoing inflammation, evidence supports a role for ferritin in modulating the immune response, via its induction of anti-inflammatory cytokines and limitation of free radical damage. Alternatively, emerging work suggests a potential causative role of ferritin in the inflammatory pathology of disease.<sup>14</sup> Transcriptional induction of the *CRP* gene mainly occurs in hepatocytes in the liver in response to increased levels of inflammatory cytokines, especially IL-6. Similar to ferritin, evidence suggests that CRP is an important regulator of inflammatory processes and not just a marker.<sup>15</sup>

Lymphopenia, the fourth marker to predict early MIV, is a common feature in patients with COVID-19 and is more pronounced in severe cases of infection. It affects mainly T cells, including CD4 Th1 and Tregs, but particularly CD8. Although circulating CD8 in patients with severe COVID-19 has exhibited phenotypes associated with abnormal functionality and exhaustion, CD4 cells have been shown to express activation markers. In addition, natural killer lymphocytes have decreased in patients with both moderate and severe cases of the disease.<sup>9,16</sup> Injured alveolar epithelial cells could lead to the infiltration of lymphocytes.<sup>16</sup>

## Conclusion

This study identifies 4 simple laboratory parameters that predict with 88% accuracy the probability of early MIV, enabling early intervention and optimization of healthcare resources. **LM**

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