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Computational simulations to dissect the cell immune response dynamics for severe and critical cases of SARS-CoV-2 infection

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Abstract

Background: COVID-19 is a global pandemic leading to high death tolls worldwide day by day. Clinical evidence suggests that COVID-19 patients can be classified as non-severe, severe, and critical cases. In particular, studies have highlighted the relationship between lymphopenia and the severity of the illness, where CD8+ T cells have the lowest levels in critical cases. However, a quantitative understanding of the immune responses in COVID-19 patients is still missing.

Objectives: In this work, we aim to elucidate the key parameters that define the course of the disease deviating from severe to critical cases. The dynamics of different immune cells are taken into account in mechanistic models to elucidate those that contribute to the worsening of the disease.

Methods: Several mathematical models based on ordinary differential equations are proposed to represent data sets of different immune response cells dynamics such as CD8+ T cells, CD4− T cells, and also CD4+ T cells in patients with SARS-CoV-2 infection. Parameter fitting is performed using the differential evolution algorithm. Non-parametric bootstrap approach is introduced to abstract the stochastic environment of the infection.

Results: The mathematical model that represents the data more appropriately is considering CD8+ T cell dynamics. This model had a good fit to reported experimental data, and in accordance with values found in the literature. The NK cells and CD4+ T cells did not contribute enough to explain the dynamics of the immune responses.

Conclusions: Our computational results highlight that a low viral clearance rate by CD8+ T cells could lead to the severity of the disease. This deregulated clearance suggests that it is necessary immunomodulatory strategies during the course of the infection to avoid critical states in COVID-19 patients.

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1. Introduction

By August 2021, SARS-CoV-2 infection had derived a global pandemic which has caused more than 200 million confirmed cases and more than 4 million deaths worldwide. People of all age can be infected, where around 20% of the cases are asymptomatic, 60% appear with mild or moderate conditions, and 20% are severe or critical cases [5]. Most of the countries have taken emergency actions, these actions include confinement of their population, travel restrictions, forced use of mask in public spaces, and even a night-time curfew. In this pandemic, epidemiological models have been important to forecast the COVID-19 pandemic and to propose mitigation strategies [18,24].

There are three coronaviruses (SARS-CoV, MERS-CoV, and SARS-CoV-2) that can cause pneumonia, which can be fatal. SARS-CoV-2 is transmitted mainly via respiratory droplets, the median incubation period is around 4 days before symptom onset [20], most of the symptomatic patients developing symptoms within 11.5 days [31]. The viral load reaches its peak within 5-6 days of symptom onset [44]. An animal model using rhesus macaques reported two peaks of viral RNA, the first peak could be an input of the virus, while the second one is due to authentic viral replication [66].

Most of the COVID-19 patients did not present any symptoms or only mild respiratory symptoms [9]. Moderate cases present principally fever, cough, and fatigue; less common symptoms are spu- tum production, headache, hemoptysis, and diarrhea [27]. Most of
the moderate patients are recovered, however, a portion of these patients are hospitalized. Approximately 20% of cases develop severe illness, requiring intensive care unit (ICU) treatment because of complications, including acute respiratory distress syndrome, arhythmia, and shock. Critical patients have symptoms of dyspnea and they are more likely to be older [61]. The bulk of patients who die had comorbidities like hypertension, heart disease, diabetes, among others. Respiratory failure is the most common cause of death, followed by sepsis, cardiac failure, hemorrhage, and renal failure. In these cases lymphopenia, neutrophilia, and thrombocytopenia were usually observed [65].

A key determinant factor of disease severity in SARS-CoV-2 is age, in particular, individuals over 65 years have the greatest risk of requiring intensive care [9]. As with other viral infections, the severity of the disease in the elderly is not directly attributed to the viral titter but the host immune response [26]. Severe patients are characterized by difficulty in breathing and low blood oxygen level; in some cases could even appear a secondary infection by bacteria or fungi that may cause respiratory failure, which is the cause of death in most fatal COVID-19 cases [9,57].

The storm of cytokines released by the immune system in response to the infection can result in sepsis that is the cause of death in 28% of fatal COVID-19 cases [65]. For influenza infection, the adaptive immune response against viral infection impairs the innate immune defense against bacterial infection [40]. Immune therapies inhibiting the viral replication and regulation of dysfunctional immune response are key to block pathologies [15,51,57]. It is still controversial whether virus persistence can increase the severity of the disease. SARS-CoV-2 viral dynamics have shown remarked differences between severe patients and non-severe patients. It has been reported that the viral load peak is higher in non-severe patients (~10^8 copies/mL) than severe patients (~10^9 copies/mL), and viral shedding time has been long in severe patients [55], even the virus is detectable until death [68]. On the other hand, it also has been reported the mean viral load of severe cases is around 60 times higher than mild cases [33]. Hasanoglu et al. [21] conducted a study to evaluate six different types of samples from 60 COVID-19 patients with different ages and clinical histories, including asymptomatic patients. They showed that asymptomatic patients could have higher viral load than symptomatic patients, and observed a significant decrease in viral load as disease severity increases.

Similar to other viral infections, adaptive immune responses have a key role in SARS-CoV-2 infection, particularly T cells [26]. It remains unclear whether T cell responses are helpful or harmful in COVID-19. Mathew et al. [37] identified three immunotypes revealing different patterns of lymphocyte response in hospitalized COVID-19 patients. Immunotype 1 was associated with highly activated CD4+ and CD8+ T cells; immunotype 2 had less CD4+ T cell activation; and immunotype 3 had lacked activated T and B cell response. Mortality occurred for patients with all three immunotypes. On the other hand, patients with severe conditions have shown lymphopenia associated with COVID-19, where CD8+ T cells have a major impact [11,38]. Direct virus killing to lymphocytes could be part of the problem, as SARS-CoV particles have been found in T cells, monocytes and macrophages [19]. Recently, it has been reported the downregulation of exhausted cytotoxic T cell in children [7] that would create a sustained inflammatory environment, which would lead to a multi-system inflammatory syndrome in children.

There are several studies around the dynamic changes of lymphocytes [32,35], showing a low level of lymphocytes in severe patients. In [64], Zhang et al. analyze the dynamic changes of lymphocyte subsets and specific antibodies in coronavirus disease. They obtained blood samples of 707 patients from Wuhan, China, which were classified into moderate, severe, and critical groups. Their results showed that the counts of total T cells, CD4+ T cells and CD8+ T cells were significantly decreased with the increased severity of illness. The levels of these lymphocytes could be helpful markers to indicate the severe illness of COVID-19 and to understand the pathogenesis of COVID-19.

Computational modeling and simulations can be pivotal to dissect the dynamics between severe and non-severe COVID-19 patients. For instance, there are machine learning approaches for discovering symptom patterns [56] where it can be identified symptoms leading to a severe disease state; or developing prognostic models for survival prediction of COVID-19 patients [54]. Likewise, epidemiological models have been of great help to follow the pandemic evolution [52], evaluate the results of different scenarios, and reveal the health measures that could help to mitigate the pandemic [4,34,48]. The use of compartment models is widespread in the study of epidemics, and its interest has been increasing in this pandemic. Models have been developed by age and sex groups to study the evolution of the pandemic, and testing which would be the best vaccination strategies [10]. Furthermore, epidemiological models have been very important in assisting public health decisions making [3,49].

Similarly, mathematical models at the within-host level can help us to elucidate the biological mechanism of innate and adaptive immune responses against a pathogen, and provide a powerful tool for testing hypotheses, as well as the evaluation of drugs and treatments. Recently, a great effort has been made to develop mathematical models to describe the dynamics of SARS-CoV-2 at the within-host level [14,17,25,45,62]. Sadria and Layton [59] studied three potential COVID-19 therapies using a calibrated within-host model with available clinical data for SARS-CoV-2 infection. Their findings suggest that therapies must be administrated no after the second- day post symptoms onset [50]. Chhetri et al. [12] developed a mathematical model and found that the combination of therapies including antiviral and immunomodulating drugs was most effective. Also, within-host models can help us to differentiate groups of patients with high mortality, Néant et al. [42] found that the viral dynamics is associated with mortality in COVID-19 patients and that strategies that consider decreasing viral load may be more effective.

While these in-host models [25,45,62] are fitted to viral data from COVID-19 patients to infer the interaction with the immune response, there has not been any study to quantify the differences between severe and critical patients with COVID-19. As far as we know, there are no models fitted to T cells data in order to examine the relation between T cell dynamic and severity of the illness. In this work, we contribute to the mathematical study of the SARS-CoV-2 dynamic and the T cell dynamics to elucidate the principal role of lymphocytes in the development of the disease between severe ad critical patients.

2. Materials and methods
2.1. Experimental data details

Here we considered the data reported by Zhang et al. [64]. They collected from 707 COVID-19 patients in Wuhan, China between February and April, 2020. The patients were classified into moderate, severe and critical groups. The moderate cases were those with fever, typical symptoms and pneumonia. 206 severe cases had respiratory distress, blood oxygen saturation less than 93%, or arterial partial pressure of O2 to fraction of inspired oxygen ratio less than 300 mmHg. 91 critical cases had respiratory failure, shock or multiple organ dysfunction needing intensive care unit treatment.

The counts of total T cells, CD4+ T cells, CD8+ T cells, B cells, and Natural Killer (NK) cells were analyzed with FACSanto flow cytometer from 50 μl of whole blood. The patients were 48.5%
males and 51.5% females. Most of the patients had fever, cough, expectoration, shortness of breath, chest distress, diarrhea at the illness onset. The most common comorbidities of the cases were hypertension (37.9%), diabetes (17.1%) and cardiovascular disease (11.1%). There were 30 deceased. The total T cells, CD4+ T cells and CD8+ T cells in moderate patients were relatively stable compared to those for several and critical cases. The severe and critical group had a lower count of lymphocyte from the illness onset but gradually recovered to the normal levels. More details can be found in the original paper [64]. The data are displayed in Fig. 1, the median is represented as points and the dashed lines represent the interquartile range (IQR). Data are reproduced from the original paper [65] using plotDigitizer.

2.2. Mathematical models

**Model 1. CD8+ T cell response:** In [25] has been reported a mathematical model to represent the interaction between SARS-CoV-2 infection and immune response dynamics. The model is given by:

\[
\frac{dV}{dt} = pV\left(1 - \frac{V}{K}\right) - cV T - CV.
\]

\[
\frac{dT}{dt} = s_T + rT\left(\frac{V^2}{V^2 + k_T^2}\right) - \delta_T T.
\]

where \(V\) is the virus level, \(T\) is the number of CD8+ T cells, \(p\) is the viral replication rate with maximum carrying capacity \(K\), and \(c\) is the rate of cleared virus. \(c V T\) represents the rate of killing of infected cell by the immune response. In this model is assumed that the activation of T cell proliferation by \(V\) is at a rate \(r\), follows a log-sigmoidal form with half saturation constant \(k_T\). The parameter \(s_T\) represents T cell homeostasis with \(\delta_T\) as the half life of T cells and \(T(0)\) the initial number of them. Fig. 2 shows a schematic representation of this model.

Contrary to [25], in this work we fitted the data of CD8+ T cells to the model (2) using the median of the CD8+ T cells count for severe and critical cases from [64]. We reduced the number of parameters to be identified to \(p\), \(c\), \(r\), \(s_T\) and \(\delta_T\). The half-life of CD8+ T cells in humans have been estimated from 34 days [39] to 255 days [60]; therefore, we take \(\delta_T = 0.01\) day\(^{-1}\). We fix \(c = 2.4\), \(K = 10^8\), and \(k_T = 1.26 \times 10^5\) for both cases; these values were taken in accordance to [25]. Also we use the initial viral level \(V(0) = 0.31\) copies/ml; this value was obtained in the previous work by using a regression model with viral load data from COVID-19 patients. However, we performed parameter estimations by varying \(V(0)\) in 0.16, 0.31, 0.47 and 100 (the detection level is around the last value) but we did not find any significant difference in parameter values. The \(s_T\) parameter for each case was fixed with the respective initial value \(T(0)\). Due to lack of data before illness onset, we assumed the initial level of T cells equal to the median of the CD8+ T cells at the day 3 \((T(0) = T(3))\) from the reported data for each case. Infection time was assumed at \(-3\) days after illness onset (daio). This infection time was also varied in \(-10\), \(-5\), \(-3\) and \(0\) daio in order to find the best value but we found no significant difference between the results. Stability analysis of this model can be found in [2].

In our model, we included CD8+ T cells in the peripheral blood of patients described above, who have a wide range of comorbidities. We only considered several and critical cases, since data from moderate cases showed no marked changes during the disease course.

**Model 2. CD8+ T cell and CD4+ T cell responses:** Similar to CD8+ T cells, there is clinical evidence of activation and/or exhaustion markers at CD4+ T cells during COVID-19 [11]. Even it has been suggested that CD4/CD8 ratio is significantly higher in critical patients than non-critical patients [43]. Because of that, we modified the Model 1. We now assumed that CD4+ helps to the proliferation of CD8+ T cells which occurs at rate \(\alpha_T\) where \(\alpha_T\) is CD4+ T cell level and \(\alpha\) is a free parameter to be estimated. We use piecewise linear fits to generate a time-dependent function \(\alpha_T(t)\) using the experimental data displayed in Fig. 1b. The modified model is
given by:
\[
\frac{dV}{dt} = pV\left(1 - \frac{V}{K}\right) - c_1VT - cV.  \tag{3}
\]
\[
\frac{dT}{dt} = s_T + rT\left(\frac{V^2}{V^2 + k_4^2}\right) - \delta_T T.  \tag{4}
\]

The fixed parameters were the same as the Model 1, and \( p, c_1, r_T, \) and \( \alpha \) were estimated in the same way by using CD8\(^+\) T cell data. We also explored two different ways to integrate CD4\(^+\) T cell data, which is shown in Model 3 and 4. The results can be found in Fig. S1 in the Supplemental Material.

**Model 3. CD8\(^+\) T cell proliferation as a function of CD4\(^+\) T cell dynamics:** In this model CD4\(^+\) T cells (and not the virus directly) are the ones that help CD8\(^+\) T cells to proliferate. The T cell response Eq. (2) of the Model 1 is changed by:
\[
\frac{dT}{dt} = s_T + rT\left(\frac{T^2}{T^2 + k_4^2}\right) - \delta_T T.  \tag{5}
\]

Here, \( r \) and \( k_4 \) parameters are estimated. In this model, the dynamics of the virus was not appreciated for severe cases, in critical cases the virus was not cleared. The results can be found in Fig. S2 in the Supplemental Material.

**Model 4. CD8\(^+\) T cell proliferation as a function of CD4\(^+\) T cell and viral dynamics:** In this model was considered that both the virus and CD4\(^+\) T cells help CD8\(^+\) T cell proliferation, this proliferation is represented by changing the T cell response Eq. (2) of the Model 1 by:
\[
\frac{dT}{dt} = s_T + rT\left(\frac{V^2}{V^2 + k_4^2}\right) - \delta_T T.  \tag{6}
\]

where \( r \) and \( k_4 \) parameters are estimated. However, in this model, the virus is not cleared. The results can be found in Fig. S3 in the Supplemental Material.

**Model 5. CD8\(^+\) T cell and NK cell responses:** Furthermore, natural killer (NK) cells are critical in the first-line defense against viral infections, and integrate innate and adaptive immune responses [59]. It has been correlated the number and function of NK during SARS-CoV-2 infection with the severity of the disease [36,67]. Therefore, we explored the viral clearance due to NK cells (N) at rate \( c_N VN \). Similar to the modification above, we use piecewise linear fits to generate \( N(t) \) using data in Fig. 1c. The modified model is given by:
\[
\frac{dV}{dt} = pV\left(1 - \frac{V}{K}\right) - c_1VT - cV - c_N VN.  \tag{7}
\]
\[
\frac{dT}{dt} = s_T + rT\left(\frac{V^2}{V^2 + k_4^2}\right) - \delta_T T.  \tag{8}
\]

The parameters \( p, c_1, r \) and \( c_N \) were estimated using CD8\(^+\) T cell data. The results shows that \( c_N \) tends to zero, thus NK cell dynamics does not play a key role in viral clearance. In severe cases the viral load does not decrease enough to be below detectable levels. These results are showed in Fig. S4 in Supplemental Material.

**Model 6. CD8\(^+\) T cell, CD4\(^+\) T cell and NK cell responses:** We also explored combining the above two models to use CD4\(^+\) T cells and NK cells data without improving the AIC value. This model is given by:
\[
\frac{dV}{dt} = pV\left(1 - \frac{V}{K}\right) - c_1VT - cV - c_N VN.  \tag{9}
\]
\[
\frac{dT}{dt} = s_T + rT\left(\frac{V^2}{V^2 + k_4^2}\right) - \delta_T T + \alpha T.  \tag{10}
\]

The results of this model are very similar to Model 2, so the NK cell response is not supported by this data set. These results can be found in Fig. S5 in Supplemental Material.

### 2.3. Parameter estimation

Ordinary differential equations were solved using a Dormand-Prince fifth-order Runge-Kutta algorithm. The estimation of the free parameters was performed by minimizing the Root Mean Square Error (RMSE) using the difference between the experimental measurement \( y_i \) and the predictive output \( \hat{y}_i \) as follows:
\[
\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (y_i - \hat{y}_i)^2}  \tag{11}
\]

where \( i \) is the corresponding sample and \( n \) is the total number of measurement. To minimize the RMSE we used the Differential Evolution (DE) algorithm [53]. We implemented a DE algorithm using GPU parallelization with code written in CUDA-C, this implementation accelerate the optimization ten times at least. Some details about a DE implementation on GPU can be found in [58]. In this DE algorithm, we used a population array of 1024 elements for each parameter to be estimated, this population is taken randomly from a uniform distribution between the minimum and maximum values of each parameter reported in the literature. To generate a new mutant array of the population we used the strategy DE/best/1/bin, which is based on mutating the best set of parameters of the population with a mutation factor of 0.8 and a crossover rate of 0.8. We selected these values because they are that one that gave the best results after testing the algorithm using different population sizes, mutation factor and crossover rates. The algorithm reaches the optimized values before 1000 iterations (it takes about 5 min on a laptop). In this parallel implementation, the 1024 elements of the population are mutated and recombined at the same time per GPU thread.

### 2.4. Akaike information criterion

To compare between different models, we used the Akaike information criterion (AIC) defined by:
\[
\text{AIC} = n \log \left(\frac{\text{RMSE}}{n}\right) + \frac{2mn}{n-m-1}  \tag{12}
\]

where \( n \) is the number of data points and \( m \) is the number of unknown parameters. A lower AIC values means a better description of the data.

### 2.5. Identifiability analysis

A mathematical model is said to be identifiable when the parameter set can be uniquely determined. Here we used the profile likelihood method proposed by Raue et al. [47]. In this method one by one the parameters are set to a range of values centered at the optimized value; the other parameters are re-optimized using the same cost function, which is the RMSE above mentioned. This methodology can detect both structurally and practically non-identifiable parameters [23]. Structural non-identifiability is related to the model structure and practical non-identifiability takes into account the amount and quality of the data. A parameter can be identified when the profile likelihood presents a concave form. However, if the data are insufficient and manifest large variabilty, the parameter could be practically non-identifiable. This can be visualized as a relatively flat valley in the profile likelihood. A structurally non-identifiable parameter has a profile that maintains a constant RMSE when the parameter is varied.
2.6. Bootstrap

The experimental data displayed in Fig. 1a present a highly variable response to SARS-CoV-2 infection, hence we performed bootstrap fits to mimic a stochastic environment of the infection. Notice that the quartiles in Fig. 1a are asymmetric, that is, that the median values of the experimental data are not in the middle of the IQR. For that reason, we generated 27 discrete data between the lower limit and upper limit of the IQR (including the median value) for each point of the daio (x-axis). We then performed a nonparametric bootstrap approach using Monte Carlo resampling. Data were resampled with replacement to have a sample of equal size to the generated values above. The parameters are estimated from the resampling. We adapted our DE code to perform 100 parameter estimations at the same time using CPU parallelization to save computational time. A total of 1000 optimizations (10 runs of our DE code) were performed using different sets of resampled data. We obtained the corresponding parameter distribution from refit our model in each of these repetitions.

3. Simulation results

The experimental data for CD8+ T cells and their respective fits of the Model 1 are displayed in Fig. 3a. The experimental data were reproduced from Zhang et al. [64]. For severe cases, the CD8+ T cell response starts about 10 to 20 daio reaching its peak between 35 to 45 daio, while for critical the CD8+ T cell response starts late, around 30–40 daio with a peak between 40 to 50 daio. Note that critical cases begin with a lower level of CD8+ T cells than severe cases (half of them), however, both reaches approximately the same level of the moderate cases at the end of the disease course. The total count of cells, that is the area under the curve (AUC), between both cases are in the same order of magnitude although is lower for critical cases (1.3 × 10^7 cells days/mL) than severe cases (2.2 × 10^7 cells days/mL).

The viral load obtained using the Model 1 with the parameter fitted to CD8+ T cells experimental data is displayed in Fig. 3b. The viral load peaks around 40 daio for critical cases and 20 daio for severe cases. There is a delay in the peak of the viral load for critical cases compared to severe cases, also critical viral load peak is around an order of magnitude lower than severe viral load peak, and even the total viral count (the AUC) is higher for severe cases (1.2 × 10^8 copies days/mL) than critical cases (4.5 × 10^6 copies days/mL). This outcome is debatable since high viral loads have been reported from patients who develop a critical case of the disease [33]. Contrarily, reported viral loads in [21] decrease as disease severity increases. In this study we used data from a group of patients with similar characteristics to the second study [21], where COVID-19 patients had a wide range of ages, some presenting comorbidities; our results are in agreement with that study.

The profile likelihood analysis was performed with the unknown Model 1 parameters. These profiles are shown in Fig. 4a–c. Critical cases show a minimum viral replication rate \( p \) and viral clearance \( c \) implying identifiability of these parameter. The likelihood profile for the CD8+ T cell proliferation rate \( r \) does not show a minimum for critical cases, this could be due to an insufficient amount of data, however, although it is not visible in the plot, there is a minimum point around 0.13. In severe cases, the profiles for \( c \) and \( r \) show a minimum while for \( p \) it is not very clear. This analysis shows the possibility to find unique values of the unknown Model 1 parameters from experimental data reported. It should be noted that the medians of the data were used in this analysis; however, there is a large variability among them, so the parameters could be practically non-identifiable.

The best fitted parameters are presented in Table 1 for CD8+ T cells and the two cases of illness severity. The viral replication rate \( p \) for critical cases is half of that for severe cases. The viral clearance \( c \) for critical cases is approximately one third of that for severe cases, while the CD8+ T cell proliferation rate \( r \) is higher for critical cases. These results suggest that the rapid proliferation of CD8+ T cells may compensate for the low clearance rate, which could be a key aspect in the development of the disease.

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**Table 1**

| Parameter | Value (95% CI) | Value (95% CI) |
|-----------|---------------|---------------|
| K         | 10^9 copies/mL| 10^9 copies/mL|
| c         | 2.4 day⁻¹     | 2.4 day⁻¹     |
| kᵣ        | 1.26 × 10⁶ copies/mL | 1.26 × 10⁶ copies/mL |
| δᵣ        | 0.01 day⁻¹ | 0.01 day⁻¹ |

| Critical cases | | |
|----------------|------------------|
| Parameter      | Best fit | Median | CI (95%) |
| p [day⁻¹]      | 3.50 | 3.55 | (3.45 - 3.81) |
| c [10⁻⁸ day⁻¹ cell⁻¹] | 0.596 | 0.595 | (0.384 - 0.636) |
| r [day⁻¹]      | 0.131 | 0.097 | (0.033 - 0.137) |

| Severe cases | | |
|--------------|------------------|
| Parameter    | Best fit | Median | CI (95%) |
| p [day⁻¹]    | 6.99 | 6.67 | (6.25 - 7.63) |
| c [10⁻⁸ day⁻¹ cell⁻¹] | 1.47 | 1.34 | (1.16 - 1.54) |
| r [day⁻¹]    | 0.020 | 0.021 | (0.014 - 0.024) |
Due to the high variability of the data, we performed bootstrap fits to obtain the confidence interval of the Model 1 parameter estimated. Fig. 4d–f shows the distribution in parameter values for severe and critical cases. The three free parameters show clear differences between cases. The viral replication rate $p$ in critical cases decreased 45% concerning severe cases, the rate of killing of infected cells by immune response $c_T$ decreased 53% for critical cases; whereas that CD8$^+$ T cell proliferation rate $r$ was four times the rate for severe cases. Table 1 shows the median and 95% confidence interval of each parameter. The median values presented for critical cases are consistent with the values for the best fit, except for $r$. Severe cases median values show the opposite behavior; only $r$ is following the value for the best fit. This discrepancy could be attributed to high variability of the experimental data used.

In order to explore the dependencies of the parameters, we displayed scatter plots in Fig. 4g–i. These plots reveal that there are no strong correlations between $p$, $c_T$, and $r$. However, we can notice a slight inter-dependence between $p$ and $r$ parameters for critical cases; and $p$ and $c_T$ parameters for severe cases. In the former, increasing $r$ decrease $p$; and in the latter, increasing $p$ increase $c_T$.

We explored the modification of the Model 1 by adding CD4$^+$ T cells and NK responses as mentioned above. Fitting these models to the data revealed that including CD4$^+$ T cells as a promoter of the proliferation of CD8$^+$ T cells does not improve the fits, similarly for the NK response. Table 2 shows the AIC number for each model. The parameter $c_N$ in Models 5 and 6 tends to have small values ($10^{-10}$), almost the same dynamics of Model 1. In Models 3 and 4, we added CD4$^+$ T cell helper as a log-sigmoidal form; however, in these models the virus does not reach undetectable levels. Notice that they have the highest AIC values.

The Model 2 has the lowest RMSE value considering both groups of patients, but its complexity increased compared to the Model 1. In fact, the Model 2 shows that the viral load could be three orders of magnitude lower than Model 1 for both groups of patients, and therefore far from the value of the viral peaks reported in the literature. Thus, the Model 1 has the lowest
AIC number and consequently the best explanation to the data sets.

### 4. Discussion

The role of the immune system during SARS-CoV-2 infection is fragmented. The T cell kinetics seem to be decisive in the resolution of severe or non-severe patients [32]. CD8+ T cells are relevant for killing infected cells during viral infections [46]. Furthermore, a defective immune response may lead to further accumulation of immune cells in the lungs causing overproduction of cytokines, resulting in a cytokine storm leading to multi-organ damage [16,57]. Therefore, an abnormal proliferation of T cells could lead to a critical state in the COVID-19 patient. Quantification of the dynamics of these T cells could help to identify the critical cases in the early stage of the disease.

In a recent study [6], patients with hematologic cancer show that higher CD8+ T cell counts is associated with improved survival. Also, robust CD4+ T cell response in conjunction with diminished CD8+ T cells is key in survival patients. Our simulations highlight a clear difference between the parameters that model critical cases and those that model severe cases. The principal difference is in the rate of T cell proliferation r, this rate is high in critical cases. This is in accordance with [29,30,63], suggesting a hyperactivation and overaggressive CD8+ T cell response. However, it is still unclear whether the T cells in COVID-19 patients are exhausted or just highly activated [11]. In severe patients with COVID-19, lymphopenia is present, where CD8+ T cells have the lowest count. It has been suggested that this state of lymphopenia is due to T cell apoptosis and migration of T cells from the peripheral blood to other tissues. It is also known that IL-7 is an important factor in T-cell homeostasis, and that it is regulated by lymphocytes; in severe patients with low lymphocyte levels, there are high levels of IL-7, which subsequently leads to T cell proliferation in the late stages of infection. This suggests that lymphopenia-induced proliferation could play a key role in disease severity. These observations have already been reported by Adamo et al. [1] and support the results we have obtained, where critical patients have a lower level of lymphocytes than severe patients and therefore a CD8+ T cell proliferation rate higher at the end of the disease.

On the other hand, fitting results show a viral clearance rate \( c_2 \) for severe cases is higher than that for critical cases. The viral replication rate \( p \) for severe cases is also higher than that for critical cases which translates to a higher viral peak. Therefore, although the severe cases have a low production of CD8+ T cells compared with that critical cases, these cells clear faster the virus. Notice that viral shedding takes approximately the same time for both types of cases, this suggests that critical cases may have a dysfunctional immune response with an excessive infiltration of T cells which could cause widespread inflammation and multi-organ damage.

In [64], initially, both critical and severe cases do not have any significant difference for CD4+ T cell levels. Nevertheless, there is a tendency for low levels of CD4+ T cells in critical patients. Note that in normal conditions, IL-2 improves transcription of FOXP3 which is widely recognized as a suppressor of the T cell response. However, in severe COVID-19 patients, activated T cells fail to express FOXP3 [28]. This T cell dysregulation promotes prolonged inflammation and tissue destruction. Model selection was not able to show that CD4+ T cells play an important role in viral clearance or CD8+ T cell proliferation.

Here, we also explored the innate immune response against viral infection adding to our model NK cell response, nevertheless, this did not improve the AIC as shown in Table 2. This may be attributed that NK cells play an important role at the beginning of the infection, and such dynamics can not be capture in the used data set [64]. It has been reported that the upregulation of human inhibitory receptors is one more strategy that SARS-CoV-2 uses to modulate NK cell cytotoxicity. It is clear that increase expression of such receptors leads to NK cell exhaustion and decreases their ability to clear a viral infection. The mechanisms that drive NK cell exhaustion are poorly understood [8]. Several upregulated genes in peripheral blood from COVID-19 patients are involved in the apoptosis pathways, suggesting lymphopenia is due to apoptosis by SARS-CoV-2. In SARS-CoV-2 infection NK cells exit the peripheral blood, contributing to local inflammation and injury. NK cells that remain in the circulation show an exhausted phenotype that facilitates virus spread [36].

The Model 1 is the best to describe the data of CD8+ T cell dynamics. The best fits show a delay viral peak for critical cases, the difference is 20 days; while CD8+ T cell levels peak approximately in the same time for both cases and with almost the same level. Critical cases have the T cell response peak 5 days after their viral load peak. It is worth to mention that experimental data taken in [64] have samples with different comorbidities and ages, our results could be different if we took data from an homogeneous group. This work aimed to generalize the dynamics of the immune system response, comorbidities were not taken into account, partly because of the lack of data, and because several clinical studies take heterogeneous populations where we could compare our results. The variations between different patients were simulated by generating data with the nonparametric bootstrap approach to elucidate the distribution of the parameters of interest. This modeling approach may be of interest to represent populations in multiscale simulations where population heterogeneity is of interest.

Fig. 3a does show that CD8+ T cells and the virus start to replicate earlier in severe patients with respect to the critical one. However, while the CD8+ T cell is delayed in the critical patients, it reaches similar levels as the severe patients. This difference between severe and critical COVID-19 patients can be attributed to the effects of aging on the immune systems which are highly altered during viral infections [22,26]. Previous mathematical modeling work [26] had formulated that the slower viral growth presented in aged individuals may lead to less immune stimulation [13].

The deregulated T cells proliferation shown in our result suggests that antiviral treatment strategies could be insufficient for severe cases, and especially critical ones. It is necessary to develop immunomodulatory strategies, such as cytokine and anti-cytokine therapies [41], during the second week of illness. Due to the heterogeneity of the patients, each of them needs a different therapeutical approach.

### Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
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