FROM ABM TO CONTINUOUS, A TUBERCULOSIS ALVEOLAR INFECTION MODEL

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Abstract Tuberculosis is an infectious disease mainly caused by the bacterium *Mycobacterium tuberculosis*. Computational and mathematical models are a good tool to understand the disease and its mechanism.We developed an Agent-Based Model (ABM) simulating a tuberculosis infection to obtain lysis rate as a function of the number of intracellular bacilli. Using a fitting of the lysis rate in a continuous model, we were able to obtain comparable results that allowed us to have a simpler model and save computational time.

I. INTRODUCTION

A. Tuberculosis

Tuberculosis (TB) is one of the top 10 death causes worldwide. In 2016 1.7 million people died from the disease mostly in low- and middle-income countries. People infected with *Mycobacterium tuberculosis* (*Mtb*) have a 5-15% lifetime risk of falling ill. In addition, when the patient's immunity system is weakened by a second factor- such as malnutrition or HIV- the risk of falling ill increases [1].

B. Natural history

The infection starts when *Mtb* arrives at the pulmonary alveolus. Each alveolus has a type of white blood cell (macrophage) which is constantly laundering this space from pathogens coming from the air that we breathe.

However, some bacteria such as *Mtb* are able to evade the bactericidal properties of phagosomes or even use it as an invasion strategy. Due to this fact, bacilli are able to replicate inside the macrophages. Each bacillus duplicates every 24 hours until a concentration between 32-64 bacilli is achieved inside a macrophage [2]. Then, macrophage's lysis takes place, releasing the intracellular bacilli to the alveolus. Extracellular bacilli infect new macrophages that enter the alveolus trying to stop the multiplication of bacilli. This flux of macrophages inside the alveolus is called non-specific immune response and it is a direct consequence of the macrophages' death. In addition, bacilli that have not been engulfed by macrophages can also multiply using neutrophils as replication support.

This cycle ideally ends once the specific inmune response appears and the TB lesion is controled and calcified.

C. Computational model

Mathematical and computational models can be used to improve the understanding of TB infection. In this project we work with a computational model, developed by *M. Català* and *S. Alonso*, that reproduces the relations between the different elements inside the alveolus.

There are five elements that are considered in the infection inside an alveolus: extracellular and intracellular bacilli, infected and uninfected macrophages and neutrophils. A bacillus is extracellular (b_E) if a macrophage has not engulfed it, otherwise, it is intracellular (b_I). The macrophages can be infected (m_I) if they have bacilli inside or uninfected (m_U) otherwise. Finally, there are also neutrophils (n) which are used by extracellular bacilli to replicate outside.

Bacilli, intracellular and extracellular, grow proportionally to the number of bacilli of each type present in the alveolus. In addition, they saturate at a certain value αm_I for intracellular and δn for extracellular.

$$\dot{b_I} = \mu b_I \left(1 - \left(\frac{b_I}{\alpha m_I}\right)^3\right) \tag{1}$$

$$\dot{b_E} = \mu b_E \left(1 - \left(\frac{b_E}{\delta n_T}\right)^3\right) \tag{2}$$

Extracellular bacilli are engulfed by uninfected macrophages proportionally to the number of extracellular bacilli and uninfected macrophages at a rate γ.

$$\dot{b_I} = -\dot{b_E} = \dot{m_I} = -\dot{m_U} = \gamma b_E m_U \tag{3}$$

Infected macrophages also engulf extracellular bacilli. The maximum number of bacilli that a macrophage can host inside is taken into account.

$$\dot{b_I} = -\dot{b_E} = \gamma b_E m_I \left(1 - \left(\frac{b_I}{\alpha m_I}\right)^3\right) \tag{4}$$

There is a flux of uninfected macrophages (σ) and neutrophils (η) entering the alveolus because of the

non-specific immune response. This flux depends on the occupied volume inside the alveolus and it is null when the alveolus is full.

The system of equations describing the behaviour of each element is shown in equations 5-9.

$$\dot{b_I} = \mu b_I \left(1 - \left(\frac{b_I}{\alpha m_I}\right)^3\right) + \gamma b_E m_U + \\ + \gamma b_E m_I \left(1 - \left(\frac{b_I}{\alpha m_I}\right)^3\right) - \alpha \cdot lysis \tag{5}$$

$$\dot{b_E} = \mu b_E \left(1 - \left(\frac{b_E}{\delta n_T}\right)^3\right) - \gamma b_E m_U - \\ - \gamma b_E m_I \left(1 - \left(\frac{b_I}{\alpha m_I}\right)^3\right) + \alpha \cdot lysis \tag{6}$$

$$\dot{m_U} = g\sigma - \gamma b_E m_U \tag{7}$$

$$\dot{m_I} = \gamma b_E m_U - lysis \tag{8}$$

$$\dot{n} = g\eta \tag{9}$$

Lysis term is not mathematically characterized, because the relation between the number of bacilli inside a macrophage and the lysis of the macrophage is in general unknown. At this point, it could be reasonable to ask if a correlation between the different agents and the number of lysis can be found.

D. Objectives

The objectives of this project are:

- To develop an Agent-Based Model (ABM) that reproduces the process of a TB infection inside an alveolus.

- Fit the lysis function with the results of the ABM to implement it in a continuous model and obtain similar results.

- To examine how the results change under the variation of some parameters.

II. THE MODEL

The development of the ABM and the continuous model has been done using MATLAB®, the code is Open Source and it can be found in the following link.

A. From the ABM to the continuous model

The ABM has the same terms as the continuous model described above (engulfment of macrophages, growth of bacillus and macrophages, and entrance of macrophages and neutrophils). The main agent is the macrophage with only one property: the concentration of bacilli.

b_I vector: The model has a b_I vector with every component representing a macrophage. Each component is the number of bacilli inside that macrophage. These numbers vary every time step because new bacilli are incorporated to the macrophage from the process of engulfment or growth. When a concentration α' of bacilli is achieved the macrophage is lysed.

Engulfment of bacilli: There cannot be more engulfed bacilli than the total amount of bacillus present in the alveolus. Then,

$$engulfment(m_I) + engulfment(m_U) < b_E$$

where the term *engulfment(m_i)* is referred to the number of bacilli that a macrophage (infected or uninfected) is able to engulf.

Alveolar saturation: The alveolus has a finite volume that needs to be considered in order to limit the number of macrophages (both dead or alive) and neutrophils that can enter our system. Therefore, the parameter g was defined as:

$$g = 1 - \frac{m \cdot V_m + n \cdot V_n}{V_a} \tag{10}$$

Where V_m, V_n and V_a are the corresponding volumes of the macrophage, the neutrophil and the alveolus respectively and m and n are the number of macrophages and neutrophiles.

This parameter goes from 1 to 0 as the total volume of macrophages and neutrophils increases and multiplies the entrance flux of the immune response.

B. Discretization

Every time step, the simulation computes the variation of the elements present in the alveolus: dm_I, dm_U, db_E, db_I, dn. These variables are integers (with b_I a vector of integers) while their variation is not. To fix that, a system of probabilities was established in order to determine the new variation of the elements.

The system of probabilities consists in comparing the variation with a uniformly distributed random number between 0 and 1. If the random number is lower than the variation, then is considered another individual.

It is known that lysis takes place when there is a concentration of approximately $\alpha = 60$ bacilli inside a macrophage. In real biology, this number is an approximation and the condition for a macrophage to lyse depends on each macrophage: one could lyse at 55 and the other at a 68 bacilli inside.

C. Input parameters

The outcome of the model depends on the following set of parameters. Most of them were obtained from experimental data in TB infection [2][3]:

Time of simulation, T_{max}: This time determines the end of the simulation. After that time, simulation's results are analyzed. It was set T_{max}=100 days.

Time step, dt: which is in days and with a value dt=0.1 days.

Macrophage's capacity of bacilli, α': It determines the maximum number of bacilli inside an infected macrophage before lysis. It was set α' as a random number from a normal distribution with mean parameter α=60 and four units of standard deviation. Each macrophage has its own capacity of bacilli assigned since their entrance in the alveolus.

Capacity of bacilli on neutrophils' surface, δ: Number of bacilli that can replicate at neutrophils' surface. In the model, δ=30.

Engulfment rate, γ: Macrophages engulf bacilli at a velocity of γ=0.01 $days^{-1}$.

Macrophages entrance rate, σ: When the infection takes place and macrophages start lysing, new uninfected macrophages enter in the alveolus with a rate σ=20$days^{-1}$.

Neutrophils entrance rate, η: Neutrophils start entering into the alveolus after the infection at a velocity η=80$days^{-1}$.

Alveolar volume, V_a: As it has been discussed before, the alveolus has a finite volume. In the simulation it was set at V_a=5·10^6 μm^3 [4]

Macrophage's volume, V_m: It was set at V_m=4990 μm^3 [5]

Neutrophil's volume, V_n: The volume occupied by neutrophils is considered as V_n=299 μm^3 [6]

At the beginning of the simulation, a number of initial Bi, Be, Mi, Mu and n is established but the output variables do not depend sensitively on these values. The simulation starts when the infection has been produced but not in a very advanced state.

D. Outcome variables

At the end of the simulation, the following outcome variables are obtained:

Lysed macrophages per day, $Lysis$: when the intracellular bacillus in a macrophage reach a certain threshold value (different for each macrophage) the macrophage is lysed. This occurs each time step dt. In order to obtain the rate of lysis per day, the obtained lysis has to be multiplied by $1/dt$.

Intracellular bacillus, Bi: Total number of bacillus inside macrophages, calculated as $sum(b_i)$

Infected macrophages, Mi: Number of infected macrophages in the alveolus.

Uninfected macrophages, Mu: Number of uninfected macrophages present in the alveolus.

Extracellular bacillus, Be: Total number of bacillus that grow outside macrophages.

Neutrophils, $Nvector$: Number of neutrophils in the alveolus.

Time vector, Ti: a vector containing the time evolution to plot the different variables against time.

III. RESULTS

A. ABM simulation

ABM model was correctly implemented, the obtained results can be seen at Figure 2. In the first days the flux of un-infected macrophages is nearly constant as g is close to 1 (the alveolus is empty). After a few days the number of infected macrophages rise as the first lysis and the multiplication of the extracellular bacillus takes place. Until the uninfected macrophages are extinct there are no extracellular bacillus because they are constantly being engulfed by macrophages. As the parameter g tends to its minimum value, the flux of macrophages and neutrophils decreases. For that reason, all the macrophages are infected and tend to 0 as the are lysed, causing an increase in the amount of extracellular bacillus.

B. Lysis function. Fitting

In order to adjust a continuous function to the lysis data obtained with the ABM, the simulation has been executed N = 500 times. Finally, an average has been done with the N set of points.

The type of function needed for the fitting and the set of variables more suitable is unknown. The fist step is to decide the combination of variables. To do so, the R-square error and the RMSE of each set of variables has been computed for a one grade polynomial. The results of each linear regression are shown in Table 1. The best values correspond to the pair Intracellular Bacillius and Infected Macrophagues.

TABLE I. R^2 and RMSE error when adjusting lyisis rate as a function of different independent variable combination

Independent variables	R^2	RMSE
Bi, Mi	0.75906	5.2962
Mu, Mi	0.22599	9.4925
Mu, N	0.36093	8.6254
Mu, Bi	0.58181	6.9774
N, Bi	0.56172	7.143
N, Mi	0.21974	9.5307

The adjust made with a plane was improved using a second grade polynomial. With this, the error improves significantly. At Figure 1 it can be seen the results and the adjusted two grade polynomial.

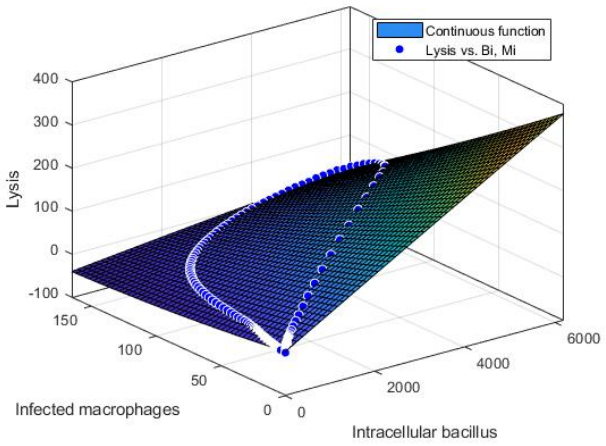

FIG. 1. Lysis rate points (in blue) and the second grade polynomial. The errors are a $R^2 = 0.9800$ and $RMSE = 1.8189$. Increasing the degree of the polynomial we achieve a better error at the expense of obtaining a non-desired behaviour outside the data domain.

C. Continuous model vs ABM

The polynomial fitting was used as a approximation to the lysis term in the continuous model. Figure 2 shows the comparison between the two models.

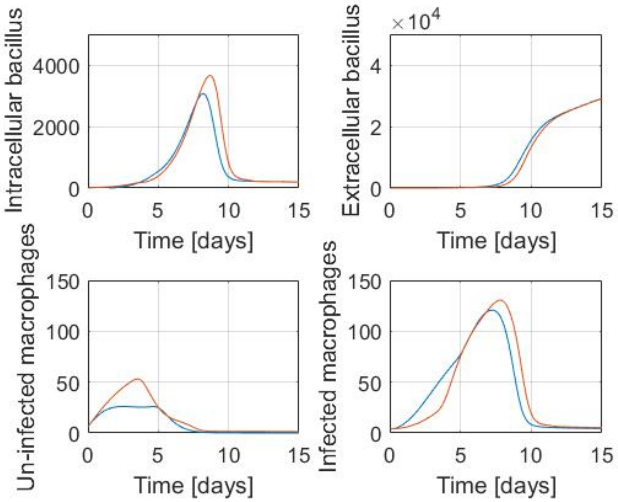

FIG. 2. Comparison of the results of the ABM (orange) and continuous model (blue). The two models present the same evolution. The biggest difference appears in *Mu*, with a peak reduction of 50%. *Bi* and *Mi* just show a small peak discrepancy and reduction. *Be* does not vary significantly.

D. Sensitivity analysis

As the value of α is one of the most uncertain parameters[2], it can be used to know how sensitive our model is when changing its value. To evaluate the sensitivity, the value of the constant has been changed to $\alpha = 30$. In figure 3, it can be seen a comparison between both results.

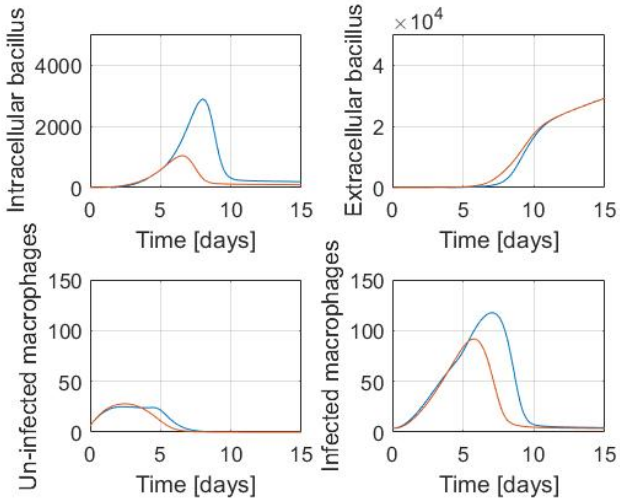

FIG. 3. Comparison of two simulations with $\alpha = 60$ (blue) and $\alpha = 30$ (orange). Every variable changes differently with α. *Bi* presents the maximum variation, decreasing nearly about 50%. The values of *Mu* and *Mi* variate a bit, the first one increasing with the reduction of α and the second one decreasing. *Be* has a minimum α dependence.

IV. CONCLUSIONS

- It was developed a continuous model from the ABM using a fitted lysis function.

- The ABM yield nearly the same results as the continuous model.Working with a continuous model is easier. While the ABM model needs to solve both the differential equations and additional conditions to work accurately, the continuous model allows the resolution of the system just solving the differential equations.

- The output variables depend on the value of the macrophage's capacity of bacilli: α although the evolution with respect to time is the same.

[1] World Health Organization. Fact sheets. Tuberculosis, (2018).

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[3] Segovia-Juarez J.L., Ganguli S., Kirschaner D. *Identifying Control Mechanisms of Granuloma Formation during M. Tuberculosis Infection using an Agent-Based Model.* Journal of Theoretical Biology, 2004.

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