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PERSPECTIVE

How mechanistic in silico modelling can improve our understanding of TB disease and treatment

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SUMMARY

TB is one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent. Decreasing the length of time for TB treatment is an important step towards the goal of reducing mortality. Mechanistic *in silico* modelling can provide us with the tools to explore gaps in our knowledge, with the opportunity to model the complicated within-host dynamics of the infection, and simulate new treatment strategies. Significant insight has been gained using this form of modelling when applied to other diseases – much can be learned in infection research from these advances.

KEY WORDS: TB modelling; disease models; statistical modelling; within-host mechanistic model

TB IS AN INFECTIOUS disease that accounts for over one million deaths globally each year.[1] This high mortality rate occurs despite the fact that effective treatments for TB have existed for decades. One factor underpinning this mortality burden is thought to be to be due to latent TB: the bacteria can exist in a variety of cell states, and slowly growing or non-replicating cells appear harder to kill.[2] It is also a reason why treatment is so long: the standard regimen for drug-susceptible TB (DR-TB) requires administration of multiple antibiotics for at least six months. This duration is difficult for patients to manage, with non-adherence leading to relapse and increasing the risk of emergent antibiotic resistance.[3] Given the growing global threat of DR-TB, there is a critical need to devise new treatment strategies of shorter duration to improve patient adherence and thus reduce the number of relapse cases and emergence of antibiotic resistance.[3]

Licensing a new regimen is difficult and expensive. With several new drugs now available many new approaches exist,[4] but it would be impossible to perform clinical trials to explore all of them with no guarantee of a successful result. The number of possible combinations has been estimated to be 9.9×10 by Cicchese et al.[5] The risk of an unsuccessful trial can be mitigated by the predictive power of modelling—if we can use existing patient and biological data to predict the novel regimens most likely to succeed, we could prioritise investment for these specific clinical trials.

Here we highlight the advances in mechanistic in silico TB modelling. We outline the use and impact of statistical modelling of trials data, and show how mechanistic models can supplement these modelling efforts. Mechanistic models that accurately reflect the pathological and pharmacological conditions within a patient during infection could make a significant contribution to our understanding of TB.

MODELLING OF TB CLINICAL TRIAL DATA

The TB drug development process involves numerous stages. Preclinical in vitro and animal experiments form the traditional cornerstone of this process; the bacteriological effect of single or combined antibiotics is measured by the reduction in bacterial load within in vitro models or harvested organs. Mathematical analyses relating drug exposure to bacteriocidal effect are taken from these data and used to estimate optimal pharmacokinetic-pharmacodynamic parameters for each antibiotic. This work is key to identifying potentially useful agents prior to use in humans.

The main statistical approaches used to predict outcome from Phase II TB treatment studies are based on modelling bacterial clearance of *Mycobacterium tuberculosis,* the causative agent of human TB, from the sputum of patients with pulmonary disease. There

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Table A comparison of methods of describing bacterial clearance in patients used to test different regimens in Phase II trials

Method	Description	Examples
Culture conversion	Patient sputum is tested using a TB detection assay and the proportion of patients converting from positive to negative is reported, with greater conversion at 2 or 3 months intended to indicate a more favourable treatment outcome	• Smear microscopy • Culture on a range of media • Molecular bacterial load assay techniques[6]
Time to culture conversion	Serial sputum collections are analysed through laboratory detection techniques to indicate the time until assay conversion for each patient The cumulative probability of conversion on different treatment regimens is calculated and regimens with relatively higher probability of clearing *M. tuberculosis* during a given time interval can be prioritised	• Kaplan-Meier method[7] • Cox proportional hazard regression[8] • Parametric models such as Weibull regression[9]
Calculation of bacterial elimination rates	Quantitative microbiology is performed on serial sputum samples. Regimens with greater drop in bacterial load in the first 8 weeks have been proposed to carry the greatest treatment shortening potential[10]	• Counting of CFUs on solid media • Recording time to positivity of liquid culture • Estimating CFU counts from the cycle threshold of molecular tests • Mathematical modelling, where linear or non-linear equations are fit to longitudinal bacterial load datasets[11]

CFU = colony-forming unit.

is a strong rationale for this: surrogate markers of treatment response should be biologically plausible and killing of the infecting pathogen fulfils this condition. Biomarkers should also have measurable statistical properties, which can be achieved through three methods: a comparison of methods describing bacterial clearance in patients is presented in the Table. Statistical approaches such as these are crucial tools to directly chart patient responses to TB treatment.

Limitations of current statistical models used for TB

When modelling bacterial load decline in sputum samples, there are obvious limitations to using statistical approaches. For example, it is possible that the *M. tuberculosis* cells which are critical for defining patient relapse are not be present in the sputum. They may be sequestered to isolated sites within the lung, such as the closed lesions described by Canetti,[12] and thus be undetectable in sputum samples. Understanding the location of bacteria is important: recent work has suggested that heterogeneous penetration by components of drug regimens into pathological TB granulomas can result in insufficient concentrations of some drugs for bacterial elimination.[13–15] This may have contributed to relapse in the experimental arms of the REMoxTB trial (a global Phase 3 clinical trial using a moxifloxacin-containing TB treatment regimen).[16] For non-sputum based markers (such as positron emission tomography/computed tomography scans), or possible blood-based markers, statistical modelling may help to provide insight to overcome these limitations.

M. tuberculosis can exist in a state where many of its metabolic processes are down-regulated, which is often associated with the presence of a lipid body in the mycobacterial cell.[2] These cells are postulated to be a cause of relapse in treated patients,[17] as the presence of lipid inclusions is associated with a significant increase in the minimum inhibitory concentration for all antibiotics. These bacteria are thus more likely to survive treatment and cause future relapse.[2] Many possible influencing factors have been postulated as the driving force behind this switch to a slower or non-replicating state,[18] but the exact determinants are uncertain. As current TB statistical models are typically based only on standard drug susceptibility tests that measure lipid-poor cells (which are phenotypically more susceptible to antibiotics), they do not account for the whole spectrum of bacterial phenotypes.

Statistical models that are fitted to data are often derived without reference to the underlying disease mechanisms or biology of the infecting organism. Thus, using these methods, less can be learned about the pathological interactions of the disease. This is the most important limitation of this modelling approach as predictions will be limited by the data the model is derived from. If we are to understand more about TB disease dynamics we will need to build more effective mechanistic within-host models, which capture the spectrum of biological processes, to supplement statistical models.

Mechanistic modelling can provide valuable insight

Mechanistic modelling allows us to model events as they occur in vivo, through mathematical or computational means. The overall system is broken down into constituent parts, each of which can be simulated: overall system dynamics emerge as a result of the combination of interactions of individual

components. It has been shown that even simple dynamics can lead to complex behaviours and that many processes found in nature can be approximated by simple models of this type.[19] Mechanistic models represent a conceptual view of the complex real-world systems they simulate, but can still provide insight into those events and the parameters that influence important outcomes. This abstract approach affords us the power to describe events even in cases where laboratory diagnostic tools or treatment monitoring tools are not available.

Developing mechanistic models can be a lengthy process, but they are inexpensive to produce compared to a clinical trial or in vitro experiment. They can also produce results in hours rather than weeks or years, can be run repeatedly (at no extra cost) and are easily adapted to account for new data, which is one of the major advantages over statistical modelling techniques. Mechanistic models can also help us improve our understanding of the mechanisms of disease. Each parameter of the model must have a biological basis and, thus, we can identify knowledge gaps.

Challenges in mechanistic modelling

Systems biology approaches are built upon current understanding of the disease, which raises an important question: "What if current knowledge is wrong? Will the models not be inherently flawed?" It actually presents an additional opportunity for mechanistic models: they can help to shed light on aspects that are not understood fully and also allow us to challenge current dogma. In this sense, modelling allows us to gain insight into disease and treatment dynamics, and provides evidence in support (or in opposition) of our beliefs about disease mechanics. Close collaboration with experimentalists and trialists is essential as model outcomes must be viewed through the lens of clinical disease to test the plausibility of predictions.

The relative paucity of knowledge regarding the processes of a disease such as TB presents a challenge when developing mechanistic models: if we don't know how the disease develops in the body, how do we determine realistic parameter values for the model? All parameters used within the model will be based on biological or clinical experiments that are inherently variable and can have wide confidence intervals. It is therefore challenging to understand the impact of different experimental definitions and methods on the results produced. This can make it hard to assign reliable parameter values, which further highlights the importance of working closely with experimentalist collaborators.

One way to mitigate this difficulty is through sensitivity analysis, where multiple simulations are run using a range of values across the parameter space, and metrics can be used to determine how uncertainty in the model parameters impacts on the model outputs. A useful review on sensitivity analyses is by Marino et al.[20]

Mechanistic modelling of in-host dynamics and applications to TB

Mechanistic in silico models have long been applied to the study of TB, but have predominantly focused on the spread of TB epidemics.[21] These models are essential in determining effective intervention strategies,[22] but if we wish to reduce TB mortality, mechanistic modelling that focuses on the in-host dynamics must also be utilised to a greater extent so that we make the gains seen within other fields. For example, within-host mechanistic models in cancer research have led to important insights into how the spatial structure of cancer cells impacts the progression of the disease: it has been shown that a low oxygen concentration in some regions can result in cancer cells in those locations becoming more resistant to chemotherapy. This is a relatively new concept for TB, in comparison to other diseases, and has only recently started to gain momentum (review articles of in-host TB modelling have been recently published[23,24]).

Mechanistic models can be applied to TB at different scales of the disease. Lesional level models simulate the interactions between host and bacteria on a cellular scale, looking at the individual factors that impact the development and progression of a single lesion of infection. At a larger scale, whole-organ or whole-body models can explore how variance of biology within and between hosts can impact both the disease and its treatment. Finally, at a clinical trial level, entire simulated populations of patients could be modelled and 'virtual' clinical trials could be run to determine how likely the success of a new regimen would be. These models could incorporate national-level data (such as HIV co-infection rates and social influences) to create realistic populations. The Figure shows the various levels of in-host TB simulation.

Over the past 15 years, Kirschner et al. have created an Agent Based Model, called *GranSim*, which describes the formation of a granuloma arising from a TB infection.[25] This initial model has been expanded upon subsequently, introducing multiple scales of modelling,[26-28] including other organs.[29] These models exploit the power of mechanistic modelling to make predictions about the outcome of infection and the impact of different dosing strategies.[30] Simulations from this model have provided insights to aid understanding of the disease. For example, they have shown that the anti-inflammatory cytokine interleukin-10 is a key determinant of infection outcome, and experiments demonstrated potential to harness it for immunotherapy.[28]

Data from *in vitro* experiments on lipid-body

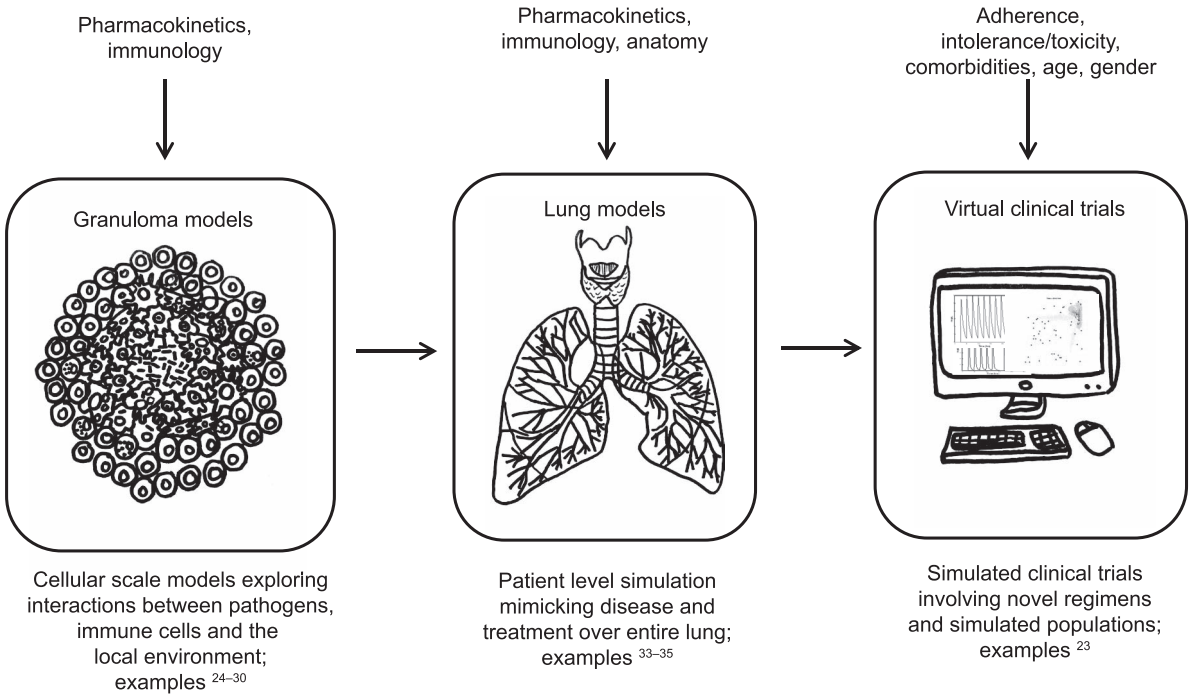

Figure The different biological scales of in silico modelling of in-host dynamics and their applications.

accumulation during TB infection has been integrated into an individual-based model to investigate the relative importance of bacterial cell state and location of bacterial load in disease outcome.[31] *GranSim* has been extended, and recent models have begun to investigate the disease process over the whole lung and associated lymphatics.[32] TB disease affects various parts of the lung, with the initial primary disease typically occurring at the basal regions of the lung, whilst post-primary disease occurs at the apices.[33] The environment within the lung is heterogeneous, with differential oxygen tensions and blood perfusion rates between the apex and the base. Understanding how this impacts TB progression will be important in understanding where bacteria reside during infection and how to target bacteria in occult locations. Pitcher et al.[34] have developed a complex network-based metapopulation model of the whole lung to demonstrate how environmental differentials can impact disease. This shows that even modest heterogeneity within the lung can dramatically impact bacterial load when latency is established.

These findings show the power of this modelling approach. By incorporating the spatial locations of cells within the lesions and across the lung, practical clinical questions can be addressed and valuable insight provided into the inner workings of TB infection. These models could allow theories of relapse causation to be explored, or to test potential therapies aimed at preventing this unfavourable outcome. As modelling capacity is developed, running virtual clinical trials to test drug regimens becomes a more realistic option.

CONCLUSION

TB is an ancient disease requiring modern solutions. Although greater amounts of data on the disease are being produced, there remains a significant lack of understanding into how the data correlates with the underlying mechanics. If new, shorter treatment regimens are to be developed, this knowledge gap presents a major hurdle, which must be overcome. Statistical modelling, such as modelling of clinical trial data, is essential to determine the efficacy of a trialled treatment regimen for TB, but may be limited by some essential data that is not available with current technology. Mechanistic models can reflect the within-host dynamics that occur during infection to bring new insight: these models can supplement current statistical models to further understanding of the disease and aid the development of new treatment regimens.

The development of within-host mechanistic models of TB are invaluable tools alongside statistical modelling as they allow us to investigate the factors within the body that lead to phenomena such as cavitation and disease transmission. Increased understanding resulting from these models will aid the creation of treatment regimens that target these important disease mechanisms. Within-host mechanistic models for TB are making increasingly significant contributions to the field. Novel mathematical and computational models have allowed the study of spatial dimensions of infection and better understanding of how the environment within the body impacts the progression of disease. These models

enable multiple simulations to be run in a fraction of the time it would take to test comparable scenarios in the real world.

Currently, most work has focused on a lesional scale and provided valuable insight into the factors that contribute to individual lesions. However, during an infection, many lesions can develop in a variety of spatial locations within the lung. Therefore, larger scale models to evaluate the whole lung and whole body are needed, investigating how individual lesions coalesce to form cavities for bacterial dissemination between people. New work in this area is emerging.[34,35] These models can then be expanded into the simulated populations needed for a virtual clinical trial. Doing so would create an invaluable tool for TB research: the ability to de-risk large, expensive clinical trials by excluding regimens that are unlikely to succeed based on the model predictions. Greater use of mechanistic models can provide us with better data that will supplement our existing statistical models; using these two complimentary fields to their full potential could empower us with the tools we need to finally eradicate TB.

The important findings that these models are capable of producing has been highlighted but it must be stressed that this can only be achieved with close and continued collaboration between modellers, clinicians and experimentalists.

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RÉSUMÉ

TB est l'une des 10 premières causes de mortalité dans le monde et la cause principale de mortalité liée à un agent infectieux unique. Raccourcir le traitement de la TB est une étape importante vers l'objectif de réduire la mortalité due à la maladie. Une modélisation mécaniste *in silico* peut nous fournir les outils permettant d'explorer les failles de nos connaissances, avec l'opportunité de modéliser les dynamiques complexes de l'infection à l'intérieur de l'hôte et simuler de nouvelles stratégies de traitement. Nous avons beaucoup appris de cette forme de modélisation quand elle est appliquée aux autres maladies ; nous avons beaucoup à apprendre en matière de recherche sur l'infection grâce à ces avances.

RESUMEN

La TB es una de las primeras 10 causas de muerte en todo el mundo y la principal causa muerte por un agente infeccioso único. Acortar el tratamiento de la TB constituye un paso importante hacia la meta de reducir la mortalidad por esta enfermedad. La modelización mecanicista por computadora aporta herramientas para examinar las lagunas del conocimiento y ofrece la oportunidad de modelizar las complejas dinámicas de la infección en el huésped y simular nuevas estrategias terapéuticas. Se ha logrado un progreso considerable en la comprensión de otras enfermedades al aplicar este tipo de simulación; la investigación de las infecciones podría aprender mucho con estos avances.