In silico design of clinical trials: A method coming of age

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Objective: **To determine the feasibility and potential usefulness of mathematical models in evaluating immunomodulatory strategies in clinical trials of severe sepsis.**

Design: **Mathematical modeling of immunomodulation in simulated patients.**

Setting: **Computer laboratory.**

Measurements and Main Results: **We introduce and evaluate the concept of conducting a randomized clinical trial *in silico* based on simulated patients generated from a mechanistic mathematical model of bacterial infection, the acute inflammatory response, global tissue dysfunction, and a therapeutic intervention. Trial populations are constructed to reflect heterogeneity in bacterial load and virulence as well as propensity to mount and modulate an inflammatory response. We constructed a cohort of 1,000 trial patients submitted to therapy with one of three different doses of a neutralizing antibody directed against tumor necrosis factor (anti-TNF) for 6, 24, or 48 hrs. We present cytokine profiles over time and expected outcome for each cohort. We identify subgroups with high propensity for being helped or harmed by the proposed intervention and identify early serum markers for each of those subgroups.**

The mathematical simulation confirms the inability of simple markers to predict outcome of sepsis. The simulation clearly separates cases with favorable and unfavorable outcome on the basis of global tissue dysfunction. Control survival was 62.9% at 1 wk. Depending on dose and duration of treatment, survival ranged from 57.1% to 80.8%. Higher doses of anti-TNF, although effective, also result in considerable harm to patients. A statistical analysis based on a simulated cohort identified markers of favorable or adverse response to anti-TNF treatment.

Conclusions: **A mathematical simulation of anti-TNF therapy identified clear windows of opportunity for this intervention as well as populations that can be harmed by anti-TNF therapy. The construction of an *in silico* clinical trial could provide profound insight into the design of clinical trials of immunomodulatory therapies, ranging from optimal patient selection to individualized dosage and duration of proposed therapeutic interventions. (Crit Care Med 2004; 32:2061–2070)**

Key Words: sepsis; immunomodulation; inflammation; computer simulation; clinical trial; anti-tumor necrosis factor

The management of conditions associated with an intense inflammatory response such as severe trauma and sepsis represents a major challenge in the care of the critically ill. There is an emerging consensus that the acute inflammatory response to major stress might be inappropriate or lead to undesirable outcomes in patients initially resuscitated successfully. In the last two decades, much has been learned regarding cellular and molecular mechanisms of the acute inflammatory response. This progress has led to considerable efforts and resources to develop interventions that modulate the acute inflammatory response and positively affect outcome in these patients. Except for recombinant human activated protein C (drotrecogin alfa [activated]) (1) and low-dose steroids (2), this knowledge has not led to effective immunomodulatory therapies; consequently, a significant effort to address the issue of target confirmation and trial design has ensued (3–9). This situation is especially vexing considering that a reasonable therapeutic rationale was supported by animal and early phase human studies for dozens of interventions that failed when evaluated in phase III (10–12).

Several researchers have proposed a variety of reasons to explain the incongruence between results and expectations. We propose that a key reason for this conundrum is the difficulty of predicting the impact of modifying single components of the highly complex, nonlinear, and redundant inflammatory response (13–18). The consequences of failing to take a systems-oriented approach to understanding and predicting the time-course of complex diseases are various and significant. Indeed, prediction of the behavior of such systems derived from localized insights gathered from limited experiments or observations pertaining to individual components on such systems may be impossible, however accurate these isolated observations may be. Meteorologists, engineers, physicists, and other scientists examining complex

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systems make extensive use of models, simplified representations of those complex systems, to shed useful insight on the behavior of such systems.

We sought to adopt a similar approach and conduct a practical demonstration of modeling a clinical trial *in silico*, by examining a therapy that had initial great promise in the setting of animal models of sepsis but failed in large, randomized clinical trials to meet generally accepted criteria for efficacy. Accordingly, we focused on the consequences of the administration to sepsis patients of a neutralizing antibody directed against the proinflammatory cytokine tumor necrosis factor (anti-TNF) (19). After promising nonhuman primate results, pooled outcome of no fewer than 11 clinical trials in 7,265 patients showed a consistent absolute reduction in mortality of approximately 3.2% ($p = .006$) favoring treatment with anti-TNF antibodies, a disappointing result in light of the effect expected from preclinical studies (20, 21). Efforts to select populations that would demonstrate a convincing benefit from anti-TNF have not met expectations either (20, 21).

We wish to illustrate insights that mathematical models could provide in elucidating the reasons for the disappointing results of this particular agent and, more generally, in the design of future trials, especially regarding drug dosing, duration of therapy, and interaction among cointerventions.

METHODS

Overview. We initially designed a mechanistic model of the acute inflammatory response based on information available from the existing literature on the roles of key cellular and molecular effectors in response to a bacterial pathogen (22, 23). We constructed a population of virtual patients differing in their initial bacterial load, bacterial virulence, time of initiation of intervention, and genetic ability to generate effectors in response to stress. We compared outcomes across several treatment arms and identified determinants of favorable and unfavorable outcomes.

Modeling the Human Inflammatory Response. Because the acute inflammatory response is comprised of a large number of components that each have specific roles yet are highly interactive, we chose to model this dynamical system with a system of differential equations, one for each component that we chose to simulate (Appendix). Each equation describes the level or concentration of components over time resulting from their interaction with other components following the principle of mass-action. We chose to represent the system at this level because serum levels of cytokines, for example, are well known to correlate with outcome in septic patients (24–28), clinical measurements are usually obtained from blood, and chemotherapeutic interventions are typically administered intravenously. Limitations resulting from this choice are discussed subsequently. The strengths of such an approach are several, in that it a) provides an intuitive means to translate mechanistic concepts into a mathematical framework; b) can be analyzed using a large body of existing techniques; c) can be numerically simulated easily and inexpensively on a desktop computer; d) provides both qualitative and quantitative predictions; and e) allows expansion to higher levels of complexity.

Initial values for rate constants were determined empirically so that the model would qualitatively reproduce observed literature data in mice administered endotoxin or subjected to cecal ligation and puncture (23, 29). Some rate constants, such as cytokine half-lives, were directly extracted from the literature (30–32).

Constructing a Clinical Trial of Anti-TNF. We generated a study population of 1,000 virtual patients. Pathogen characteristics (growth rate and initial load) were chosen to result in a survival of approximately 60%. We varied the delay before medical consultation, and thus eligibility for treatment, reasoning that the distribution of the delays to medical consultation after onset of infection was related to initial pathogen load and virulence (i.e., sicker patients would generally consult earlier). To simulate genetic diversity of the study population, we randomly varied individual propensity of immune cells to generate effector molecules (proinflammatory such as TNF and interleukin [IL]-6), anti-inflammatory, and nitric oxide synthase activity) from ±25% of baseline as dictated by literature data (33). Those variations were sufficient to explain wide swings in individual serum levels of effectors.

Optimizing Trial Design. We wished to illustrate the application of mathematical modeling to optimizing the design of a clinical trial. We achieved this demonstration in two steps. First, we identify administration strategies that would result in the best outcomes for the entire cohort. Second, we illustrate how the simulation can help with patient selection, given a treatment administration regimen. Importantly, our goal was specifically not the optimization of treatment regimen to individuals, although this

Table 1. Time to detection of disease and 7-day survival by quartile[a] of the population

Baseline Characteristics	7-Day Survival, %	Mean Detection Time, Hrs
Overall population	62.9	20.8
Host factors		
TNF responsiveness[a]		
Q1	76.4	20.8
Q2	67.2	20.9
Q3	61.2	20.5
Q4	46.8	21.0
Anti-inflammatory responsiveness[a]		
Q1	56.4	20.1
Q2	58.8	19.9
Q3	64.4	21.9
Q4	72.0	21.3
iNOS responsiveness		
Q1	63.6	21.2
Q2	58.0	21.1
Q3	64.8	21.5
Q4	65.2	19.4
Pathogen factors		
Pathogen inoculum[b,c]		
Q1	68.8	43.1
Q2	63.6	18.7
Q3	65.2	12.5
Q4	54.0	8.9
Pathogen virulence[b]		
Q1	100	19.7
Q2	97.2	21.2
Q3	49.2	20.8
Q4	5.2	21.4
Time to detection[b,c]		
Q1	57.6	7.4
Q2	59.6	12.2
Q3	65.2	19.1
Q4	69.2	44.5

TNF, tumor necrosis factor; iNOS, inducible nitric oxide synthase.

[a]Quartiles are from lowest values (Q1) to highest (Q4) for effector cell propensity to elaborate products for a given stimulus, for pathogen initial inoculum, for pathogen virulence, and for time to detection of disease; [b]$p < .01$ between quartiles for mortality; [c]$p < .01$ between quartiles for difference in earliest detection time.

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constitutes another potential application of our simulation.

To identify optimal dosing and duration of administration strategies, we submitted the virtual cohort of 1,000 patients to nine interventions with anti-TNF. We varied the duration of administration of anti-TNF (6, 24, or 48 hrs). Comparatively, the half-life of anti-TNF antibodies in naïve patients is 40–50 hrs (34, 35) We simulated the binding of serum TNF with three different "doses" of anti-TNF (2, 10, and 20 arbitrary units). Depending on dose, TNF neutralization varied from 18.6% to 55.5% of total TNF produced in controls. A clear correlation with published reports is difficult as these do not typically report areas under the curve and do not always distinguish between biologically active TNF, TNF bound by antibody, and TNF bound by specific soluble receptors (35). Death was determined by the inability of the individuals to clear more than 50% of maximal sustained tissue dysfunction at one week. Such a definition segregated the population into two outcome groups (see the Results).

Trial optimization involves selecting a dosing strategy that optimizes outcome in a cohort of patients and then selecting patients who would benefit from treatment while avoiding treating patients for which treatment would either have no effect or cause harm. The optimal treatment administration scheme has already been determined as part of prior results (see preceding section). To select patients who would most benefit from this treatment, we constructed a multinomial logistic model with a four-valued outcome variable: a) is helped by treatment (survives but would have died without treatment); b) survives irrespective of treatment; c) dies irrespective of treatment; and d) is harmed (dies because of treatment). Independent variables were chosen at the time of disease detection (the earliest possible treatment opportunity) and 60 mins later, reflecting the possibility of using short-term trends in analytes and assuming rapid diagnostic capabilities. Variables included serum TNF, anti-inflammatory activity, long-acting proinflammatory cytokine (IL-6), their ratios and products, activated protein C, thrombin, as well as blood pressure and cell counts of activated neutrophils. The statistical model was validated in a different population of 1,000 simulated cases. All predictions from the statistical model relate to the validation population.

We wrote our own software for the simulations and analyses (JB, RK, GC). Statistical analyses and multivariate statistical models were conducted in SPSS (Chicago, IL).

RESULTS

Baseline Population

Characteristics of the baseline population and outcome are detailed in Table 1. Mean time to medical consultation was 20.1 hr from the onset of infection and was shorter in patients with high pathogen load and high virulence. Survivors had lower peak and cumulative TNF, IL-6, nitrites/nitrates, and global tissue dysfunction than nonsurvivors (Fig. 1 and Table 1). Overall mortality rate was 37.1% as determined by tissue dysfunction at 168 hrs divided by maximal dysfunction value during the first 168 hrs (Fig. 2.). We chose this variable as a proxy to unfavorable outcome because there was a clear bimodal distribution of this variable after 72–96 hrs. Therefore, the model offered a "natural" separation of favorable and unfavorable outcomes. There is a logical physiologic correlate to this observation: After reaching a maximum, tissue dysfunction tended to improve significantly and rapidly in some cases (survivors) or remained elevated at a substantial fraction of maximum in all other cases (nonsurvivors). Mortality rate was higher with high bacterial load, high virulence, and high TNF production potential by effector cells. Previously suggested predictors of outcome, such as TNF/IL-10 ratio and TNF/IL-6 ratio, indeed discriminated between survivors and nonsurvivors (36–39). However, this discrimination was only moderate and highly time-dependent (Fig. 3.).

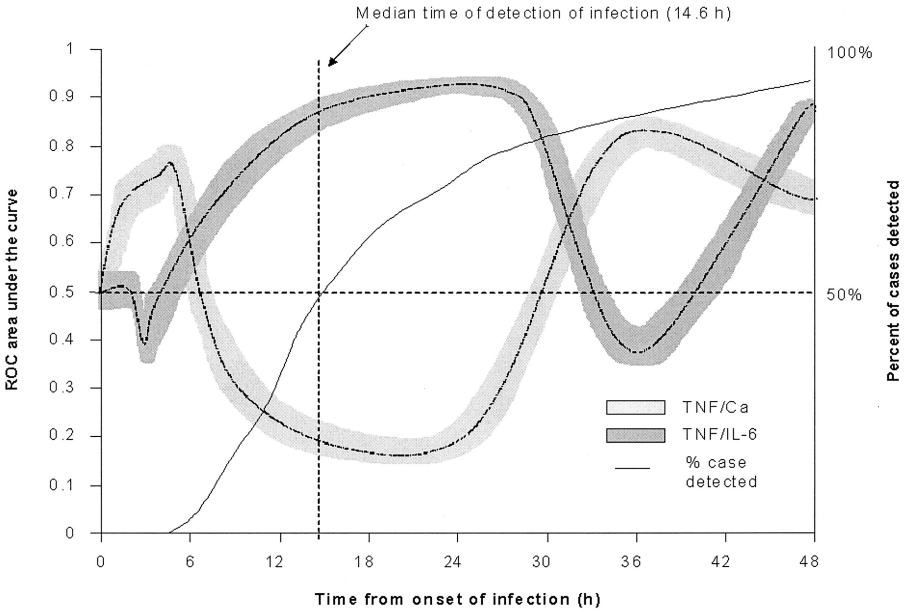

Figure 1. Time course of receiver operating characteristic (*ROC*) area under the curve for tumor necrosis factor(*TNF*)/interleukin (*IL*)-6 and TNF/anti-inflammatory activity (*Ca*) ratios. The ability of TNF/IL-6 and TNF/Ca ratios to discriminate survivors from nonsurvivors as determined by ROC area under the curve varies significantly in time. Given that the predictive values of those ratios would be most useful early in the course of infection and that cases do not necessarily present early, the usefulness of those ratios as discriminators of outcome is questionable.

Optimizing Trial Design

Impact of Drug Dose and Duration of Administration on Outcome. We observed a saturation of the effect of anti-TNF on peak and cumulative concentration of mediators and tissue dysfunction. Markers of inflammation were higher with the lowest dose of anti-TNF, but there was little difference between the two higher doses (Table 2). In fact, the higher treatment dose was associated with higher cumulative serum TNF levels as measured by area under the curve. The reason for this paradoxical effect seems to be a rebound in proinflammatory mediators, presumably because of decreased generation of anti-inflammatory mediators, and therefore decreased inhibition of proinflammation after anti-TNF is discontinued.

Despite relatively modest differences in circulating levels of effectors, the simulations suggest that modifications in treatment intensity and duration result in large differences in survival (Table 3). Survival is highest when the lowest dose of anti-TNF is administered for 48 hrs and worst when the highest dose is administered for 48 hrs. A surprising yet key finding is that anti-TNF treatment helps a significant percentage of individuals but also harms many. Thus, the beneficial effect of this therapy is reduced considerably if it is administered in a randomized fashion (Fig. 4). This balance is

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also very dependent on drug dose and duration. The proportion of patients helped by anti-TNF increases with dosage, but so does the proportion of patients harmed. Duration also had a significant impact on survival, but less so than dose. There was therefore a clear, but nonintuitive, interaction between dose and duration to affect overall survival.

Improving Case Selection in a Clinical Trial

To illustrate the implications of using modeling to improve case selection in clinical trials, we selected as an intervention the administration of anti-TNF at an intermediate dose for 24 hrs. We chose this intervention as opposed to the optimal one (low-dose anti-TNF administered for 48 hrs) because we wished to illustrate the potential of modeling to identify patients harmed by an intervention. Under this intervention, 242 cases were helped by treatment, whereas 181 were harmed, for a global survival advantage of 6.1% in the anti-TNF arm. Cases helped by anti-TNF treatment had higher peak TNF, IL-6, and anti-inflammatory levels. They also tended to have higher initial pathogen load and higher pathogen virulence. Nonsurvivors who were not helped by treatment had overwhelming infection and were treated late, and TNF levels tended to be low at onset of treatment. Patients harmed by therapy tended to have infections of moderate severity but were low TNF responders and high anti-inflammatory responders (Fig. 5). Interestingly, low production of TNF and high production of IL-10, determined by specific genetic polymorphisms, have been shown to be beneficial in transplant patients (40). However, potentially independent determinants of outcome such as pathogen load, virulence, and vigor of effector cells to produce TNF are not accessible to clinicians having to decide whether to administer treatment.

We therefore constructed a statistical model in which the independent variables were based on levels of effectors and cell counts measured at the beginning and 60 mins into the earliest treatment window, and we assessed the ability of this statistical model to identify patients potentially helped or harmed by the intervention. Actual and predicted outcomes from this statistical model are presented in Table 4. This model also demonstrated that several variables other than mediators were predictive of response to therapy. Specifically, baseline circulating TNF, IL-6, TNF to anti-inflammatory activity ratio, neutrophils circulating thrombin, and activated protein C were predictive of treatment efficacy ($p <$ .01). Sixty-minute trends in TNF, IL-6, TNF to IL-6 ratio, TNF to IL-6 product, and TNF to anti-inflammatory activity ratio were also predictive.

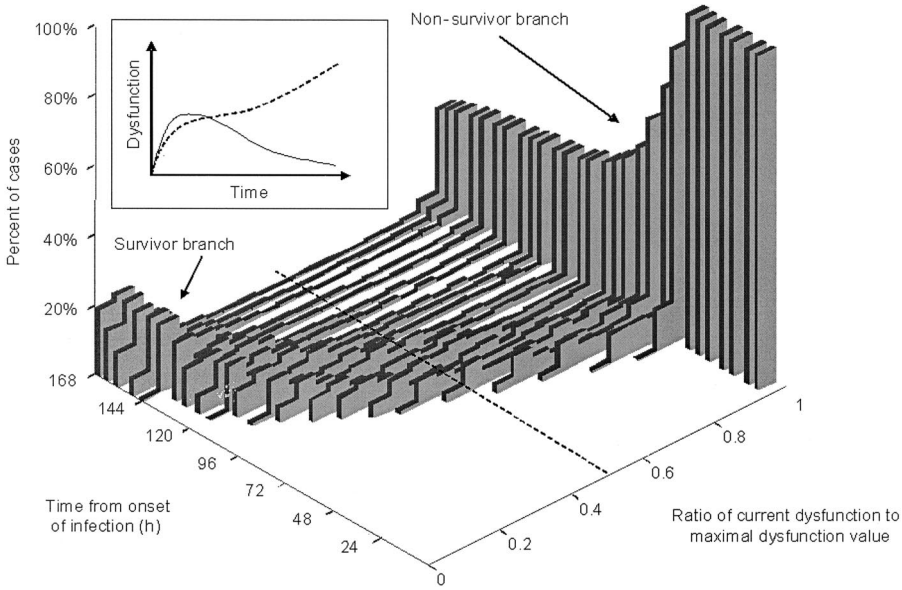

Figure 2. Frequency histogram of the time course of tissue dysfunction in the control cohort. Over the first 48–72 hrs (time is on the y-axis), tissue dysfunction is increasing. Accordingly, the ratio of current level of dysfunction to maximal dysfunction (x-axis) since onset of disease is 1. As their clinical condition improves, ultimate survivors witness a decrease in tissue dysfunction, and the ratio of current level of dysfunction to maximum level is now <1. This is depicted in the inset, where a typical survivor (*solid line*) sees resolution of dysfunction, whereas a nonsurvivor (*dashed line*) sees progressive worsening of dysfunction. The population segregates, between 72 and 96 hrs, into a nonsurvivor branch where dysfunction remains high (ratio close to 1) and a survivor branch where dysfunction improves with time (ratio gradually decreasing). The dotted line in the main figure represents the threshold of 50% of current to maximal dysfunction discriminating survivors from nonsurvivors.

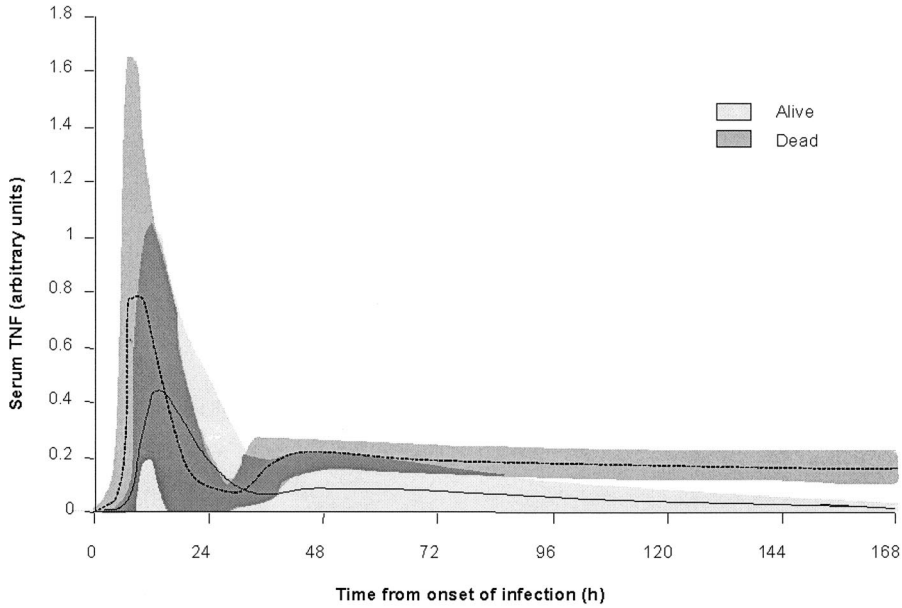

Figure 3. Time course of serum tumor necrosis factor (*TNF*) and outcome. Survivors generally have mean lower serum TNF levels (*pale gray area*, 95% confidence interval of mean) that peak later than in nonsurvivors (*dark gray area*, 95% confidence interval of mean). There is considerable overlap early in the clinical course, rendering TNF measurement a poor discriminator of outcome.

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Table 2. Cumulative and peak analytes levels across different treatment arms (arbitrary units)

	Controls	Duration of Anti-TNF Therapy		
		6 Hrs	24 Hrs	48 Hrs
Cumulative TNF				
Controls	97			
Anti-TNF, dose				
2		83	72	67
10		75	58	51
20		76	64	56
Peak TNF				
Controls	0.54			
Anti-TNF, dose				
2		0.46	0.41	0.41
10		0.42	0.30	0.30
20		0.43	0.27	0.27
Cumulative IL-6				
Controls	336			
Anti-TNF, dose				
2		259	207	182
10		211	143	112
20		220	155	124
Peak IL-6				
Controls	0.62			
Anti-TNF, dose				
2		0.54	0.50	0.50
10		0.51	0.38	0.38
20		0.53	0.36	0.36
Cumulative dysfunction				
Controls	803			
Anti-TNF, dose				
2		686	598	564
10		616	475	429
20		631	501	458
Peak dysfunction				
Controls	1.25			
Anti-TNF, dose				
2		1.15	1.06	1.05
10		1.10	0.90	0.88
20		1.11	0.86	0.83

TNF, tumor necrosis factor; IL, interleukin.

Because these results are derived from a predetermined, but arbitrarily large, sample size, all differences could potentially be made statistically significant and, therefore, no tests of significance are reported.

Table 3. Impact of treatment dose and duration on cohort survival at 7 days

	Controls	Duration of Anti-TNF Therapy		
		6 Hrs	24 Hrs	48 Hrs
Survival (%)				
Controls	62.9			
Anti-TNF, dose				
2		72.6	76.9	80.8
10		77.9	69.0	72.8
20		76.5	56.3	57.9

TNF, tumor necrosis factor antibody.

The lowest dose administered for 48 hrs resulted in the best survival, whereas higher doses for longer times had worse survival than placebo.

If the decision to treat had been based on this statistical model, adopting a strategy to treat only patients predicted to be helped by treatment would have resulted in 265 (26.5%) patients treated. Of these, 233 (positive predictive value = 87.2%) would indeed have been helped, whereas only two (0.2%) patients would actually have been harmed by treatment; withholding treatment for the other 735 patients would have resulted in harming a further 22 patients while preventing 87 patients from being harmed. In summary, in a separate validation cohort, following the statistical model's recommendation for treatment would have harmed 24 patients whereas indiscriminate administration would have harmed 148 patients. Adopting a strategy to administer treatment in those predicted to be helped and also in those predicted to die irrespective of treatment would have resulted in treating 397 patients, harming 12 patients, yet sparing 136 lives (Table 4).

Two recent clinical trials that restricted administration of anti-TNF to patients with IL-6 levels greater than 1000 pl/mL favored anti-TNF treatment over placebo (20, 21). When restricted to patients in the upper two quartiles of IL-6 at the time of detection, our model predicts a comparable mortality of 57%. The model also predicts a survival advantage of 26.4%, with only 6.8% of patients harmed by treatment. The reported 28-day adjusted absolute mortality differences in these trials were 4.0% and 6.9%, favoring anti-TNF treatment (20, 21). In our simulated cohort, the discrimination of serum IL-6 levels for mortality at the time of detection as expressed by receiver-operating characteristic area under the curve (ROC) was 0.814 (0.786–0.847). ROC for discrimination of patients harmed by treatment was 0.715 (0.693–0.757). Comparatively, the discrimination of a statistical model was almost perfect for mortality (ROC = 0.999) and excellent to identify patients who would be harmed (ROC = 0.979 [0.971–0.986]). Our model confirms the relevance of selecting patients based on IL-6 levels but also points out how the selection process could be improved.

DISCUSSION AND CONCLUSIONS

The almost universal failure of immunomodulatory therapies has called into question the basic nature of how antisepsis trials are designed and carried out (9, 41). Furthermore, assuming that a prospective drug demonstrates benefit over placebo in a randomized trial, how is this drug to be used as part of a multidrug regimen? In the absence of clear physiologic insight, it appears hazardous to compare or combine prospective therapies to recombinant human activated protein C without adopting a factorial design. Indeed, the combination of a prospective drug with recombinant human activated protein C might well prove worse than standard care without recombinant human activated protein C. The recent iteration of the Helsinki convention on the safety of patients dictating that randomized clinical trials should compare new

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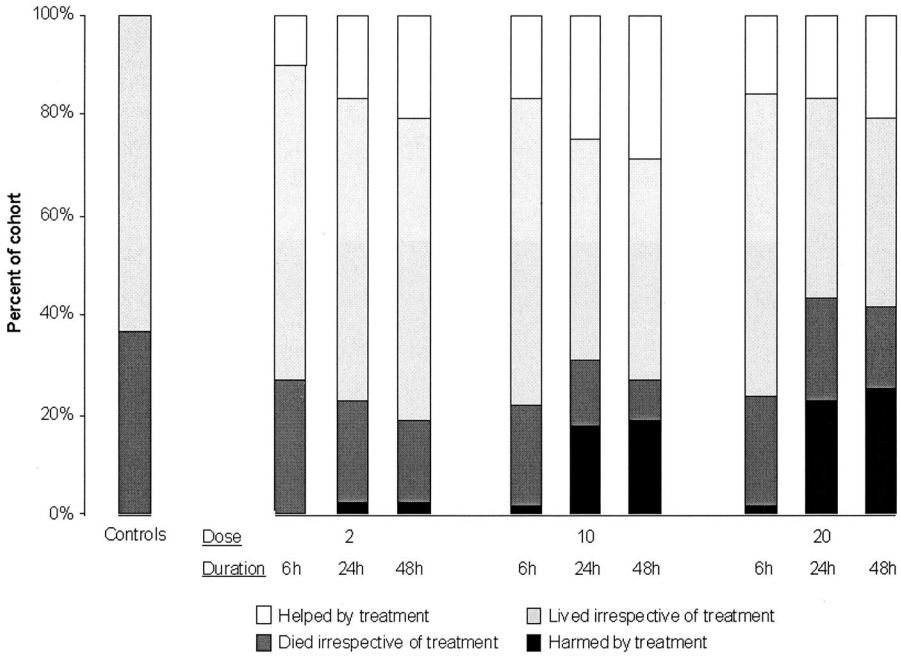

Figure 4. Mortality rate at 7 days according to treatment regimen. There is a dose response relationship with all durations of administration of anti-tumor necrosis factor: More patients are helped by treatment with higher doses. However, longer duration of administration is also associated with increasing harm, where people who would have survived in the placebo arm are effectively killed by treatment.

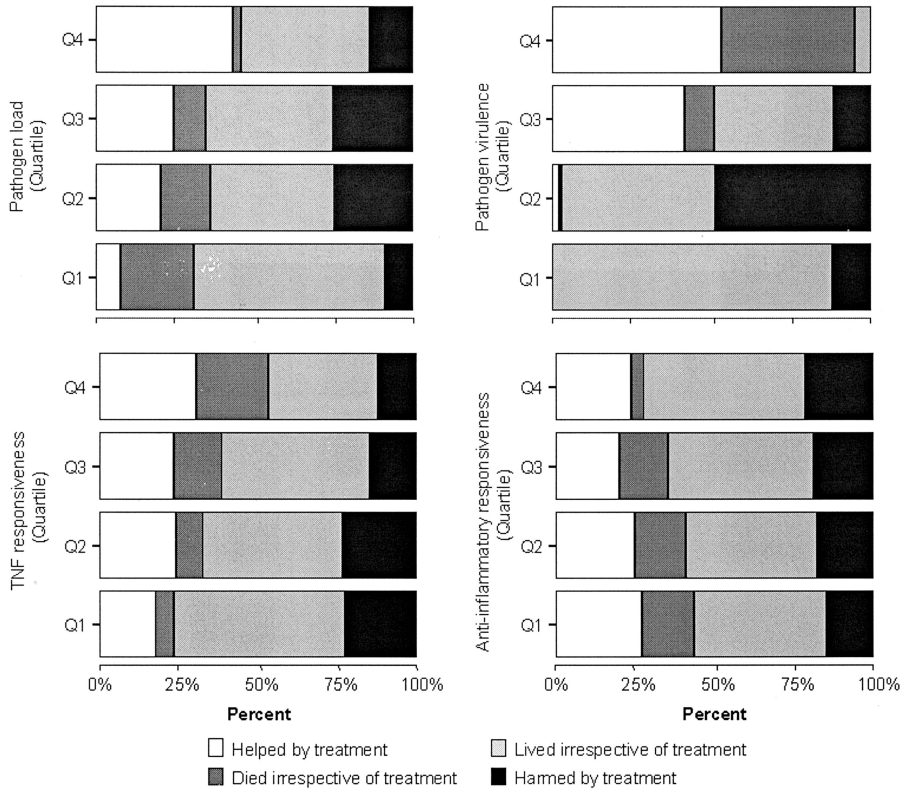

Figure 5. Determinants of outcome. For each baseline feature, representing host (ability of immune effector cells to elaborate tumor necrosis factor [*TNF*] and anti-inflammatory (CA) given a standard stimulus) and pathogen (bacterial load and virulence) determinants, the study population is divided into quartile of increasing value. The impact of anti-TNF treatment, administered for 24 hrs at a dose of 10, is depicted. Anti-TNF appeared particularly useful where there was a higher pathogen load, high pathogen virulence, higher TNF responsiveness, and lower IL-10 responsiveness. Conversely, harm was more probable in those with high CA responsiveness or low TNF responsiveness and those infected with pathogen of lower virulence.

The construction of an in silico *clinical trial could provide profound insight into the design of clinical trials of immunomodulatory therapies, ranging from optimal patient selection to individualized dosage and duration of proposed therapeutic interventions.*

compounds to the current "best" does not trivially apply to the problem of sepsis (6). Insights as to how to improve the design of such trials, including reducing the need for an unrealistically large number of patients yet preserving enough sensitivity to detect clinical benefit, are yet to emerge. There is growing sense of the need for a paradigm shift in this regard and that the concept of the randomized controlled trial, as we know it, might face insurmountable challenges in the context of multimodal therapies of complex disease. We propose that an intense effort at modeling of complex physiologic processes and their modulation might become an essential tool in the design of clinical trials of emerging therapies in this context. Although basic scientists and clinicians perceive the need for a more comprehensive approach to understanding physiologic dynamics and their modulation, significant advances in the implementation of practical tools derived from this "complex systems" approach have not been forthcoming. Reasons for this include the unfamiliarity of medical scientists with the necessary analytical tools involved, the perceived complexity of the processes to be modeled, the incomplete knowledge of the mechanisms involved, the difficulty in generating results that are perceived useful by interested parties, and lack of insight as to the limits and biases of conventional statistical approaches and reductionism-driven hypothesis testing (13, 14, 17).

We present such an implementation as a practical approach to the vexing problem of immunomodulation in patients with severe sepsis. The results of the simulation presented herein reproduce many observations characteristic of this disease process.

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Table 4. Comparison of simulated outcome and prediction of a statistical model regarding the benefit of anti-tumor necrosis factor (TNF) before treatment administration

	Predicted Outcome				
Observed Outcome	Helped by Treatment	Alive Irrespective of Treatment	Dead Irrespective of Treatment	Harmed by Treatment	Total Percentage
Helped by treatment	231	*6*	12	*4*	25.3
Alive irrespective of treatment	17	409	3	38	46.7
Dead irrespective of treatment	15	0	117	0	13.2
Harmed by treatment	**2**	59	0	87	14.8
Total percentage	26.5	47.4	13.2	12.9	100

The sum of the rows corresponds to observed simulated outcome for anti-TNF administered at a dose of 10 units for 24 hrs, whereas the sum of the columns corresponds to predicted outcome from a logistic multinomial model. Adopting a strategy to treat patients predicted to be either helped by anti-TNF or patients predicted to die irrespective of treatment would result in 397 patients treated (sum of the first and third column). In ten patients, the model recommended withholding treatment while it would be beneficial, thus resulting in an error of omission (italicized numbers). Of those in whom treatment is recommended by the statistical model, two of 265 would actually be harmed (boldface number), thus resulting in an error of commission. With indiscriminate administration, 148 patients would, in fact, have been harmed by treatment. Following the recommendation of a statistical model would, therefore, save 136 lives (148–2–10) and avert the administration of drug in 603 patients.

For example, in a disease where tissue dysfunction results from excessive immune stimulation, it is reasonable to find that there is dose-response behavior to intervention in patients who are helped. It is also reasonable that excessive suppression of appropriate inflammation, especially if an individual has a limited capacity to generate inflammation, results in harm. Yet, the simulation furnishes nonintuitive predictions as to how an intervention would have to be designed to optimize clinical response. Our results are humbling in that there clearly appear to be opposing effects of immunomodulation in severe sepsis, where patients are both saved and killed, that are not apparent in the outcome of clinical trials as currently measured. However, our simulation also suggests that modeling could assist in further refining enrollment criteria for trials or treatment criteria for approved interventions. As shown, an intervention targeted to all those individuals predicted to be helped, based on measured circulating analytes, physiologic parameters, and possibly genetic polymorphisms, could be successful.

Further methodological development might improve on the ability to predict likely response to treatment. We present herein a statistical model to achieve this goal. The intuition, however, is that the same mathematical model that describes the likely course of illness in individual patients could be used to obtain improved predictions in individual patients based on sequential measurements of several analytes. How to accomplish this goal is an analytical problem yet to be explored. We suggest that a diagnostic device that would measure relevant analytes repeatedly and in near-real time, coupled to a mathematical model such as the one presented herein, could help predict the dynamics of inflammation of a particular patient and thereby guide intervention. An immediate extension of such a development could be real-time monitoring of response to intervention, resulting in modification of the intervention before harm occurs or becomes irreversible.

There are several limitations to our approach. Our model remain essentially qualitative, although we are currently conducting efforts to calibrate the simulation using animal data (42, 43). A more quantitative comparison to data from existing trials would certainly be possible and would provide further validation of the predictive ability of the model, assuming that longitudinal measurements of analytes were obtained. Although our simulations reproduce several observations from clinical trials of anti-TNF treatment, we cannot at this stage address nonattributable mortality or attributable mortality beyond a limited time horizon. It is likely that the success of treatments for severe sepsis is limited by factors, such as burden of chronic disease and parameter organ physiologic reserve, which are not easily modeled. Our approach does not take such factors into consideration. Equation-based models include a large number of parameters that express the relative importance and time-scale of interaction and processes involved. The values of these parameters are typically difficult to extract from existing literature and therefore attributed in such as way that the model reproduces known kinetics of as many variables as possible under a variety of experimental conditions. This qualitative fitting exercise is time-consuming and does not guarantee that the resulting set of parameters is either unique or optimal. The relationship between gene polymorphism and outcome of patients with sepsis has been difficult to document (33, 44–50). Although models such as the one presented herein provide clear predictions, these are difficult to confirm in the absence of consistent clinical and epidemiologic studies. One expects systematic advances in the next few years as substantial efforts are underway to elucidate the relationship between sepsis outcome and polymorphisms of effectors of the acute inflammatory response.

In recent years, modeling complex biological systems has become increasingly useful and is moving toward practical applications (18, 51, 52). Companies have offered similar approaches to simulate clinical trials (www.entelos.com) pertaining to asthma and diabetes but not to critical illness. It appears essential to continued progress that complex biological problems such as sepsis be approached through collaborative efforts to enhance the face validity and acceptance of the process for all those involved. It is therefore necessary to call on the resources and insights of clinicians, bench scientists, and mathematicians to address this problem. Importantly, efforts to construct models applicable to humans would greatly benefit from unpublished data collected in phase I and II trials. This is a nontrivial endeavor that requires close collaboration between academicians of disparate backgrounds and industry (13–15). It is our hope that given such an effort, we will see a new generation of therapeutics to successfully address the seemingly intractable problem of sepsis and organ dysfunction.

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APPENDIX: Components of the mathematical model

Model Component	Symbol	Comment
Pathogen	P	A generic Gram-negative pathogen.
Lipopolysaccharide	PE	Immunostimulant derived from Gram-negative bacteria or administered exogenously.
Resting macrophage	MR	Circulating monocyte or local macrophages that acts as a cellular pool for activated macrophages. The total count of resting monocyte/macrophages can increase in proportion to the total inflammatory activity.
Activated macrophages	MA	Activation triggered by lipopolysaccharide (LPS), tumor necrosis factor (TNF), interleukin (IL)-6, tissue trauma, and tissue dysfunction. Activation is down-regulated by anti-inflammatory cytokines.
Activated neutrophils	NA	Activation triggered by LPS, TNF, IL-6, and tissue dysfunction.
Nitric oxide synthase (NOS) activity	NOD	Combines the activities of constitutive and inducible NOS. Normally participates in blood pressure homeostasis. Increased by LPS and TNF in activated neutrophils and macrophages. Decreased by anti-inflammatory cytokines.
Circulating NO_2^-/NO_3^-	NO	A measure correlating with cumulative NOS activity.
Tissue necrosis factor	TNF	A major early proinflammatory cytokine secreted mainly by activated macrophages but also by activated neutrophils.
Interleukin-6	IL6	A proinflammatory cytokine with additional anti-inflammatory effects.
Generic anti-inflammatory activity	CA, CAR, CAI	Represents the combined long-lived anti-inflammatory activity of IL-10, steroids, transforming growth factor-β, soluble receptors to proinflammatory cytokines, and intracellular products with anti-inflammatory activity, such as heat-shock molecules. As a group, their anti-inflammatory activity is triggered by TNF, IL-6, and nitric oxide.
Activated protein C	PC	Acts as an antithrombotic and anti-inflammatory agent.
Tissue factor	TF	Promoted by proinflammation, increases global dysfunction.
Thrombin	TH	Represents global procoagulant/anticoagulant balance, also participates in blood pressure and tissue dysfunction.
Blood pressure	B	Homeostasis depends mainly on NO. The blood pressure equation contains a "restoring" term representing autonomic autoregulation.
Tissue damage	D	Can be caused by hypotension, the action of proinflammatory cytokines, tissue microthrombosis. Nitric oxide is tissue-protective, and there is a slow natural repair process.

Functions included in the equations

The model includes functions expressing saturating dynamic similar to Michaelis-Menten kinetics:

$$f(v,y) = \frac{y \cdot v}{v + y}$$

$$f2(v,y) = \frac{y^2 \cdot v^2}{v^2 + y^2}$$

Inhibitory functions expressing down-regulation or exhaustion kinetics:

$$fs(v,y) = \frac{y}{v + y}$$

$$f2(v,y) = \frac{y^2}{v^2 + y^2}$$

Step/square functions:

$$square(ton, toff) = heav(t - ton) - heav(t - toff)$$

where heav (x) = $1, x > 0$

$= 0, x \leq 0.$

Equations

The equations were generated from "influence diagrams," expressing current knowledge regarding interactions between components included in the model. We documented the vast majority of these interactions, although data on relative quantification are scarce.

$$P' = k_{pg} \cdot P \cdot (1 - k_{ps} \cdot P) \cdot heav(P - P0) - (k_{pm} \cdot MA + k_{pno} \cdot NO + k_{po2} \cdot O2 + k_{ab} \cdot heav(t - tab)) \cdot P$$

$$PE' = k_{pp} \cdot P + k_{pg} \cdot P \cdot (1 - k_{ps} \cdot P) \cdot heav(P - P0) - (k_{pm} \cdot MA + k_{pno} \cdot NO + k_{po2} \cdot O2 + k_{ab} \cdot heav(t - ta - k_{pe} \cdot PE$$

$$MR' = -(k_{mp} \cdot P + k_{mpe} \cdot PE + k_{md} \cdot D) \cdot (S_m + f2(TNF, x_{tnf}) \cdot k_{m6} \cdot f2(IL6, x_{il6}) \cdot fs2(CA, x_{ca})) \cdot MR + k_{mm} \cdot f(s_{noa} \cdot TNF + s_{noa} \cdot PE + NO, x_t) - k_{mr} \cdot MR + S_m$$

$$MA' = (k_{mp} \cdot P + k_{mpe} \cdot PE + k_{md} \cdot D) \cdot (S_m + f2(TNF, x_{tnf}) \cdot k_{m6} \cdot f2(IL6, x_{il6}) \cdot fs2(CA, x_{ca})) \cdot MR - k_{ma} \cdot MA$$

$$NA' = (k_{np} \cdot f(P, x_{t2}) + k_{npe} \cdot f(PE, x_{t2}) + k_{ntnf} \cdot f(TNF, x_{tnf}) + k_{n6} \cdot f(IL6, x_{il6}) + k_{nd} \cdot f(D, x_{t2})) \cdot NA \cdot (1 - k_{ns} \cdot NA) - ((k_{nno} \cdot NO/s_{noa} + k_{no2} \cdot O2) \cdot NA - k_n \cdot (fs(TNF, x_{tnf}) + fs(IL6, x_{il6})) \cdot NA + S_n$$

$$NOD' = (k_{non} \cdot NA + k_{nom} \cdot MA) \cdot fca(CA, x_{ca}) \cdot (f(TNF, x_{tnf}) + f(IL6, x_{il6})) - k_{nod} \cdot NOD$$

$$NO' = k_{no} \cdot (NOD \cdot s_{noa} - NO)$$

$$O2' = ((k_{o2n} \cdot N + k_{o2m} \cdot MA) \cdot (f(TNF, x_{TNF}) + k_{026} \cdot f(IL6, x_{il6})) + k_{02np} \cdot NA \cdot f(P, x_t)) \cdot fs2(CA, x_{ca}) - k_{o2} \cdot O2$$

$$TNF' = (k_{tnfn} \cdot NA + k_{tnfmr} \cdot MR + k_{tnfma} \cdot MA) \cdot fs2(CA, x_{tnfca}) \cdot (1 + k_{tnftnf} \cdot f(TNF, x_{tnf}) - k_{tnf} \cdot TNF - k_{atnf} \cdot square(tiatnf, tiatnf + dur) \cdot TNF$$

$$IL6' = k_{6m} \cdot MA \cdot (1 + k_{6th} \cdot f(TH, x_t) \cdot fs2(CA, x_{t2}) - k_6 \cdot IL6$$

$$CAR' = (k_{can} \cdot N + k_{cam} \cdot MA) \cdot (k_{catnf} \cdot f(TNF, x_{tnf}) + k_{ca6} \cdot f(IL6, x_{il6}) + k_{cano} \cdot f(NO, x_t) + k_{cao2} \cdot f(O2, x_t)) - k_{car} \cdot CAR$$

$$CAI' = CAR - k_{ca} \cdot CAI$$

$$CA = CAI \cdot k_{capc} \cdot PC$$

$$TF' = (k_{tfpe} \cdot PE + k_{tftnf} \cdot TNF + k_{tf6} \cdot IL6) \cdot fs(PC, x_t) - k_{tf} \cdot TF$$

$$TH' = (k_{th1} + k_{thn} \cdot TH) \cdot TF - k_{th} \cdot TH$$

$$PC' = k_{pcth} \cdot TH - k_{pc} \cdot PC$$

$$B' = k_b \cdot (B_a - B) - ((k_{bno}/s_{noa}) \cdot NO \cdot fs(O2, x_t) + k_{btnf} \cdot TNF + k_{bth} \cdot TH) \cdot B$$

$$D' = k_{db} \cdot (1 - (B/B_a)) + k_{dtnf} \cdot TNF + k_{do2} \cdot O2 + (k_{dno}/s_{noa}) \cdot NO \cdot fs(NO, x_{t2} \cdot s_{noa})/s_{noa} + k_{dth} \cdot TH + k_{deq} \cdot O2 \cdot e^{-10 \cdot (NO - s_{noa} \cdot O2)^2/s_{noa}^2} - k_d \cdot D$$

Continued

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Model parameters

Model variables are divided into several categories: initial conditions, rate constants, and saturation constants determining the behavior or the modulating functions *f* and *fs* described previously. Variables labeled as floating determined either trial conditions (dose and duration of anti-tumor necrosis factor treatment) or individual case characteristics.

Fixed initial conditions (where initial conditions dependent on patient or trial characteristics [see next table], the corresponding parameter is said to be floating).

P(0) = floating	PE(0) = 0	MR(0) = 1	MA(0) = 0	NA(0) = 0.01	NOD(0) = 0
NO(0) = 50	O2(0) = 0	TNF(0) = 0.001	IL6(0) = 0	CAR(0) = 0	CAI(0) = 0
TF(0) = 0	TH(0) = 0	PC(0) = 0	B(0) = 1	D(0) = 0	

Rate constants (hr^{-1} unless otherwise specified)

kpg = floating	kps = 0.001	kpm = 0.1	kpno = 0.5	kpo2 = 1.0	kab = 0.0
kpp = 1.0	kpe = 0.2	kmp = 0.05	kmpe = 0.02	kmd = 0.04	kmr = 0.05
km6 = 0.8	kmm = 0.1	kma = 0.2	knp = 0.2	kntnf = 0.4	kn6 = 2.0
knd = 0.2	kns = 0.1	knno = 1.0	kno2 = 6.0	kn = 0.04	knpe = 1.5
knon = 0.25	knom = 0.2	knod = 0.1	kno = 0.1	ko2n = 0.2	ko2m = 0.1
ko26 = 0.1	ko2np = 0.0	ko2 = 0.5	ktnfn = floating	ktnfmr = 5e-4	ktnfma = floating
ktnftnf = 0.7	ktnf = 2.5	katnf = floating	k6m = floating	k6th = 0.4	k6 = 0.1
kcan = floating	kcam = floating	kcatnf = 0.1	kca6 = 0.04	kcano = 0.1	kcao2 = 0.1
kcar = 0.5	kca = 0.02	kcapc = 0.1	ktfpe = 0.01	ktftnf = 0.1	ktf6 = 0.1
ktf = 0.1	kth1 = 0.05	kthn = 0.004	kth = 0.1	kpbth = 0.1	kpc = 0.1
kb = 0.2	kbno = 0.02	kbtnf = 0.02	kbth = 0.2	kdb = 0.1	kdtnf = 0.01
kdo2 = 0.02	kdth = 0.05	kdno = 0.01	kdeq = 0.1	kd = 0.03	

Other model constants (units)

Ba = 1.0	snoa = 870	Sm = 0.1 (h-1)	Sn = 0.001 (h-1)	P0 = 10-4
dur = floating (h)		tiatif = floating (h)		tab = floating (h)

Saturation constants

xtnf = 1.0	xt = 1.0	xil6 = 1.0	xca = 10.0	xt2 = 10.0	xtnfca = 1.0

Trial and cases conditions

Trial variables (varied for each treatment arm)

	Comment	Range
k_{atnf}	Intensity of treatment	2, 10, or 20
dur	Duration of administration	6, 24, or 48 hrs

Case variables (varied for each case, uniform distribution unless specified otherwise)

P(0)	Initial bacterial load	1.6	(0.3–3.0)
k_{pg}	Bacterial doubling rate	0.104	(0.01–0.2)
tiatnf	Time of initiation of therapy	20.8	(4.2–107.5)[a]
k_{tnfn}[b]	TNF production by activated neutrophils	0.5	(0.4–0.6)
k_{tnfm}[b]	TNF production by activated macrophages	0.5	(0.4–0.6)
k_{can}[b]	Anti-inflammatory effector production by neutrophils	0.1	(0.08–0.12)
k_{cam}[b]	Anti-inflammatory effector production by activated macrophages	0.04	(0.032–0.048)
k_{nom}[b]	Nitric oxide production by activated macrophages	0.2	(0.16–0.24)
k_{non}[b]	Nitric oxide production by activated neutrophils	0.25	(0.2–0.3)

[a]$tiatnf^{-1}$ is a uniform distribution obtained from the P(0) distribution; [b]for each patient, variables are co-varied.

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