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A mathematical model of hematopoiesis: II. Cyclical neutropenia

Caroline Colijn[a], Michael C. Mackey[b,*]

[a]*Department of Mathematics and Centre for Nonlinear Dynamics, McGill University, 3655 Promenade Sir William Osler, Montreal, QC, Canada H3G 1Y6*

[b]*Departments of Physiology, Physics & Mathematics and Centre for Nonlinear Dynamics, McGill University, 3655 Promenade Sir William Osler, Montreal, QC, Canada H3G 1Y6*

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Abstract

Cyclical neutropenia is a dynamical disease of the hematopoietic system marked by an oscillation in circulating leukocyte (e.g. neutrophil) numbers to near zero levels and then back to normal. This oscillation is also mirrored in the platelets and reticulocytes which oscillate with the same period. Cyclical neutropenia has an animal counterpart in the grey collie. Using the mathematical model of the hematopoietic system of Colijn and Mackey [A mathematical model of hematopoiesis: I. Periodic chronic myelogenous leukemia. Companion paper to the present paper.] we have determined what parameters are necessary to mimic laboratory and clinical data on untreated grey collies and humans, and also what changes in these parameters are necessary to fit data during treatment with granulocyte colony stimulating factor (G-CSF). Compared to the normal steady-state values, we found that the major parameter changes that mimic untreated cyclical neutropenia correspond to a decreased amplification (increased apoptosis) within the proliferating neutrophil precursor compartment, and a decrease in the maximal rate of re-entry into the proliferative phase of the stem cell compartment. For the data obtained during G-CSF treatment, good fits were obtained only when parameters were altered that would imply that G-CSF led to higher amplification (lower rate of apoptosis) in the proliferating neutrophil precursors, and a elevated rate of differentiation into the neutrophil line.

Keywords: Neutropenia; Mathematical model

1. Introduction

All blood cells originate from the hematopoietic stem cells (HSC) in the bone marrow. These stem cells differentiate and proliferate, forming the three major cell lines: the leukocytes, the platelets, and the erythrocytes. The known peripheral regulatory loops all have a negative feedback nature and are mediated by a variety of cytokines including erythropoietin (EPO), which mediates the regulation of erythrocyte production; thrombopoietin (TPO), which plays the same role in the platelet line (but may also affect other lines); and granulocyte colony-stimulating factor (G-CSF), which regulates leukocyte numbers.

In Colijn and Mackey (2004), we have presented a comprehensive mathematical model of the regulation of hematopoiesis by linking together models for the regulation of the HSC and the differentiated cells (leukocytes, platelets and erythrocytes) in which the cell numbers are regulated by negative feedback loops mimicking the actions of these cytokines. This modelling was motivated by the existence of several hematological diseases that display a highly dynamic nature characterized by oscillations in one or more of the circulating progeny of the HSC (Haurie et al., 1998b). These include, but are not limited to, cyclical neutropenia (CN), periodic chronic myelogenous leukemia (PCML), cyclical thrombocytopenia, and periodic hemolytic

*Corresponding author. Tel.: +1 514 398 4336; fax: +1 514 398 7452.
E-mail address: mackey@cnd.mcgill.ca (M.C. Mackey).

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anemia. Of particular interest in this paper is cyclical neutropenia, while periodic chronic myelogenous leukemia was dealt with in the previous paper (Colijn and Mackey, 2004). In both of these diseases, the oscillations in peripheral blood cell counts all occur at the same period in a given patient.

Cyclical neutropenia is characterized by oscillations that are most prominent in the neutrophils. Neutrophil numbers fall from normal or above normal levels to almost zero, and rise again, with a period of about 19–21 days in humans (Guerry et al., 1973; Haurie et al., 1998b; Hammond et al., 1989). The disease also occurs in grey collies, with a shorter period of 11–16 days (Haurie et al., 1999b). Interestingly, the platelet numbers typically oscillate as well, with the same period as the neutrophils, but with a mean around the normal platelet level. Reticulocyte levels may also oscillate, again with the same period as the neutrophils and platelets.

The origins of oscillations in cyclical neutropenia are unclear. While many have modelled CN as arising from oscillations in peripheral control loops that regulate neutrophil numbers (for example, King-Smith and Morley, 1970; Morley et al., 1969), the work of Hearn et al. (1998) cast doubt on this explanation. As an alternative, Mackey (1978) and Haurie et al. (1998b) have suggested that the oscillations originate in a loss of stability in the hematopoietic stem cells, but Bernard et al. (2003) suggests that the origin of CN lies in a destabilization of the combined HSC and peripheral neutrophil control system. The matter remains unresolved, and the model results presented here offer some insight into this question.

The hypothesis that oscillations originate in the stem cells is related to the fact that these oscillations occur in more than one cell line. However, in many earlier models, only one cell line, or one line coupled to the stem cells, was represented. In this context it is not possible to examine the effects of a destabilization in one line or in the stem cell compartment on the other lines. In the work of Bernard et al. (2003), they were able to duplicate various features of CN. However, since their model included neither erythrocyte nor platelet control, it is unknown if their hypothesis would be consistent with observed platelet and reticulocyte data.

The present model provides a framework in which these questions can be addressed. Specifically, we wish to use a comprehensive model for the regulation of hematopoiesis to deduce what must be different in the model parameters to explain the dynamic behaviour seen in CN as manifested in the grey collie and in humans. To do this, we take a G_0 model for the stem cell compartment, based on Mackey (1978) and Pujo-Menjouet et al. (2001), and couple it to a leukocyte model based on the work of Bernard et al. (2003), as well as to two simplified models representing the control of platelet and erythrocyte production. Our model is presented in some detail in the preceding paper (Colijn and Mackey, 2004), along with a detailed discussion of the relevant parameter estimation. Therefore, in Section 2.1, we give only a summary of the mathematical formulation, and in Section 2.2, we present the normal steady-state parameters without the detailed discussion in Colijn and Mackey (2004). In Section 4, the simulations generated by the model are compared with observed dog and human neutropenia data.

2. A model of the hematopoietic system

2.1. Model formulation

In this paper, we link together models of the neutrophil, platelet and erythrocyte lines, coupling them via the hematopoietic stem cell compartment. The pluripotential, non-proliferating stem cells are represented in the model by Q (see Fig. 1). The circulating neutrophils, erythrocytes and platelets are denoted N, R and P, respectively. The model is the same as presented in Colijn and Mackey (2004), with a neutrophil compartment in place of the leukocyte compartment, and with no reticulocyte compartment. The erythrocyte

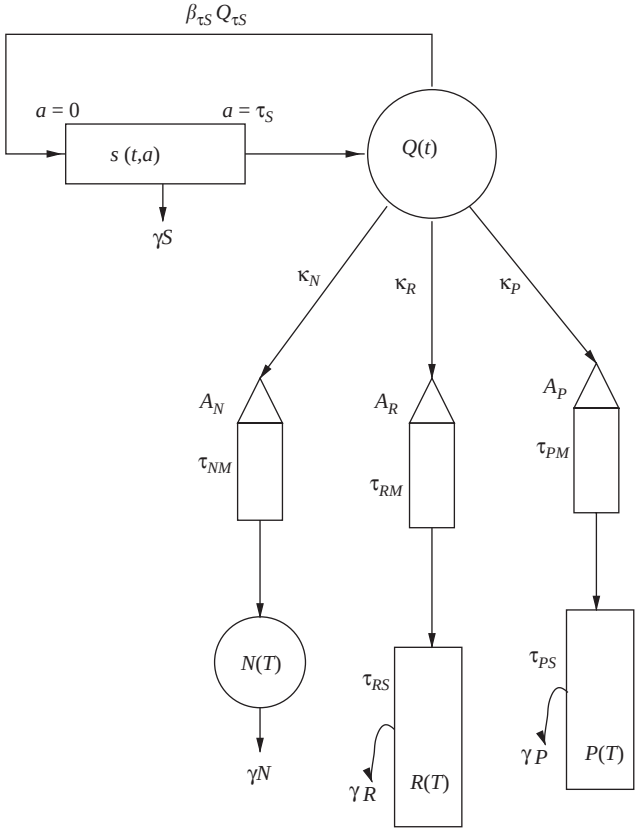

Fig. 1. The model in block form. See the text and Colijn and Mackey (2004) for further details.

compartment is as it is in Colijn and Mackey (2004), and is as given in Eq. (1). These minor changes were made because the patient and grey collie data available for this cyclical neutropenia study are different than the data for periodic chronic myelogenous leukemia that we analysed in the previous paper. See Colijn and Mackey (2004) for all of the model details, which are only given in a telegraphic form in this section.

The rates of differentiation into the three lines are given by $\kappa_N(N)$, $\kappa_R(R)$ and $\kappa_P(P)$, respectively. The dependence of these rates on the numbers of circulating cells of the relevant type represents the feedback between the circulating cell numbers and the production. As in Colijn and Mackey (2004), for the sake of simplicity we will use subscript notation to denote delays: $Q_{\tau_S} \equiv Q(t-\tau_S)$, and similarly for the other functions of time, and the other delays.

With this notation, the equations composing the model are given by

$$\begin{aligned}
\frac{dQ}{dt} &= -\beta(Q)Q - (\kappa_N+\kappa_R+\kappa_P)Q + 2e^{-\gamma_S\tau_S}\beta(Q_{\tau_S})Q_{\tau_S},\\
\frac{dN}{dt} &= -\gamma_N N + A_N\kappa_N(N_{\tau_N})Q_{\tau_N},\\
\frac{dR}{dt} &= -\gamma_R R + A_R\{\kappa_R(R_{\tau_{RM}})Q_{\tau_{RM}}\\
&\quad - e^{-\gamma_R\tau_{RS}}\kappa_R(R_{\tau_{RM}+\tau_{RS}})Q_{\tau_{RM}+\tau_{RS}}\},\\
\frac{dP}{dt} &= -\gamma_P P + A_P\{\kappa_P(P_{\tau_{PM}})Q_{\tau_{PM}}\\
&\quad - e^{-\gamma_P\tau_{PS}}\kappa_P(P_{\tau_{PM}+\tau_{PS}})Q_{\tau_{PM}+\tau_{PS}}\},
\end{aligned} \tag{1}$$

where

$$\begin{aligned}
\beta(Q) &= k_0\frac{\theta_2^s}{\theta_2^s+Q^s},\\
\kappa_N(N) &= f_0\frac{\theta_1^n}{\theta_1^n+N^n},\\
\kappa_P(P) &= \frac{\bar{\kappa}_p}{1+K_pP^r},\\
\kappa_R(R) &= \frac{\bar{\kappa}_r}{1+K_rR^m}.
\end{aligned} \tag{2}$$

2.2. Parameter estimation

2.2.1. The stem cell compartment

The relevant parameter estimation has largely been described in Colijn and Mackey (2004). However, there are several small changes in our steady-state values reflecting the fact that we are trying to understand cyclical neutropenia data rather than periodic chronic myelogenous leukemia (PCML) data. First, the model of Bernard et al. (2003) was successful in duplicating some of the features of CN. For this reason, it is useful to begin our simulations from the starting point used in that paper. Also, in Colijn and Mackey (2004) there was motivation to seek long-period solutions typical of PCML, while there is no such necessity here as the periods in CN are much shorter. We therefore are free to choose the rate of apoptosis in the stem cell compartment, γ_S, to be 0.07/day and k_0, the coefficient of the Hill function in the stem cells to be 8.0, following Bernard. A summary of the parameters used in the model is given in Table 1. Throughout, the subscript $*$ indicates a steady-state value. The steady-state values are obtained (and related to other parameters) by setting the rates of change in (1) to zero: $\dot{Q} = \dot{N} = \dot{R} = \dot{P} \equiv 0$.

Table 1
Normal steady state parameters

Parameter name	Value used	Unit	Sources
Stem cell compartment			
Q_*	1.1	$\times 10^6$ cells/kg	1
γ_S	0.07	days^{-1}	1
τ_S	2.8	days	1,2
k_0	8.0	days^{-1}	1
θ_2	0.3	$\times 10^6$ cells/kg	1
s	4	(none)	1
Neutrophil compartment			
N_*	3.55–7	$\times 10^8$ cells/kg	2,3
γ_N	2.4	days^{-1}	1,4,5
τ_{MN}	3.5	days	1
A_N	752	100's	1,3
f_0	0.40	days^{-1}	(calculated)
θ_1	0.36	$\times 10^8$ cells/kg	1
n	1	(none)	1
Erythrocyte compartment			
R_*	3.5	$\times 10^{11}$ cells/kg	6
γ_R	0.001	days^{-1}	6
τ_{RM}	6	days	6
τ_{sum}	120	days	6
τ_{ret}	2.8	days	3
A_R	5.63	10,000's	3,7
$\bar{\kappa}_r$	1.17	days^{-1}	(calculated)
K_r	0.0382	$(\times 10^{11}$ cells/kg$)^{-m}$	6
m	6.96	(none)	6
Platelet compartment			
P_*	2.94	$\times 10^{10}$ cells/kg	8
γ_P	0.15	days^{-1}	8
τ_{PM}	7	days	8
τ_{PS}	9.5	days	8
A_P	28.2	1000's	3
$\bar{\kappa}_p$	1.17	days^{-1}	(calculated)
K_p	11.66	$(\times 10^{10}$ cells/kg$)^{-r}$	8
r	1.29	(none)	8

Sources: 1 = (Bernard et al., 2003), 2 = (Abkowitz et al., 1988), 3 = (Beutler et al., 1995), 4 = (Deubelbeiss et al., 1975), 5 = (Haurie and Mackey, 2000), 6 = (Mahaffy et al., 1998), 7 = (Novak and Necas, 1994), 8 = (Santillan et al., 2000).

3. Data

We compare the model simulations to data previously analysed in Haurie et al. (1998a, b) for humans and dogs.

Our dog data were supplied by Dr. David Dale (University of Washington School of Medicine, Seattle). The dogs were kept in temperature-controlled environments, and blood specimens were drawn daily. In Haurie et al. (1999b), the authors use Lomb periodogram analysis to test data from the 9 cyclical neutropenic grey collies. We have data for the same 9 dogs before treatment, and for 7 of the 9 undergoing G-CSF treatment through daily injections. In all of the untreated dogs, the data show significant periodicity in the neutrophils and/or the platelets, according to the Lomb periodogram analysis.

Our human data are for pediatric and adult patients that were diagnosed with severe idiopathic neutropenia, cyclical neutropenia or congenital neutropenia (Dale et al., 1993), but all have been shown to be oscillating (Haurie et al., 1999a). Patients were divided into two groups; in one group, G-CSF therapy was administered after a 4-month observation period, and in the other, the therapy was begun immediately on diagnosis. We examined data from patients from the first group, for the purposes of comparing the treated and untreated time periods. The G-CSF was administered subcutaneously, in doses sufficient to raise the minimal neutrophil counts to between 1.5×10^9 and 10×10^9 cells/L. These data have been previously analysed for periodicity by Haurie et al. (1999a), and we only worked with data showing significant periodicity at the $p = 0.05$ level or better in any of the cell types for which data were available.

4. Fitting and simulation

As mentioned above, the origins of cyclical neutropenia are somewhat obscure, and various models have been proposed to explain the onset of oscillations (see, for example, the review in Hearn et al. (1998a, b)). One point of view is that oscillations are induced by a destabilization of a feedback loop at the level of neutrophil precursors, as has been suggested by a number of authors, for example Morley et al. (1969), Morley and Stohlman (1970), King-Smith and Morley (1970), Morley (1970), Reeve (1973), MacDonald (1978), Kazarinoff and van den Driessche (1979), von Schulthess and Mazer (1982), Shvitra et al. (1983), Wichmann et al. (1988), and Schmitz et al. (1995, 1990). However, it was argued in Hearn et al. (1998) that it is unlikely that a destabilization of such a feedback loop could account for the nature of the oscillations in neutropenia, and in addition, it could not account for the observed oscillations in other cell lines. As an alternative, it has been proposed by Bernard et al. (2003) that the origin of the oscillations in cyclical neutropenia is an increase in apoptosis in the early neutrophil precursors which leads to a destabilization of the combined HSC plus neutrophil production system. If this were to occur, it would result in oscillations in the platelets and the erythrocytes.

In this context, and following Bernard et al. (2003), we began to simulate cyclical neutropenia with a reduction in the amplification parameter in the neutrophil line (A_N) to mimic an increase in apoptosis in the neutrophil precursors. We found that the model does generate stable oscillations when $0 \leqslant A_N \leqslant 267$. (The normal value is 752. When $A_N = 0$, the neutrophil level is constant at $N = 0$ but the stem cells oscillate.) The overall features of these oscillations are similar to those found in cyclical neutropenia, with periods ranging from 13 days to 17 days, except that the neutrophil numbers are invariably much lower than their steady-state values. In some of the data, the neutrophils oscillate up to a maximum that is several times the normal steady-state value, which cannot be accounted for in the model by changing A_N alone.

This motivated our decision to try automated parameter fitting procedures (as in our examination of PCML in Colijn and Mackey (2004)) to determine both to what extent the model is capable of duplicating the qualitative and quantitative features of the observed data, and what parameter adjustments are necessary for this agreement to be achieved.

Our approach is as follows. We first fit the data for untreated dogs and humans, changing the parameters necessary to match the observed data. Then, we start with a reasonable untreated parameter set as a starting point for the fitting of the treated (G-CSF) data, changing only the parameters that have been linked to the effects of G-CSF. In this way, we can first explore what is required to simulate untreated neutropenia, and then examine what has to change to explain the effects of the G-CSF treatment.

The parameters we used to fit the untreated data were: A_N, f_0, A_P, $\bar{\kappa}_P$, γ_S, k_0 and K_P. The basis for this choice is as follows. First, since there are two clear ways to reduce the neutrophil levels in the model we expected that a decrease in A_N would be the primary change necessary for the simulation of cyclical neutropenia, and that it might also be necessary to decrease f_0, which scales the overall differentiation into the neutrophil line. Secondly, since we are comparing the model simulations to data for the neutrophils as well as for the platelets, and because there is considerable variability in mean platelet counts in both the cyclical neutropenia data and in normals, we allowed A_P, $\bar{\kappa}_P$ and K_P to change while fitting the model to the untreated data. This was necessary to match the overall platelet mean

and amplitude of oscillation. The parameter K_P adjusts the steady-state platelet value P_*. Any value of K_P between 3 and 13×10^{10} cells/kg body mass reflects a P_* value in the range $(1.4, 4) \times 10^{10}$ cells/kg body mass. These platelet compartment parameters were held fixed for the simulations of the treated data, as G-CSF was assumed not to affect the platelet compartment. Finally, we also allowed γ_S to change in the treated and untreated simulations; the steady-state value of this parameter is not precisely known (Mackey, 2000), and in the model it affects the period and qualitative features of the oscillations. It was changed in both treated and untreated simulations. k_0 was allowed to change in the simulations as well, as it is has been hypothesized (see above) that cyclical neutropenia involves a destabilization of the stem cell compartment.

The parameters changed in the simulations of the data during G-CSF treatment were: A_N, θ_1, γ_S, τ_S and τ_{NM}. It is known that G-CSF interferes with apoptosis (Basu et al., 2002), which we simulate by allowing A_N to change. Bernard et al. (2003) argued for changing the others, commenting that θ_1 depends on the production (or administration) of G-CSF. Finally, it is well established that G-CSF decreases the neutrophil maturation time τ_{NM} (Price et al., 1996).

4.1. Dog data

As in Colijn and Mackey (2004), we have applied both a Marquardt–Levenberg and simulated annealing approach to fitting the parameters for the dog data. Briefly, the Marquardt–Levenberg algorithm is initiated at a point in parameter space, from which it computes the sum of squares difference between the simulation and the data:

$$S = \sum_{i=1}^{M} (y_s - y_i)^2, \tag{3}$$

where y_i are the observed data points, y_s are the simulated data points and M is the total number of points available. The algorithm takes the path of quickest descent in S, altering the parameter set used to generate the simulation as it goes.

As discussed in Colijn and Mackey (2004), the Marquardt–Levenberg approach has a problem dealing with multiple local minima. Since the convergence algorithm is a 'greedy' one, taking the fastest route possible to a minimum, in many cases it converges to a local minimum of S. However, with the dog data (which is of unusually good quality, having been taken daily), it was possible to obtain a reasonable fit to the data. This was not possible with the human data, in part because of the sparse sampling and in part because the variability of the data made it impossible to guess where to start the simulations.

It should be noted that we were not always able to obtain a good fit, or even convergence, with the Marquardt–Levenberg approach using all of the fitting parameters mentioned above. For example, in the treated data, the algorithm would generate negative values of the delay times τ_S and τ_{NM} when they were included in the procedure, and as a result, they were set manually. Therefore, the parameters used with the Marquardt–Levenberg fitting approach are not always identical to those listed above. A plot of the data from Dog 113 and the resulting Marquardt–Levenberg fit is shown in Fig. 2 for the untreated case, and in Fig. 4 during treatment.

The results of the parameter fitting from the Marquardt–Levenberg algorithm are shown in Table 2 for the untreated data, and in Table 4 for the dogs undergoing treatment with G-CSF. In both of these tables, the errors shown in percent (in brackets) are based on the width of the S^2 convergence basin, and not on its depth, and are computed from the covariance matrix returned by the Marquardt–Levenberg algorithm. Thus, these errors are often comparatively small, when there are nearby local minima. When an error of (0.00) is listed, this means that the parameter was not included in the fit procedure, so that no covariance terms were available.

For the untreated dogs, the parameter A_N is lower than the normal steady-state value. The average value of A_N in our simulations is approximately one third of the normal default value, which would correspond to a loss of between one and two effective divisions in the neutrophil precursors in the grey collie relative to a normal dog. This would mirror an increase in apoptosis

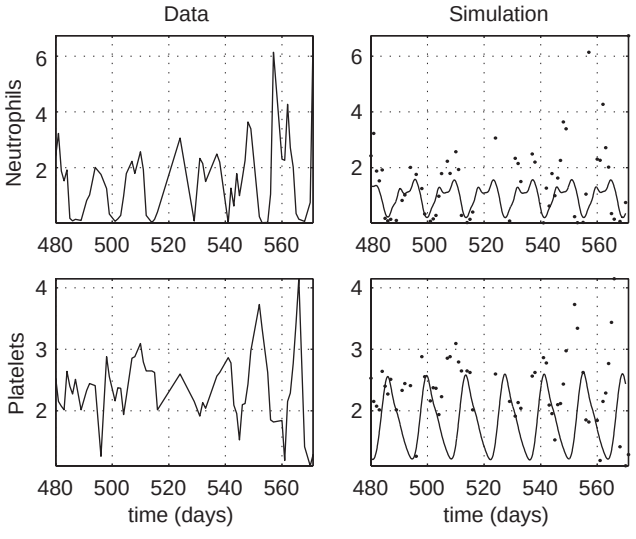

Fig. 2. Serial Neutrophil and Platelet Data: untreated Dog 113. The left-hand side figures show the observed data, and the right-hand side figures show the data (points) with the simulation resulting from the Marquardt–Levenberg fitting method (solid line). Neutrophils are in units of $10^8\,\mathrm{kg}^{-1}$, while platelets are in units of $10^{10}\,\mathrm{kg}^{-1}$.

Table 2
Parameter estimates for the 9 untreated dogs, based on the Marquardt–Levenberg method

Default	A_N		f_0		A_P		$\bar{\kappa}_P$		γ_S		τ_S		K_P	
	752.00		0.40		28.00		1.17		0.07		2.8		11.66	
Dog 100	500.0	(6.40)	0.30	(1.46)	19.15	(0.00)	1.20	(0.00)	0.07	(2.05)	2.5	(0.00)	3.40	(4.13)
Dog 101	134.5	(23.78)	0.94	(0.47)	32.96	(0.43)	0.93	(0.15)	0.06	(0.00)	2.5	(0.00)	11.66	(0.00)
Dog 113	43.0	(9.96)	0.55	(10.34)	8.96	(8.38)	2.80	(5.26)	0.07	(0.00)	2.4	(1.35)	11.66	(0.00)
Dog 117	634.5	(3.07)	0.23	(2.62)	19.15	(0.00)	1.20	(0.00)	0.03	(0.00)	2.5	(0.00)	3.40	(0.00)
Dog 118	67.8	(12.08)	0.65	(11.57)	43.07	(36.08)	0.93	(39.95)	0.04	(0.00)	2.5	(0.00)	11.66	(0.00)
Dog 126	324.0	(9.34)	0.22	(15.16)	11.45	(12.85)	1.20	(20.04)	0.05	(0.00)	2.5	(0.00)	3.40	(0.00)
Dog 127	10.1	(2.50)	1.22	(1.43)	25.29	(39.87)	0.10	(21.05)	0.07	(0.00)	2.5	(0.00)	0.85	(67.14)
Dog 128	110.0	(2.23)	0.94	(4.39)	26.39	(6.09)	0.50	(6.48)	0.07	(0.00)	2.4	(0.00)	3.40	(0.00)
Dog 125	400.0	(0.00)	0.30	(0.00)	19.15	(0.00)	1.20	(0.00)	0.07	(0.00)	2.5	(0.00)	3.40	(0.00)
Mean	247.1	(7.71)	0.59	(5.27)	22.84	(11.52)	1.12	(10.33)	0.06	(0.23)	2.5	(0.15)	5.87	(7.92)
St. Dev.	224.9	(7.29)	0.37	(5.60)	10.57	(15.69)	0.74	(13.92)	0.01	(0.68)	0.0	(0.45)	4.42	(22.25)

The top line of the table gives the normal values from Table 1 for comparison. Error estimates, in percent, are shown in brackets.

in the primitive neutrophil precursors. f_0 was slightly higher than the normal on average, but since the range for the default value is 0–0.8 (Bernard et al., 2003), this difference is probably not significant.

The platelet parameters did not differ significantly from their normal values in the Marquardt–Levenberg fits. The rate of apoptosis in the stem cell compartment, γ_S, was slightly lower than the default value in these fits; however, in this parameter there were many local minima and γ_S was often chosen by inspection rather than by the algorithm, as convergence was obtained at many different points. The values of τ_S and K_P were also obtained by inspection rather than by the fitting algorithm.

We used the results of the Marquardt–Levenberg fitting procedure as a starting point for a simulated annealing approach to fitting the dog data. The simulated annealing procedure is the same as in Colijn and Mackey (2004), where a detailed description was given. In brief, the approach combines a random walk in parameter space with a decreasing probability of taking a step that increases the 'energy' function.

Our energy function is similar to a sum of squares; it is given by

$$E = \sqrt{\sum_{i=1}^{M}\left(\frac{(N_i^s - N_i)^2}{\bar{N}^2} + \frac{(P_i^s - P_i)^2}{\bar{P}^2}\right)} + \beta\left[\left(\frac{(\bar{N}^s - \bar{N})^2}{\bar{N}^2} + \frac{(\bar{P}^s - \bar{P})^2}{\bar{P}^2}\right)\right] + \beta\left[\frac{(\mathrm{var}(N^s) - \mathrm{var}(N))^2}{\mathrm{var}(N)^2} + \frac{(\mathrm{var}(P^s) - \mathrm{var}(P))^2}{\mathrm{var}(P)^2}\right], \quad (4)$$

where M is the total number of data points available, N and P refer to neutrophils and platelets, s indicates simulation, and the lack of a superscript indicates observed data. The bars indicate that the mean has been taken, and 'var' refers to the variance. β is a small positive number less than one. This energy function is discussed in more detail in Colijn and Mackey (2004).

The use of the simulated annealing approach that starts from the parameter sets found with the Marquardt–Levenberg confirms that these results are near a global minimum. If they were not, the annealing procedure would generate very different results. However, because we began the annealing at the Marquardt–Levenberg parameter values, it is not reasonable to draw conclusions about the relative effectiveness of the two procedures based on these results. Furthermore, as noted above, we were not able to fit the same sets of parameters with the two methods. Some comments about comparison between the methods are, however, to be found in Colijn and Mackey (2004).

The parameter sets found with the simulated annealing procedure for the untreated and treated dogs are given in Tables 3 and 5. The errors here, again shown in parentheses, represent the percentage change in the relevant parameter necessary to effect a change of 10 percent in the energy function. Because this is a wider range than the typical depth of the local minimum in S found with the Marquardt–Levenberg procedure, these errors tend to be larger, but this does not necessarily imply that the fit results are poorer.

At this point, it is possible to compare the results of the annealing method and the Marquardt–Levenberg method for the untreated neutropenic dog data. Interestingly, the mean value of A_N is the same to within error for the two methods, as is the value of f_0. Again, the platelet parameters A_P and κ_P did not change much from their normal default values.

k_0 scales the overall amount of re-entry into the proliferative phase in the stem cell compartment. Due to

Table 3
Parameter estimates for the 9 untreated dogs, based on the simulated annealing method

Default	A_N		f_0		A_P		$\bar{\kappa}_P$		γ_S		k_0		K_P	
	752.00		0.40		28.00		1.17		0.07		8.0		3–13	
Dog 100	488.1	(14.03)	0.31	(10.08)	21.64	(2.45)	1.38	(5.70)	0.06	(8.91)	1.46	(5.43)	3.4	(10.42)
Dog 101	135.8	(3.26)	0.81	(2.80)	91.74	(31.38)	0.33	(19.82)	0.05	(27.19)	1.04	(24.29)	8.0	(25.73)
Dog 113	51.5	(14.05)	0.48	(22.11)	6.15	(13.12)	3.47	(14.07)	0.01	(95.00)	1.80	(11.62)	11.6	(41.93)
Dog 117	659.5	(17.74)	0.17	(9.53)	14.01	(4.99)	0.69	(4.26)	0.05	(8.75)	1.59	(13.98)	3.8	(5.63)
Dog 118	73.4	(12.82)	0.69	(12.01)	49.38	(64.30)	1.16	(62.95)	0.03	(98.13)	1.21	(28.76)	10.8	(51.00)
Dog 126	394.1	(12.49)	0.24	(15.08)	20.66	(3.43)	1.24	(13.57)	0.07	(7.27)	1.14	(18.43)	3.7	(19.36)
Dog 127	320.8	(36.92)	0.52	(11.73)	8.53	(18.68)	1.54	(9.83)	0.06	(6.41)	1.41	(7.60)	2.8	(29.21)
Dog 128	18.8	(13.87)	1.44	(8.90)	30.88	(60.66)	0.26	(72.01)	0.01	(65.00)	1.34	(8.24)	2.5	(99.26)
Dog 125	74.6	(17.90)	1.20	(28.09)	53.25	(63.49)	0.31	(52.50)	0.07	(31.72)	1.21	(9.61)	5.0	(42.00)
Mean	246.3	(15.90)	0.65	(13.37)	32.91	(29.17)	1.15	(28.30)	0.05	(38.71)	1.36	(14.22)	5.7	(36.06)
St. Dev.	228.6	(13.45)	0.44	(14.94)	27.60	(28.30)	1.00	(38.32)	0.02	(55.52)	0.24	(14.29)	3.5	(45.96)

The top line of the table gives the normal values from Table 1 for comparison. Error estimates, in percent, are shown in brackets.

the different capabilities of the two methods, it was possible to include k_0 in the fit parameters using the annealing method, where it was not possible with the other method. Note that in Table 3, k_0 is significantly lower than the normal steady-state value. This indicates that in our model, at least in the fits to the dog data, neutropenia is best simulated not only by a decrease in amplification (an increase in apoptosis) in the neutrophil precursors but by a decrease in the maximal rate of cellular reentry from the G_0 phase of the stem cells into the proliferative phase of the cell cycle. In Section 4.2, we will compare these trends with the corresponding fits to the human data. Here, we turn next to the dog data where there was G-CSF treatment.

Plots showing the result of the simulated annealing fit procedure for the same dog (Dog 113) as in Figs. 2 and 4 are shown in Figs. 3 and 5. Note that in Fig. 2, the platelet oscillations reach a minimum well below the minimum of the data, and the neutrophils do not reach a high enough maximum. Both of these are much improved in the simulation shown in Fig. 3. In the data shown in Figs. 4 and 5, note that there is a qualitative difference between the neutrophil behaviour in the first and second halves of the data, which is not duplicated by the model. This is because the model simulations each have a constant set of parameters, so that the model is largely unable to accommodate large qualitative changes. The only way that the fitting algorithms can duplicate such changes is by preserving transient behaviour for longer than usual, and indeed remaining transient behaviour is seen in the first parts of Figs. 4 and 5. Note also that the simulated annealing fit is visibly better than the Marquardt–Levenberg fit, though neither fit duplicates the rapid changes in the data; these are probably due to fluctuations of unknown origin.

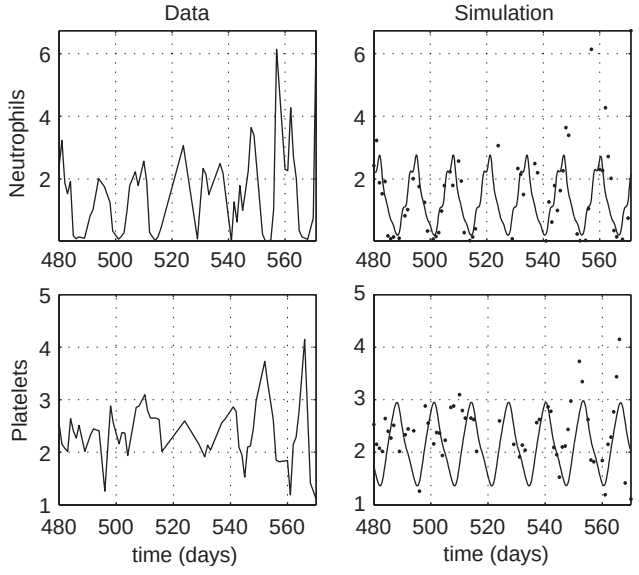

Fig. 3. Serial Neutrophil and Platelet Data: untreated Dog 113. The left plot shows the observed data, and the right plot shows the data (points) with the simulation (solid line) using parameters derived from the simulated annealing method. Neutrophils are in units of $10^8\,\mathrm{kg}^{-1}$, while platelets are in units of $10^{10}\,\mathrm{kg}^{-1}$.

For the treated dogs, Tables 4 and 5 show the parameter sets resulting from the Marquardt–Levenberg and simulated annealing fits, respectively. In both sets of results, it is clear that G-CSF has increased the amplification factor in the neutrophil line, A_N. In both sets of results for the data during G-CSF treatment, τ_S decreased (consistent with more rapid proliferation in the stem cell compartment) and θ_1 increased (consistent with an increase in the presence of G-CSF, Bernard et al. (2003)).

What was not anticipated in these results is the slight *increase* in the rate of stem cell apoptosis, γ_S. However,

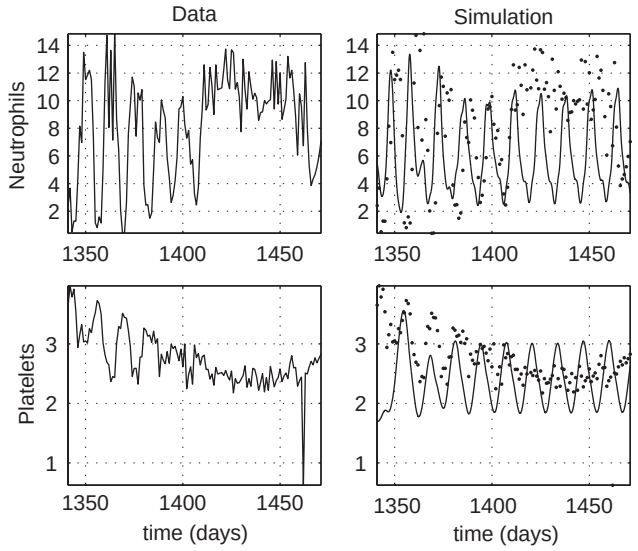

Fig. 4. Serial Neutrophil and Platelet Data: with G-CSF treatment in Dog 113. The left plot shows the observed data, and the right plot shows the data (points) with the simulation (solid line) using parameters derived from the Marquardt–Levenberg method. Neutrophils are in units of $10^8\,\mathrm{kg}^{-1}$, while platelets are in units of $10^{10}\,\mathrm{kg}^{-1}$.

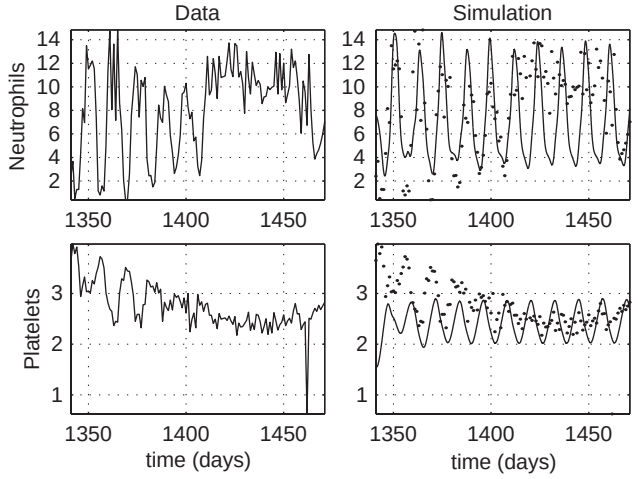

Fig. 5. Serial Neutrophil and Platelet Data: with G-CSF treatment for Dog 113. The left plot shows the observed data, and the right plot shows the data (points) with the simulation (solid line) using parameters resulting from the simulated annealing method. Neutrophils are in units of $10^8\,\mathrm{kg}^{-1}$, while platelets are in units of $10^{10}\,\mathrm{kg}^{-1}$.

note that for the dogs, γ_S did not increase as much from the normal steady-state value in the annealing method as it did in the Marquardt–Levenberg method, and the annealing method's fits were in general better, when compared using a cost function similar to a χ^2 test (see Colijn and Mackey (2004) for a discussion of this approach to comparing the fit quality). Furthermore, in several cases the range of γ_S for which the simulation was in the 10 percent energy range was very large; in these cases we do not have meaningful results for the value of γ_S. More comments about this parameter are made in Section 4.2 with reference to the human data.

4.2. Human data

For the reasons mentioned above, we have not applied the Marquardt–Levenberg approach to analyse the human data, but used only the simulated annealing method. The energy function used here is the same as that given in Eq. (4).

As for the grey collies, for the data from untreated humans we begin by fitting the parameters A_N, f_0, A_P, $\bar{\kappa}_P$, γ_S, k_0 and K_P. In each patient, these results are then used as a starting point for fitting the G-CSF (treated) data, changing parameters A_N, θ_1, γ_S, τ_S and τ_{NM}. The parameters found for the untreated data are shown in Table 6.

Several notable patterns emerge. First and most obviously, in untreated human cyclical neutropenia patients the parameter A_N is even more drastically reduced relative to the normal value than it was in the dog data. This reduction in the amplification is by a factor of approximately 30 compared to the normal value, equivalent to a loss of five effective divisions in the neutrophil precursors. This is consistent with the fact that in the human data, the neutrophil levels are often an order of magnitude lower than they are in the dog data; while in the dogs, neutrophil levels often oscillated between almost zero and several times the normal value, in the human data they more typically oscillated between almost zero and 1/10–1/3 of the steady-state value.

As in the dog data, the platelet parameters generally are not significantly different from their normal steady-state values, though a fair degree of variability in these parameters is needed to match the variable platelet data among the patients. γ_S, the rate of apoptosis in the stem cell compartment, is just slightly lower than the steady-state value of 0.07, with a standard deviation of 0.02. And, as in the dog data, k_0 is lower than the steady-state value, indicating that neutropenia is simulated here, in part, by decreasing the maximal rate of re-entry into the proliferative phase in the stem cell compartment.

For the data for human patients treated with G-CSF, the parameter values are shown in Table 7. Again, most notable are the values of A_N, which, as expected, have increased significantly and are now close to the normal steady-state value. Also, θ_1 has increased, and τ_S has decreased slightly, though the change is not significant when the error and standard deviations are taken into account.

Some comments need to be made regarding the fit results for τ_{NM}. This parameter was included in the simulated annealing approach because it was suggested in Bernard et al. (2003) that it would decrease under G-CSF treatment (Price et al., 1996). When attempts

Table 4
Parameter estimates for the 7 treated dogs, based on the Marquardt–Levenberg method

Default	A_N		f_0		A_P		$\bar{\kappa}_P$		γ_S		τ_S		K_P		θ_1	
	752		0.4		28		1.173		0.07		2.8		3–13		0.36	
Dog 100	881.4	(12.39)	0.69	(36.44)	10.57	(6.95)	5.16	(4.70)	0.13	(1.83)	2.1	(0.00)	11.66	(0.00)	0.36	(42.57)
Dog 101	740.9	(3.41)	1.96	(7.94)	23.47	(6.22)	2.70	(4.39)	0.13	(1.21)	2.1	(0.00)	11.66	(0.00)	0.36	(11.46)
Dog 113	1106.2	(21.00)	0.34	(32.30)	10.73	(9.62)	3.97	(5.21)	0.13	(200.00)	2.1	(0.00)	11.66	(0.00)	0.58	(43.18)
Dog 117	982.6	(5.91)	1.55	(5.45)	20.46	(4.79)	3.41	(4.24)	0.13	(0.79)	2.0	(0.00)	11.66	(0.00)	0.40	(0.00)
Dog 118	1266.2	(2.56)	0.34	(3.88)	10.26	(1.21)	3.18	(2.86)	0.12	(1.51)	2.1	(0.00)	11.66	(0.00)	0.80	(4.38)
Dog 127	95.0	(13.35)	0.76	(1.66)	20.00	(84.40)	0.23	(73.69)	0.10	(9.41)	2.1	(0.00)	3.50	(0.00)	2.56	(19.40)
Dog 128	422.9	(4.65)	1.57	(4.79)	25.05	(13.42)	1.20	(8.83)	0.12	(2.17)	2.1	(0.00)	11.66	(0.00)	0.40	(0.00)
Mean	785.0	(9.04)	1.03	(13.21)	17.22	(18.09)	2.84	(14.85)	0.12	(30.99)	2.1	(0.00)	10.49	(0.00)	0.78	(17.28)
Std	406.7	(6.78)	0.66	(14.63)	6.50	(29.49)	1.66	(26.01)	0.01	(74.59)	0.0	(0.00)	3.08	(0.00)	0.80	(18.76)

The top line of the table gives the normal values from Table 1 for comparison. Error estimates, in percent, are shown in brackets.

Table 5
Parameter estimates for the 7 treated dogs, based on the simulated annealing method

Default	A_N		θ_1		γ_S		τ_S		τ_{NM}	
	750.00		0.36		0.07		2.80		3.50	
Dog 100	908.2	(20.59)	0.57	(30.17)	0.11	(85.00)	1.08	(90.00)	5.12	(16.64)
Dog 101	666.9	(24.53)	1.01	(28.22)	0.09	(95.47)	1.44	(14.94)	0.67	(236.33)
Dog 113	1107.8	(10.75)	0.78	(7.87)	0.13	(4.45)	2.02	(1.35)	3.73	(8.85)
Dog 117	1009.2	(32.55)	1.04	(47.23)	0.10	(12.11)	2.03	(7.20)	0.22	(110.00)
Dog 118	1268.5	(31.65)	1.80	(40.69)	0.12	(16.80)	2.14	(3.81)	1.32	(40.14)
Dog 127	94.4	(0.00)	2.63	(0.00)	0.09	(0.00)	2.09	(0.00)	3.64	(0.00)
Dog 128	401.1	(11.15)	0.50	(13.30)	0.01	(80.00)	2.28	(15.11)	0.60	(110.00)
Mean	779.4	(18.75)	1.19	(23.93)	0.09	(41.98)	1.87	(18.92)	2.18	(74.57)
St. Dev.	416.6	(23.23)	0.77	(30.84)	0.04	(61.92)	0.44	(35.81)	1.94	(116.23)

The top line of the table gives the normal values from Table 1 for comparison. Error estimates, in percent, are shown in brackets.

were made to fit it with the Marquardt–Levenberg method, the algorithm would often fail to converge, or τ_{NM} would become negative or otherwise unrealistic. Here, our simulated annealing algorithm contains restrictions so that parameters may not run below zero. However, we did not fix an upper bound, and some of the results for τ_{NM} are so large as to be biologically unrealistic.

A visual inspection of simulations over ranges of τ_{NM} indicates that there is no significant advantage to changing this parameter. Thus, the large values of τ_{NM} in Table 7 are the result of the annealing process performing a large number of steps of a random walk, and *not* effectively selecting for τ_{NM} because changes in τ_{NM} do not change the energy significantly. Note, however, that changing τ_{NM} does change the *phase* of the oscillations, so that locally, the energy does change with τ_{NM}. This accounts for the errors shown in Table 7. In any case, we cannot base conclusions about the values of τ_{NM} in neutropenia on these results, because they are often unrealistically large.

Interestingly, γ_S has increased compared to the normal steady state in these fits as well, and unlike in the dogs, there is evidence here that it increased significantly compared to the steady-state values. This was not an anticipated effect of G-CSF treatment, but has emerged from these simulations in both the human and dog data. It would be of interest to systematically explore the dynamical behaviour of this model and its dependence on γ_S as well as on A_N and θ_1; however, such a study is beyond the scope of the current paper.

Plots of the data and the resulting fits for one of the patients are shown in Figs. 6 and 7. The human data were much more sparsely sampled than the dog data; we show not only the data and the full simulation, but the simulation sampled at the times when there was observed data. Note that this changes the appearance of the fit. The fitting procedure is of course unable to distinguish between simulations that differ primarily at points where there is no data. This accounts for the qualitative difference between the centre and right plots

Table 6
Parameter estimates for the untreated human patients, based on the simulated annealing method

Patient	A_N		f_0		A_P		$\bar{\kappa}_P$		γ_S		k_0		K_P	
	752.00		0.40		28.00		1.17		0.07		8		3–13	
104	10.7	(5.44)	0.33	(0.20)	5.36	(4.78)	1.97	(5.44)	0.06	(0.29)	1.29	(0.58)	6.4	(4.96)
39	23.2	(8.72)	1.05	(0.07)	10.41	(4.84)	2.02	(0.64)	0.06	(0.03)	1.32	(0.06)	7.6	(2.24)
42	12.8	(0.00)	2.22	(0.00)	133.00	(0.00)	0.24	(0.00)	0.09	(0.00)	2.42	(0.00)	10.3	(0.00)
43	93.2	(0.00)	0.20	(0.00)	5.64	(0.00)	2.93	(0.00)	0.05	(0.00)	0.88	(0.00)	4.7	(0.00)
50	7.2	(0.00)	0.68	(0.00)	13.23	(0.00)	0.77	(0.00)	0.07	(0.00)	1.44	(0.00)	4.0	(0.00)
69	48.1	(21.9)	0.08	(0.02)	3.95	(0.32)	0.52	(0.05)	0.01	(0.01)	4.11	(0.83)	0.2	(0.03)
25	35.7	(0.00)	0.50	(0.00)	42.07	(0.00)	0.31	(0.00)	0.04	(0.00)	0.68	(0.00)	1.2	(0.00)
54	38.0	(0.00)	0.71	(0.00)	18.69	(0.00)	0.39	(0.00)	0.07	(0.00)	1.83	(0.00)	6.2	(0.00)
102	11.7	(1.50)	0.11	(0.02)	36.61	(3.61)	1.20	(0.07)	0.02	(0.16)	0.60	(0.15)	5.8	(0.50)
105	9.2	(0.00)	0.06	(0.00)	39.43	(0.00)	0.54	(0.00)	0.06	(0.00)	2.98	(0.00)	6.1	(0.00)
108	58.9	(0.00)	0.15	(0.00)	48.73	(0.00)	0.91	(0.00)	0.07	(0.00)	0.55	(0.00)	3.2	(0.00)
114	12.3	(0.00)	0.10	(0.00)	27.12	(0.00)	0.15	(0.00)	0.07	(0.00)	1.54	(0.00)	1.6	(0.00)
32	27.8	(8.58)	0.11	(0.04)	37.69	(33.0)	0.32	(0.31)	0.02	(0.33)	1.01	(0.50)	1.6	(0.98)
4	10.4	(6.29)	0.30	(0.03)	17.69	(11.7)	1.19	(0.24)	0.07	(0.06)	3.64	(0.23)	4.3	(0.90)
60	23.7	(0.00)	0.62	(0.00)	17.47	(0.00)	0.54	(0.00)	0.07	(0.00)	1.12	(0.00)	2.1	(0.00)
66	12.6	(0.00)	0.57	(0.00)	13.18	(0.00)	1.22	(0.00)	0.07	(0.00)	1.35	(0.00)	4.0	(0.00)
95	16.0	(0.00)	0.45	(0.00)	21.73	(0.00)	1.09	(0.00)	0.07	(0.00)	2.01	(0.00)	4.2	(0.00)
97	4.9	(0.00)	0.26	(0.00)	24.06	(0.00)	2.80	(0.00)	0.01	(0.00)	0.68	(0.00)	8.9	(0.00)
103	12.0	(0.00)	0.72	(0.00)	6.23	(0.00)	1.56	(0.00)	0.01	(0.00)	1.14	(0.00)	2.3	(0.00)
58	25.3	(2.50)	0.99	(0.02)	22.24	(3.27)	1.23	(0.07)	0.09	(0.01)	1.22	(0.01)	5.7	(0.42)
7	6.9	(0.00)	0.51	(0.00)	8.74	(0.00)	4.06	(0.00)	0.00	(0.00)	1.10	(0.00)	6.5	(0.00)
77	2.5	(0.00)	0.60	(0.00)	12.79	(0.00)	1.32	(0.00)	0.06	(0.00)	1.63	(0.00)	3.5	(0.00)
78	10.5	(1.80)	0.37	(0.04)	21.81	(4.28)	1.15	(0.40)	0.15	(0.05)	2.45	(0.13)	6.2	(2.23)
80	68.8	(17.8)	0.03	(0.01)	43.56	(11.9)	0.43	(0.22)	0.04	(0.34)	0.63	(0.33)	2.6	(2.80)
24	4.2	(0.00)	0.37	(0.00)	26.18	(0.00)	0.76	(0.00)	0.08	(0.00)	1.08	(0.00)	4.3	(0.00)
26	24.5	(11.5)	0.12	(0.05)	36.26	(5.23)	0.94	(0.13)	0.05	(0.44)	0.95	(0.63)	3.1	(0.46)
3	23.9	(0.00)	0.41	(0.00)	49.79	(0.00)	0.42	(0.00)	0.00	(0.00)	0.84	(0.00)	2.8	(0.00)
Mean	23.5	(1.64)	0.27	(0.01)	28.37	(4.83)	1.00	(0.06)	0.05	(0.05)	1.55	(0.09)	4.2	(0.24)
St. Dev.	15.7	(3.14)	0.21	(0.02)	11.64	10.91	0.74	(0.12)	0.02	(0.11)	1.04	(0.18)	2.3	(0.45)

The top line of the table gives the normal values from Table 1 for comparison. Error estimates, in percent, are shown in brackets. The first group of patients were diagnosed with cyclical neutropenia, the second with congenital neutropenia and the third with idiopathic neutropenia.

in both of the figures. For example, in Fig. 6, the simulated platelet oscillations decrease below the observed values in the full simulation, but not in the sampled simulation on the right. Also in Fig. 6, the simulation of the neutrophils does not quite reach the observed maximum, as was the case for the dog data. However, the simulations are in the correct range both for the very small-magnitude oscillations of Fig. 6, and for the large-amplitude treated data of Fig. 7, though the fit of Fig. 7 is not precise (especially for the platelets).

5. Conclusions

Several general comments can be made about the model's ability to mimic the dynamic characteristics of cyclical neutropenia. Both the qualitative and quantitative features of untreated CN can be duplicated by the model. Not only were the periods and amplitudes of the all the data sets well-matched (c.f. Figs. 3 and 5), but the 'secondary bump' previously observed on the falling phase of the neutrophil counts in the untreated cyclical neutropenia cases (Haurie and Mackey, 2000) occurs in the simulations as well (see Fig. 6). In addition, the phase offset between the neutrophils and the platelets was in general duplicated by the model, though the platelet match was not as successful in Fig. 7 as in other cases. The model did not duplicate the qualitative change in the treated data for Dog 113 (Figs. 4 and 5), though this could probably be accomplished by allowing parameters to change with time. Also, it should be noted that there is noise in the data, which affects the expected fit quality of any model.

Further, with reference to Figs. 2 and 3, the model simulation was capable of generating platelet and

Table 7
Parameter estimates for the human patients treated with G-CSF, based on the simulated annealing method

Patient	A_N		θ_1		γ_S		τ_S		τ_{NM}	
	752.00		0.36		0.07		2.80		3.50	
104	651.7	(3.48)	0.80	(3.28)	0.45	(5.76)	1.08	(4.92)	10.52	(4.99)
39	1386.5	(51.91)	0.21	(4.69)	0.04	(6.88)	3.42	(10.47)	5.52	(1.22)
42	691.9	(100.08)	0.03	(105.47)	0.01	(100.00)	0.91	(98.00)	48.07	(3.27)
43	381.4	(0.35)	5.62	(0.89)	0.44	(0.70)	1.24	(0.60)	60.16	(4.24)
50	59.1	(44.51)	11.22	(98.00)	0.02	(18.75)	18.14	(1.10)	16.41	(13.53)
69	724.5	(9.1)	0.47	(2.15)	0.39	(0.94)	1.03	(0.59)	6.54	(0.39)
25	598.6	(10.83)	0.76	(23.07)	0.37	(10.41)	1.19	(7.41)	0.60	(7.47)
54	513.6	(5.15)	0.74	(4.91)	0.37	(9.12)	1.07	(7.53)	9.80	(7.15)
102	474.9	(18.92)	1.02	(7.14)	0.51	(4.80)	1.03	(0.51)	53.60	(2.58)
105	486.6	(1.53)	0.92	(3.89)	0.03	(4.06)	1.62	(1.34)	11.91	(0.31)
108	115.5	(4.14)	1.74	(4.64)	0.08	(5.00)	2.03	(1.88)	1.79	(7.61)
114	752.1	(4.23)	0.38	(28.87)	0.71	(1.06)	0.84	(0.15)	16.50	(0.52)
32	135.8	(5.75)	2.58	(4.80)	0.01	(95.0)	0.88	(2.25)	10.26	(3.71)
4	623.8	(16.47)	0.26	(17.81)	0.40	(0.7)	1.50	(0.34)	2.49	(9.11)
60	1503.7	(10.97)	0.51	(4.65)	0.54	(4.97)	0.89	(3.99)	7.60	(7.80)
66	468.8	(7.14)	2.33	(7.38)	0.78	(1.25)	0.76	(1.55)	13.57	(7.98)
95	851.6	(7.56)	0.51	(5.63)	0.02	(4.38)	12.51	(2.44)	3.41	(2.47)
97	888.2	(33.08)	0.05	(34.69)	0.97	(100.00)	0.09	(95.00)	17.40	(6.94)
103	791.5	(22.67)	1.14	(33.03)	0.71	(80.51)	0.37	(63.44)	10.57	(4.54)
58	136.1	(3.77)	0.82	(1.55)	0.03	(10.31)	1.22	(3.91)	34.57	(1.33)
7	681.0	(4.08)	0.40	(4.14)	0.02	(22.81)	2.23	(1.23)	6.37	(1.22)
77	913.2	(21.18)	0.12	(22.58)	0.03	(30.31)	3.76	(4.24)	25.01	(4.44)
78	670.0	(0.79)	0.77	(0.04)	0.18	(0.20)	1.51	(0.31)	2.22	(0.63)
80	629.9	(10.4)	2.00	(13.51)	0.10	(5.3)	1.71	(3.41)	0.11	(110.00)
24	659.8	(23.90)	0.61	(38.66)	0.62	(131.43)	0.26	(115.18)	17.07	(1.77)
26	558.9	(9.6)	0.21	(7.97)	0.00	(0.00)	3.68	(6.25)	4.43	(16.04)
3	1345.6	(27.73)	1.05	(8.89)	0.00	(90.00)	1.44	(8.07)	10.64	(6.49)
Mean	655.4	17.0	1.4	18.2	0.3	27.6	2.5	16.5	15.1	8.8
St. Dev.	357.1	21.2	2.3	26.6	0.3	40.5	3.9	33.4	16.1	20.6

The top line of the table gives the normal values from Table 1 for comparison. Error estimates, in percent, are shown in brackets. The first group of patients were diagnosed with cyclical neutropenia, the second with congenital neutropenia and the third with idiopathic neutropenia.

neutrophil variations with the observed period and relative phase between the platelets and the neutrophils. Of particular interest are the oscillations in the platelet line; the primary change of decreasing A_N to mimic an increase in apoptosis in the neutrophil line gives a good match for oscillations in the platelet line. This indicates that the model is successful in coupling the three cell lines through the same stem cell population, and in representing the effects of changes in one cell line on the others.

The features of the treated data were also duplicated fairly well. For example, it was difficult in previous models (Haurie and Mackey, 2000; Bernard et al., 2003) to obtain neutrophil oscillations matching the data that did not have a lower bound near zero, but one significantly above zero. In our model, this was achieved by the simulated annealing fit procedure (see Figs. 4 and 7).

Interestingly, however, we were not able to induce oscillations that quantitatively agreed with the observed data for untreated neutropenia by changing A_N alone with the other parameters as given in Table 1; other critical parameters that had to be decreased below normal to consistently obtain oscillations at the required amplitudes were k_0 and γ_S, both of which determine aspects of the stem cell regulation.

There were two distinct differences between the results for the neutropenic dogs and the human patients. In the dogs, the parameter estimates were in general less extreme than in the patients. For example, in Tables 2 and 3 for grey collies, the values of A_N found are approximately 245, just under half the steady-state value. For the untreated neutropenic patients, A_N was found to be only about 23, compared to the steady-state value of 752. This reflects the lower neutrophil levels in the human data. The increase in A_N with G-CSF

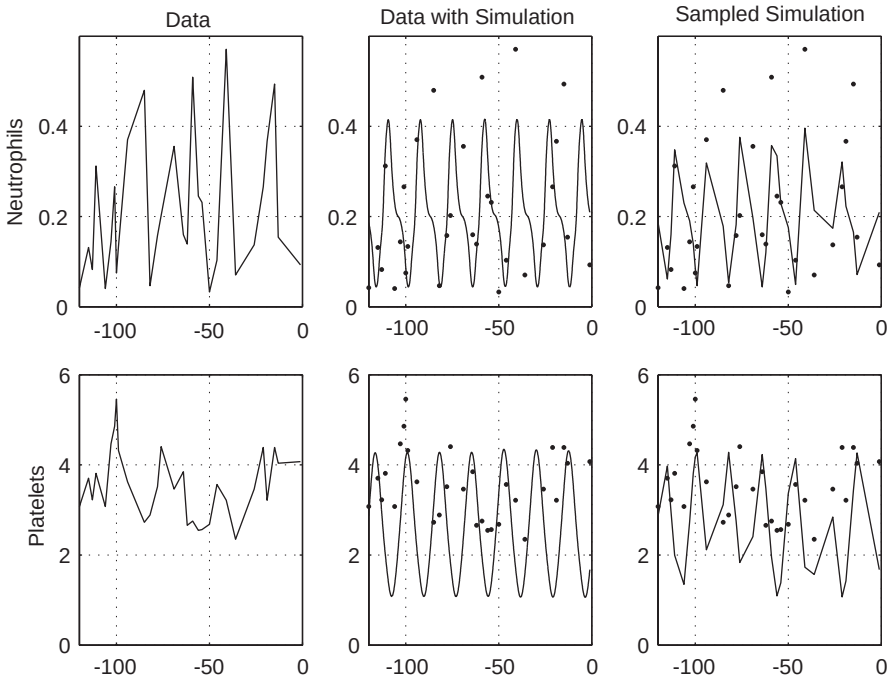

Fig. 6. Serial Neutrophil and Platelet Data: untreated Patient 114. The left plot shows the observed data, and the centre plot shows the data (points) with the simulation (solid line). The plot on the right shows the data with the simulation sampled at the times when the data were collected. Neutrophils are in units of $10^8\,\mathrm{kg}^{-1}$, while platelets are in units of $10^{10}\,\mathrm{kg}^{-1}$.

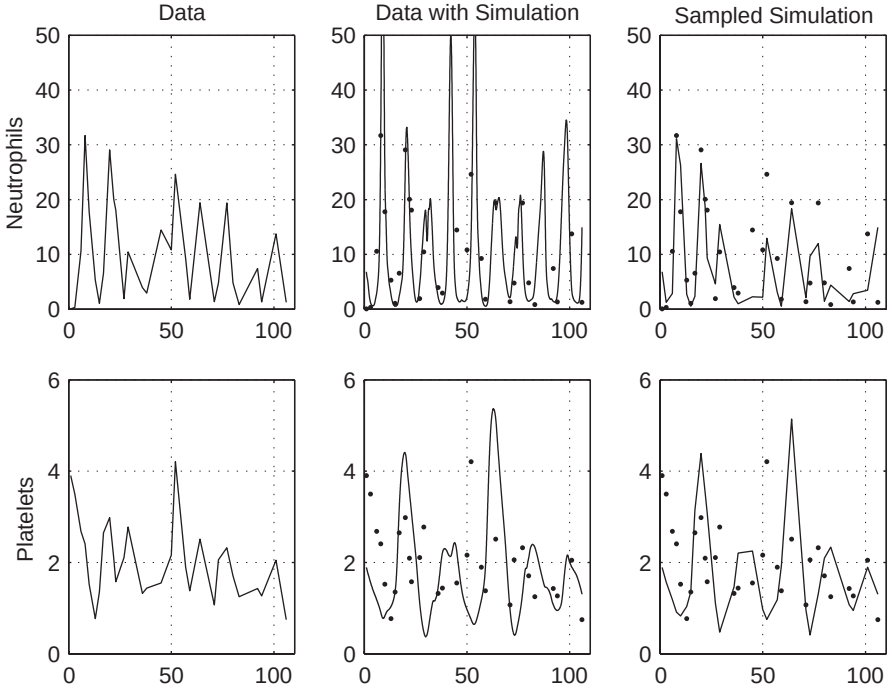

Fig. 7. Serial Neutrophil and Platelet Data: during G-CSF treatment, Patient 114. The left plot shows the observed data, and the centre plot shows the data (points) with the simulation (solid line). The plot on the right shows the data with the simulation sampled at the times when data were collected. Neutrophils are in units of $10^8\,\mathrm{kg}^{-1}$, while platelets are in units of $10^{10}\,\mathrm{kg}^{-1}$.

treatment was correspondingly much higher in the humans than it was in the dogs, as in both cases G-CSF raised A_N to approximately its steady-state value. In keeping with the more extremal changes in the humans compared to the dogs, the increase in γ_S with G-CSF treatment occurred in both, but was notably

more pronounced in humans. It is not clear what biological mechanism could link an increase in apoptosis in the hematopoietic stem cells with G-CSF, but the regulatory kinetics of HSCs are not entirely well-understood.

The results presented here lend increased credibility to the hypothesis that the origins of oscillation in cyclical neutropenia are a destabilization in the stem cell compartment, induced by changes in the neutrophil line; the oscillations are then transmitted to the other lines. In our model simulations, the decrease in A_N relative to the normal value is the most significant change recorded in our successful attempts to fit data in untreated neutropenia, and the increase in A_N is the most significant effect of G-CSF seen in our model.

Though the change in A_N takes place in the neutrophil line, the consequent destabilization of the stem cell compartment leads to oscillations that are transmitted to the other lines. This lends support for the hypothesis that oscillations in the stem cells drive the oscillations in cyclical neutropenia, as opposed to oscillations originating in a peripheral control loop regulating the neutrophil production.

To explore this hypothesis further, we decoupled the stem cell compartment from the neutrophils, platelets and erythrocytes, and ran simulations with each of the parameter sets found for untreated cyclical neutropenia, to determine whether oscillations were occurring in the (now isolated) stem cell loop. In 16/27 human patients, and 6/9 dogs, oscillations did occur, at approximately the correct periods. For the others, a slight decrease in f_0, the constant scaling the differentiation rate into the neutrophil line, recovered the oscillations. This result further supports the hypothesis that oscillations in cyclical neutropenia are connected with a bifurcation in the dynamics of the hematopoietic stem cell compartment. Essentially, there is a bifurcation in the stem cell compartment with respect to f_0, near the points in parameter space found in our simulations of cyclical neutropenia. The coupling of the other compartments affects the position of this bifurcation point, so that when those compartments are removed, some of the parameter sets are just beyond that point and do not oscillate.

In summary, compared to the normal steady-state values, we found that the parameter changes that mimic untreated cyclical neutropenia are a decreased A_N and k_0. A biological interpretation of these two changes would be a decreased amplification (increased apoptosis) within the proliferating neutrophil precursor compartment, and a decrease in the maximal rate of re-entry into the proliferative phase of the stem cell compartment. It was also necessary to vary the parameter γ_S to simulate cyclical neutropenia. Though it was not on average much different from the steady-state value, γ_S affects the period of oscillations, and particularly in the human data there was quite a bit of variability in the period.

For the data obtained during G-CSF treatment, good fits were obtained only when A_N and θ_1 were increased significantly. This would imply that G-CSF led to higher amplification (lower rate of apoptosis) in the proliferating neutrophil precursors, and there was on average a higher rate of differentiation into the neutrophil line than without the treatment. The apoptosis rate γ_S in the stem cell compartment increased significantly in many of our simulations, though not all; however, the mean rose from 0.05 to 0.3 days^{-1}. The duration of the proliferative phase of the stem cell cycle, τ_S, was also decreased, but not significantly, in our simulations of G-CSF treatment.

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