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Cost-effective G-CSF therapy strategies for cyclical neutropenia: Mathematical modelling based hypotheses

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Abstract

Using computer simulations of a mathematical model for the regulation of stem cell and neutrophil production in dogs, we have studied the efficacy of four different treatment protocols for cyclical neutropenia involving granulocyte colony stimulating factor (G-CSF). The first treatment scheme is based on the bifurcation analysis of the mathematical model and proposes a daily, phase-dependent, protocol. The second involves alternate day administration of G-CSF. The third triggers G-CSF administration whenever neutrophil levels fall below a predetermined level, and the fourth one follows a random administration protocol. The computer simulations predict that clinically desirable results can be achieved with the three last methods, using far less G-CSF than would be needed with the standard daily treatment. If the results of this modelling are borne out clinically, they will entail a considerable financial savings for patients.

Keywords: Cyclical neutropenia; G-CSF treatment; Mathematical modelling; Non-standard treatment

1. Introduction

Cyclical neutropenia (CN) is a rare haematological disease characterized by oscillations in the circulating neutrophil count. These levels fall from normal to barely detectable levels with a typical period of 19–21 days in humans (Guerry et al., 1973; Dale and Hammond, 1988; Haurie et al., 1998), even though periods up to 40 days have been observed (Haurie et al., 1998). These oscillations in the neutrophil count are generally accompanied by oscillations around normal levels in other blood cell lineages such as platelets, lymphocytes and reticulocytes (Haurie et al., 1998, 2000). All grey collies (Lund et al., 1967) are born with this congenital disease and oscillations with period on the order of 11–16 days are observed (Haurie et al., 1998, 1999a,b, 2000). This animal model has provided extensive experimental data to decipher the nature of CN.

A number of mathematical models have been proposed to explain the origin of these oscillations as well as to understand the control of neutrophil production in non-pathological cases. For a survey of previous models, see Hearn et al. (1998) and Haurie et al. (1998). In a recent mathematical modelling study (Bernard et al., 2003), it was shown that all of the characteristics of CN in the grey collie can be accounted for by an elevated level of cellular death (apoptosis) in the neutrophil precursors. This elevation of apoptosis in CN was first predicted from modelling considerations (Mackey, 1978) and has been observed clinically (Aprikyan et al., 2001). The modelling predicts that decreasing the level of

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apoptosis in neutrophil precursors can attenuate or eliminate many of the symptoms of CN.

CN is often treated using granulocyte colony stimulating factor (G-CSF) (Hammond et al., 1989) which is known to interfere with apoptosis (Koury, 1992; Park, 1996; Williams et al., 1990; Williams and Smith, 1993). Treatment protocols typically call for daily subcutaneous injection of G-CSF at 3–5 μg per kg of body weight (American Society of Clinical Oncology, 1997; Dale et al., 2003). For a 60 kg adult this currently entails a yearly cost of approximately US$ 40,000. Clearly, it would be of enormous help if the same clinical effects could be achieved with the use of less G-CSF. A few alternative treatment strategies have been reported in which various administration schemes have been used (Jayabose and Sandoval, 1994; Dicato et al., 1992; Danielson and Harmenberg, 1992; Dale et al., 2003), yet no attempt to use current knowledge of the dynamic behaviour of the haematopoietic system for designing new treatment protocols has apparently been made.

This paper uses the recent model of Bernard et al. (2003) to examine, through computer simulations, the predicted effects of different protocols for the administration of G-CSF to grey collies. The goal is to find ways of administering G-CSF less frequently than daily and still achieve the same clinical effect reached using a daily regimen. In Section 2, we describe the model for CN and its dynamic characteristics and we explain how the model can mimic the effects of CN and G-CSF treatment. The third section presents the bifurcation analysis of the model while varying the parameters from untreated CN to the set of parameters representing the effects of the G-CSF treatment on a CN patient. An interesting bistability region exists for certain parameter values, suggesting that the oscillations seen in CN could be stopped by appropriately perturbing the system. In Section 4, we illustrate the computer predicted results of four different schemes for the administration of G-CSF. The first uses the bistability property to design a daily phase-dependent protocol. The second involves giving G-CSF every other day. The third is a "threshold triggered decision" in which G-CSF is administered whenever the neutrophil count falls below a predetermined value. Finally, the fourth scheme is a random delivery protocol. The last three protocols use less G-CSF than the daily standard treatment. The paper concludes with a brief discussion in Section 5.

2. The model

2.1. The model equations

The model for neutrophil production consists of a system of two delay differential equations (DDEs). Each equation considers the net production and loss rates (termed $P(t)$ and $L(t)$, respectively) of haematopoietic stem cells and mature neutrophils

$$\frac{dS(t)}{dt} = P_S(t) - L_S(t), \tag{1}$$

$$\frac{dN(t)}{dt} = P_N(t) - L_N(t). \tag{2}$$

The net production rates are

$$P_S(t) = 2e^{-\gamma_S \tau_S} S(t-\tau_S) K(S(t-\tau_S)), \tag{3}$$

$$P_N(t) = AS(t-\tau_N) F(N(t-\tau_N)). \tag{4}$$

The delayed arguments account for the time required for cell division cycles and maturation to occur. The net loss rates are

$$L_S = S(t)[F(N(t)) + K(S(t))], \tag{5}$$

$$L_N = \alpha N(t). \tag{6}$$

The variable S represents the number of haematopoietic stem cells in the resting (G_0) phase. Cells in the resting phase can either enter the proliferative phase at a rate $K(S)$ or differentiate at a rate $F(N)$ to ultimately give rise to mature neutrophils, represented by the second variable, N. Cells in the HSC proliferative phase undergo apoptosis at a rate γ_S and the cell cycle duration is τ_S. The fraction of HSC surviving cell division is then $\exp(-\gamma_S \tau_S)$. Successive divisions amplify cell numbers in the differentiation pathway by a factor A, which is also used to account for cell loss due to apoptosis. After a time τ_N, each differentiated HSC gives rise to A mature neutrophils N, which are released into the circulation. It is assumed that mature neutrophils are eliminated at a rate α (Fig. 1).

Two feedback loops control the entire process through the neutrophil differentiation rate $F(N)$ and the HSC proliferation rate $K(S)$. G-CSF mediates neutrophil differentiation through a clearance mechanism. When the neutrophil count decreases, G-CSF levels increase due to the lack of clearance and differentiation increases. As a function of the neutrophil count, the differentiation rate is thus monotonically decreasing (here G-CSF is implicitly taken into account), and is described by a monotone decreasing Hill function,

$$F(N) = f_0 \frac{\theta_1^n}{\theta_1^n + N^n}, \tag{7}$$

where f_0 is the maximal differentiation rate (i.e. in the absence of neutrophils), n is a cooperativity coefficient and θ_1 is the neutrophil concentration at which differentiation is half-maximal. Notice that θ_1 depends on endogenous G-CSF production and G-CSF administration. HSC proliferation is assumed to be regulated through a similar mechanism. When the HSC level is depleted, intercellular signals trigger cellular replication. These signals decrease when HSC levels increase back to normal levels. The HSC

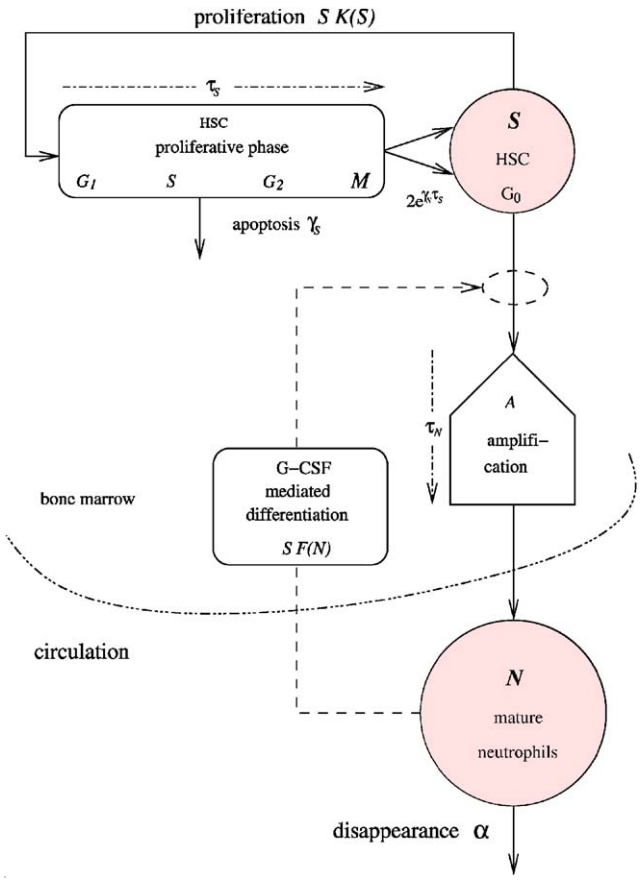

Fig. 1. Model of neutrophil production. The variable S represents the number of haematopoietic stem cells in the resting (G_0) phase. Cells in the resting phase can either enter the proliferative phase at a rate $K(S)$ or differentiate at a rate $F(N)$ to ultimately give rise to mature neutrophils N, the second variable. Cells in the HSC proliferative phase undergo apoptosis at a rate γ_S and the cell cycle duration is τ_S. Successive divisions amplify cell numbers in the differentiation pathway by a factor A, which is also used to account for cell loss due to apoptosis. After a time τ_N, differentiated cells become mature neutrophils N and are released into the blood. It is assumed that mature neutrophils die at a fixed rate α. Two feedback loops control the entire process through the proliferation rate $K(S)$ and the differentiation rate $F(N)$.

proliferation rate feedback term is thus also described by a monotone decreasing Hill function,

$$K(S) = k_0 \frac{\theta_2^s}{\theta_2^s + S^s}. \quad (8)$$

The parameter k_0 is the maximal proliferation rate, s is a cooperativity coefficient and θ_2 the HSC concentration at which HSC proliferation is half-maximal. See Bernard et al. (2003) for a detailed derivation of the feedback functions.

2.2. Simulating CN and G-CSF

Bernard et al. (2003) showed that their model can replicate the characteristics of CN and the effects of G-CSF administration by using physiologically relevant values for the 11 different parameters. The parameters used for numerical simulation are in agreement with experimental data in dogs and are presented in Bernard et al. (2003). There they concluded that the cause of the oscillations in CN is a destabilization in the HSC regulatory system due to an elevated apoptotic rate in the neutrophil precursors. Since the amplification parameter A accounts for the cell loss due to apoptosis, the onset of CN is mimicked by decreasing the parameter A. Also, mimicking the effects of G-CSF treatment on a cyclical neutropenic dog involves modifying five parameters. First, G-CSF was shown to decrease apoptosis in both the HSC (decrease of γ_S) and the neutrophil precursors (increase of A). Second, it increases the differentiation rate F by increasing θ_1, which is proportional to the production of G-CSF. Finally, G-CSF decreases the HSC proliferative phase duration τ_S as well as the neutrophil precursors transit time τ_N. Bernard et al. (2003) showed that the effects of G-CSF can be reproduced with the model when G-CSF administration is simulated with these five changes in the model parameters. Table 1 presents the parameter values used to reproduce neutrophil production in three situations of interest: in a healthy dog (without CN), in a dog suffering from CN, and a dog under G-CSF treatment. For more details on the estimation of these parameters, see Bernard et al. (2003).

Table 1
Estimated model parameters used for numerical simulations of the normal state in dogs, cyclical neutropenia in grey collies, and during daily G-CSF treatment. Values from Bernard et al. (2003)

Parameter	Unit	Health	CN	G-CSF
A	100	380	10	20
f_0	day^{-1}	0.8	0.8	0.8
θ_1	10^8 cell/kg	0.36	0.36	0.8
k_0	day^{-1}	8.0	8.0	8.0
θ_2	10^6 cell/kg	0.095	0.095	0.095
n	—	1	1	1
s	—	2	2	2
τ_N	day	3.5	3.5	3.0
τ_S	day	2.8	2.8	2.6
γ_S	day	0.07	0.07	0.05
α	day^{-1}	2.4	2.4	2.4

3. Analysis of the model

3.1. Bifurcation analysis

As presented in Section 2.2, there are three sets of parameters of interest characterizing a healthy state, the CN state, and CN during G-CSF therapy. Going from a healthy state to a CN state involves a decrease in the

amplification parameter A. The stability analysis of this case was presented in Bernard et al. (2003). In this study, we are interested in the effects of the G-CSF treatment on CN subjects and, hence, we carry out a bifurcation analysis while varying the parameters from untreated CN to the set of parameters representing the effects of the G-CSF treatment on a CN dog. Mimicking G-CSF treatment with the model involves the change of five parameters: the neutrophil amplification A, the HSC apoptosis rate γ_S, the parameter θ_1, the cell cycle duration τ_S and the maturation transit time τ_N (see Table 1).

Performing a bifurcation analysis with respect to five parameters is difficult to carry out and interpret. Therefore, we instead assume that the five parameters vary linearly between the untreated CN state and the G-CSF treated CN values. We use a new parameter T and parameterize the five parameters as a function of T such that $T = 0$ and 1 correspond to untreated CN and G-CSF treated CN, respectively. More precisely, using the values from Table 1, we parameterize A, θ_1, γ_S, τ_N and τ_S with respect to T as follows:

$$A(T) = 10(1 - T) + 20T,$$

$$\theta_1(T) = 0.36(1 - T) + 0.8T,$$

$$\gamma_S(T) = 0.07(1 - T) + 0.05T,$$

$$\tau_N(T) = 3.5(1 - T) + 3T,$$

$$\tau_S(T) = 2.8(1 - T) + 2.6T.$$

Then we perform the bifurcation analysis with respect to the parameter T. Increasing T is associated with increasing the concentration of G-CSF.

For the analysis presented in this section, we have used `DDE-BIFTOOL`, a `Matlab` package for bifurcation analysis of DDEs (Engelborghs et al., 2001). The complete bifurcation diagrams for N is presented in Fig. 2. The envelope of periodic solutions is shown by thick lines and steady states are represented by thin lines (dashed and solid lines are for unstable and stable solutions, respectively).

In Bernard et al. (2003), it was shown that the mathematical model has only one positive steady state (SS) under physiologically relevant conditions. The stability of this SS depends on the values of the different parameters of the model. For $T = 0$ (CN), the positive SS is unstable. When T is increased towards 1, the SS becomes stable at $T = 0.63$ (point O in Fig. 2), where a supercritical Hopf bifurcation occurs. At the Hopf point, point O in Fig. 2, a small amplitude stable limit cycle is created. This stable limit cycle exists only for values of T between 0.5324 and 0.6362. At point I ($T = 0.5324$), the limit cycle disappears through a reverse saddle-node bifurcation of limit cycles. Another unstable limit cycle (points I–II) also disappears at this point, which had previously appeared at $T = 1.3647$ through a saddle-node bifurcation of limit cycles, together with a large amplitude limit cycle.

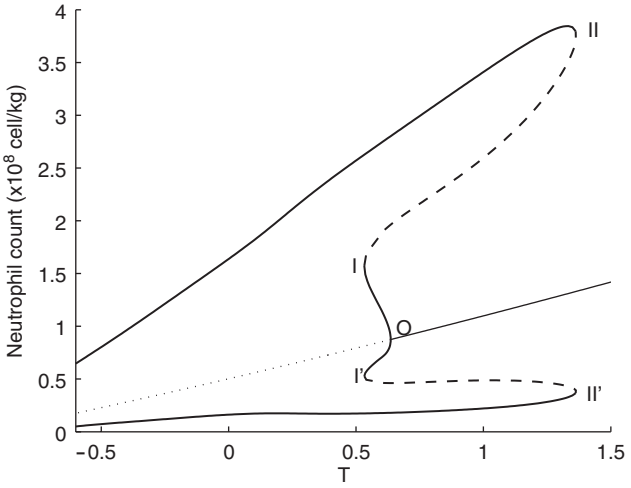

Fig. 2. Bifurcation diagram for N with respect to the new parameter T where the envelope of the solution and the fixed point are shown. A Hopf bifurcation occurs at point O ($T = 0.6362$), where the stability of the fixed point is changed and a small stable periodic orbit is created. This stable limit cycle exists between $T = 0.5324$ and $T = 0.6362$. It disappears at point I ($T = 0.5324$), through a reverse saddle-node bifurcation, together with an unstable limit cycle. This unstable limit cycle exists for T between 0.5324 (points I, I') and 1.3647 (points II, II'), where it was created through a saddle-node bifurcation. The latter also created a stable limit cycle that exists when $-0.7 \leqslant T \leqslant 1.3647$. There is bistability for T between 0.6362 and 1.3647.

3.2. Interpretation

At $T = 0$, the SS is unstable and the only stable solution is the limit cycle. Hence, the oscillations in the neutrophil count are inevitable. This agrees with the characteristic behaviour seen in CN. As T is increased towards 1, the amplitude of the stable limit cycle increases and the period of the large amplitude limit cycle decreases (see Fig. 3).

This also agrees with the experimental data. Daily G-CSF administration is generally associated with an increase of the amplitude and a decrease of the period of the oscillations (Haurie et al., 1999a,b). However, it has also been reported that in some isolated cases G-CSF therapy abolished significant oscillations (Haurie et al., 1999a,b; Hammond et al., 1989). Interestingly, the model accounts for the annihilation of the oscillations under G-CSF treatment. Indeed, for $T \geqslant 0.6362$, and in particular at $T = 1$, two stable solutions coexist and therefore two types of behaviours can occur (SS solution and large oscillations). This bistability suggests that by wisely designing a treatment procedure, it might be possible to manipulate the system into a region of parameter space corresponding to a stabilization and effectively use it to reduce the amount of G-CSF

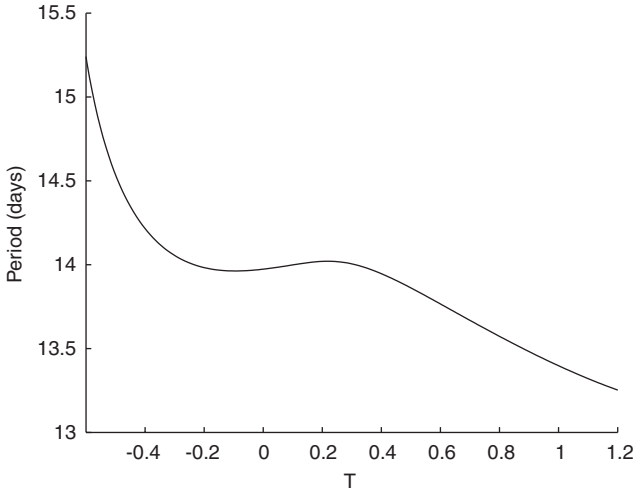

Fig. 3. Period of the stable limit cycle as a function of T.

required in treatment. The goal of this study is to use the dynamics of the model to design cost effective treatment schemes for CN. Section 4 presents different schemes of G-CSF administration, and as expected the bistability present in the G-CSF parameter set plays an important role.

4. The effects of non-standard G-CSF delivery protocols

In this section, we detail the results of computer simulations of a the mathematical model to study four non-standard G-CSF treatment protocols for CN. We first present the technical details of the numerical simulations and explain how we evaluated the efficacy of the different protocols. In Section 4.3, the simulated results for daily administration of G-CSF are reported. The bistability present in the G-CSF parameter space is used to design a phase-dependent protocol. Then, the three subsequent sections are devoted to the presentation of three non-standard G-CSF treatment protocols (namely the alternate day, the level-dependent and the random protocols) that use less G-CSF than the standard treatment.

4.1. Numerical methods

Numerical simulations were performed using the DDE solver `dde23` (Shampine and Thompson, 2001) for `Matlab`, which solves DDEs with constant delays. Mimicking G-CSF injection in a CN subject was done by changing the corresponding sets of parameters. Recall that five parameters were modified under the influence of G-CSF: A, γ_S, θ_1, τ_S and τ_N. To solve a DDE system, an initial (history) function needs to be specified. This function plays an important role and completely determines the eventual solution. In this case, we were interested in the effects of G-CSF treatment on CN subjects, and so the history function had to be an approximation of the solution in the CN case. We first computed the solution with the CN parameters for a transient period of 200 days. This allowed the system to settle down to the stable periodic solution and gives a good approximation of the behaviour in the CN situation. Then, administration of G-CSF was simulated by solving the system using the set of parameters corresponding to G-CSF. For instance, to simulate the alternate day treatment (where G-CSF is given every two days), we first integrated the DDE system using the CN parameters for one day. Then, we integrated the equations using the G-CSF parameters for the next day, switching from one parameter set to the other during the simulated treatment period. The values of the delays are modified to mimic G-CSF by assuming that the effects of G-CSF are immediate when injected, and that those effects last exactly one day before disappearing completely and instantaneously. The alternative would be to use a variable delay, but given the absence of detailed information connecting G-CSF pharmacokinetics and alterations in the delays, we have chosen the simpler alternative.

4.2. Investigation administration patterning

During the period of neutropenia (when the absolute neutrophil count (ANC) is below 0.38×10^8 cell/kg), the body is more vulnerable to bacterial and fungal infections. In investigating different patterning of G-CSF administration, the number of days of neutropenia in a cycle will serve as a benchmark for evaluating efficiency of different treatment strategies.

The estimated parameter values are for dogs and the cycle period for the CN parameters is about 14 days (see Fig. 3). Knowing that there is a bistability for the G-CSF parameters (see Section 3), we suspect that the history of neutrophil and HSC levels could have an effect on the results. Therefore, for each scheme, computer simulations were performed for every starting day in the cycle (14 runs for each administration scheme). In each case, we simulated a treatment period of 70 days (5 cycles of 14 days). The average number of neutropenic days, as well as the average number of treatment are reported in Table 2.

4.3. Phase-dependent protocol (daily)

In Section 3, we demonstrated that there is a bistability in the G-CSF parameter space. Thus, it is not surprising that computer simulations predict that daily administration of G-CSF could either lead to large amplitude oscillations with reduced period of neutropenia, or stabilization of the absolute neutrophil count

Table 2
Summary of the results of the three different protocols. Values represent means, and standard deviations are in parentheses. For the level dependent protocol, levels are in units of 10^8 cell/kg

Protocol	Neutr. days (%)	Days of treatment (%)
No Treatment	34.3	0.0
Daily	3.2 (3.4)	100.0 (0.0)
Stabilization	0.0 (0.0)	100.0 (0.0)
Oscillations	5.6 (2.5)	100.0 (0.0)
Alternate Day	4.4 (4.2)	50.0 (0.0)
Stabilization	0.0 (0.0)	50.0 (0.0)
Oscillations	7.3 (2.8)	50.0 (0.0)
Level Dependent		
level = 0.5	6.3 (2.6)	37.9 (2.6)
level = 0.7	3.0 (2.5)	47.6 (2.3)
level = 0.9	1.1 (1.7)	53.8 (4.5)
Random		
50% Treat. D.	13.0 (7.5)	50.0 (0.0)
70% Treat. D.	7.9 (4.1)	70.0 (0.0)

(ANC) (average ANC of 1.1×10^8 cell/kg, see Fig. 4). Since this stabilization is above the neutropenic level, it would constitute a desirable solution. We found that the type of response (stabilization or oscillations) depends on the history of neutrophil level on the first day of treatment. This suggests that the starting date of the treatment will affect the behaviour of the neutrophil count. For daily and alternate day therapies, this bistability can be characterized in terms of the starting day of the treatment (see Fig 5.) and both behaviours (stabilization and oscillations) are distinguished in Table 2. The treatment would be optimal when the mathematical model predicts a stabilization of the ANC. From our numerical simulations, if day 0 represents the day in the cycle at which the ANC nadir occurs, the optimal phase for starting the treatment is between day 3 and day 8 for a cycle length of 14 days. Moreover, the model predicts that starting the treatment during the neutropenic phase (days 0–1 and days 12–13) will lead to large amplitude oscillations.

4.4. Alternate day delivery

In the every-other-day therapy, the model predicts that the blood neutrophil level when G-CSF administration starts (and hence, the starting day of the treatment) also has an effect on the behaviour of the neutrophils. However, because the treatment is interrupted every other day the behaviour of the neutrophil count is influenced by the ANC every time G-CSF is injected. Consequently, a more erratic response results, but having the same general behaviour, and we can still distinguish a stabilization and an oscillatory phase (see Fig. 6 which shows a predicted stabilization

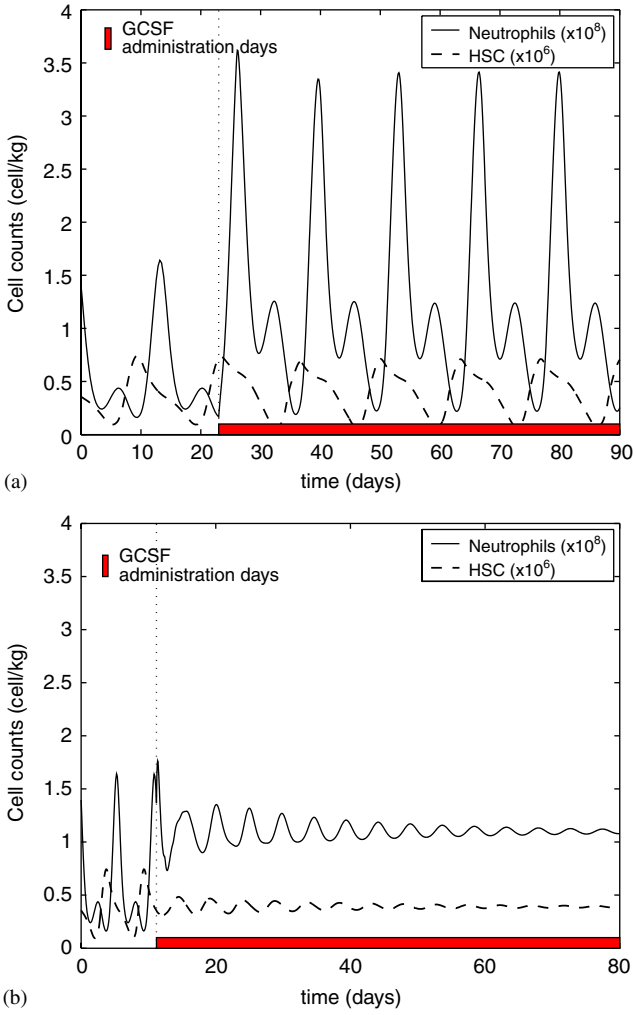

Fig. 4. Daily G-CSF treatment: two possible behaviours can be seen. (a) Large amplitude oscillations. (b) Stabilization of an oscillatory neutrophil count when starting the daily treatment right after the beginning of the falling phase of oscillation.

behaviour following the cessation of treatment). In this case, the solution is said to be in the stabilization phase if the ANC remains between 0.5 and 1.75×10^8 cells/kg. The range of ideal starting days is as broad as for daily G-CSF administration, and this scheme also efficiently increases the nadir of the neutrophil level and shortens the period of neutropenia. Therefore, it seems to achieve positive results similar to daily delivery of G-CSF. In particular, one can see from Table 2 that the results for alternate-day treatment during the stabilization phase are better than the results for the oscillating period of the daily scheme. This suggests that if one is able to localize the stabilization phase for a subject, then starting every-other-day treatment in this period would give results as good as continuous G-CSF administration in the oscillatory phase. And more importantly, it would use half the amount of G-CSF.

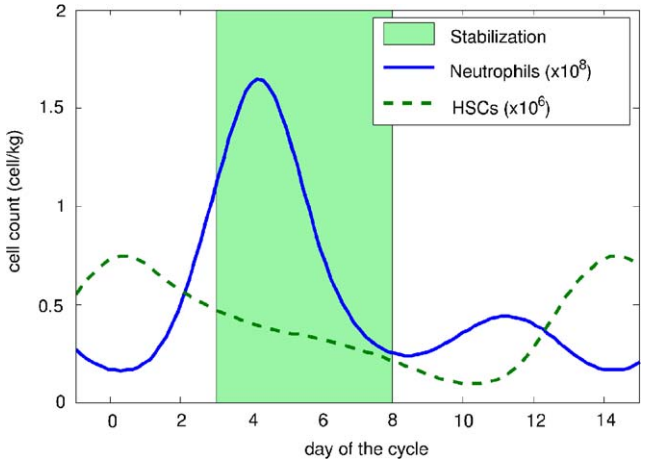

Fig. 5. Effect of starting day of treatment for cycles with a period of 14 days using the daily and alternate day schemes. Ideal starting day of treatment (in the stabilization phase) is between day three and day eight.

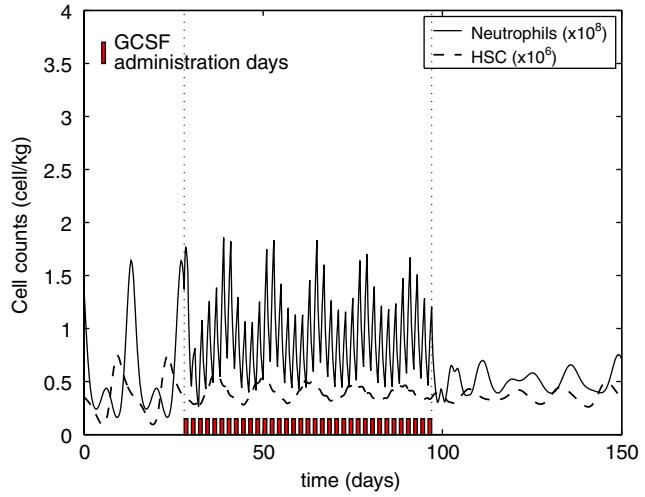

Fig. 6. Simulation of the ANC and HSC count using an alternate day regimen, representing a stabilization (the ANC remains between 0.5 and 1.75×10^8 cells/kg). Note the low-level neutrophil oscillations following the cessation of treatment.

4.5. Threshold triggered delivery

To determine the effects of threshold triggering drug delivery, we simulated G-CSF administration whenever the ANC falls below a predetermined level. Results for levels 0.5, 0.7 and 0.9×10^8 cell/kg are shown in Table 2. The bistability in the parameter space was still present in the results, but there was no obvious distinction between a stabilization and oscillatory phase as a function of starting day of treatment, as for the alternate day and daily schemes (see Fig 7.). This protocol targets specific days when G-CSF is needed and, as a result, it seems that its efficiency is good with respect to both number of neutropenic days and number of treatment days,

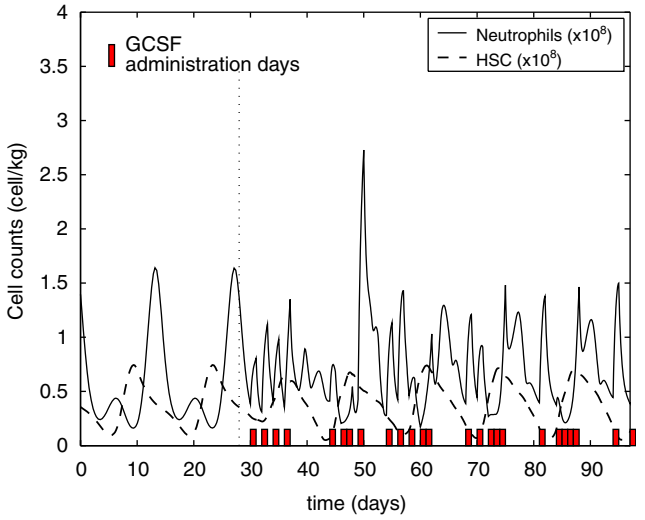

Fig. 7. Simulation of the ANC and HSC count using a neutrophil level dependent regimen (threshold level = 0.5×10^8 cell/kg). Over a treatment period of 70 days, there were 35.7% days of G-CSF administration and 5.7% days of neutropenia.

especially for high threshold levels (see for instance level 0.9×10^8 cell/kg in Table 2). Although this scheme presents very interesting theoretical results, it is impractical to implement, since it would require frequent blood work.

4.6. Random protocol

The results obtained with the level-dependent scheme suggest finding an alternative scheme that would give similar results, but that would be more easily implemented. We consider a random protocol in which G-CSF is administered randomly for a predetermined number of days. In particular, we use the number of days of administration obtained in the level-dependent method as a first indicator (average of 53.8% of treatment days for level 0.9×10^8 cell/kg). However, because the random protocol is not as targeted as the level-dependent scheme, it appears that the number of days of treatment must be higher than for threshold triggered therapy to achieve analogous results. We simulated random administration of G-CSF during 70 days for two cases (50% and 70% of treatment days) (see Fig. 8). Because the random protocol does not necessarily administer G-CSF on the first day of treatment, we only made simulations for starting day 0 and 5 (in the oscillatory and stabilization phase, respectively). In each case, we carried out 12 runs and averaged the results, presented in Table 2. The protocol with 50% of treatment days did not give good results. However, the results for the protocol with 70% of treatment days give similar results as the alternate day therapy. Table 2 gives a summary of all the methods and their results.

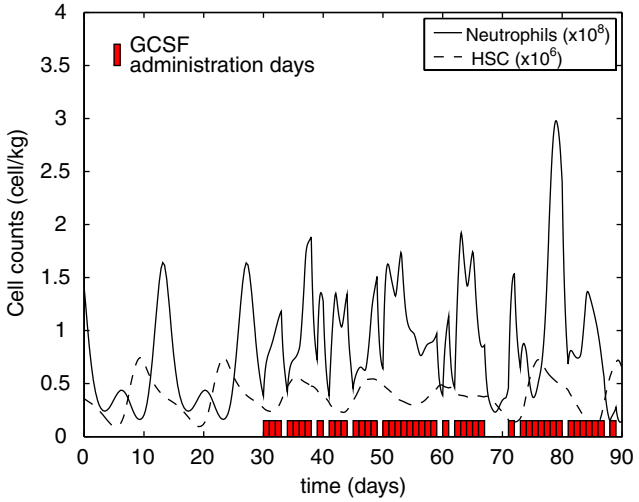

Fig. 8. Example of the effects of the random protocol with 70% of treatment days.

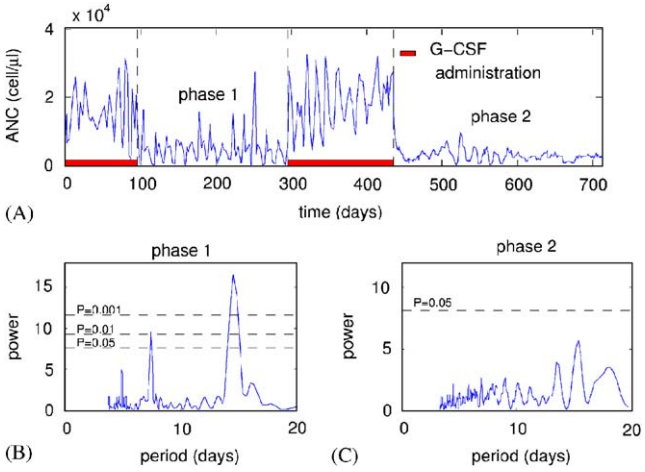

Fig. 9. (A) Absolute neutrophil count from a grey collie (Oprah, data generously supplied by Prof. David C. Dale). The red bars show periods of continuous G-CSF treatment. Notice the periodicity in Phase 1 versus Phase 2. (B) and (C) show the corresponding Lomb periodograms of Phases 1 and 2, respectively.

5. Discussion

CN has been extensively modelled in dogs due to its interesting dynamics and the availability of relatively abundant data for grey collies who have this disease. However, there have been no attempts to use its dynamic features to investigate and improve existing treatment strategies.

Using a mathematical model based on canine data, we first carried out a bifurcation analysis of the model. For a wide parameter range, the system displays bistability. In this case, depending on the *history* of neutrophil and HSC levels, the neutrophil count may either oscillate or stabilize to a SS. This bistability phenomenon suggests a potential neutrophil count, or phase-dependent treatment, dependent on neutrophil levels, as we have discussed in Section 4.3. Second, we have provided a computer simulation study of three treatment protocols that achieve essentially the same results as daily administration of G-CSF, but with smaller total G-CSF levels and/or fewer days of administration. The first treatment scheme is the alternate day (or every-other-day) regimen, which increases the nadir of the neutrophil level to clinically acceptable levels. The bistability in the system was still present for this scheme, suggesting a phase-dependent alternate day treatment. The second proposed treatment protocol, neutrophil level-dependent administration, in its current form would be difficult to implement, but an alternative random treatment scheme could easily be designed to achieve similar results. Mimicking the G-CSF administration pattern that was obtained by computer simulation is a possibility that should be considered. However, because there may be subtle differences between the grey collie model and the human disease, clinical trials will be necessary to establish efficacy of the treatments in humans.

Although there is no experimental evidence that bistability really occurs in CN patients, some experimental records (Haurie et al., 1999a,b) display different responses to G-CSF treatment applied at different times which could be indicative of bistability. Fig. 9 shows the ANC from a grey collie over 712 days. Two phases of continuous G-CSF treatment were applied (day 0–96 and day 296–436). The two phases without treatment show significant differences in the amplitude of oscillation of the ANC and periodicities. Spectral analysis using the Lomb periodogram (a generalization of Fourier spectral analysis for unevenly sampled data) has proven to be useful for analysis of periodic haematological diseases (Fortin and Mackey, 1999; Haurie et al., 1999a,b). We applied the Lomb periodogram technique to the two phases without treatment. Phase 1 (day 96–296) has a period of 14.6 days with strong significance $p \leqslant 10^{-5}$. Phase 2 also shows some periodicity to the eye (period 15.4 days) but it is not significant. This clear change in the neutrophil dynamics may indicate that the second G-CSF treatment stabilized the neutrophil level, whereas the first phase of treatment did not. Bistability, if it exists, suggests that the neutrophil count can either display periodic fluctuations with large amplitude or stabilize around an average value. In this paper, based on computer simulations we showed how the timing of the treatment affects the behaviour of the ANC.

A second potential indication of the existence of bistability in the system is provided by some experimental results on severe chronic neutropenia (SCN) (Haurie et al., 1999a,b). SCN is characterized by a persistent ANC below 0.38×10^8 cell/kg. It includes congenital neutropenia (ConN), which is usually recognized soon after birth; idiopathic neutropenia (IN),

which is acquired during childhood or adulthood; and cyclical neutropenia (CN), which is characterized by regularly occurring episodes of severe neutropenia.

We hypothesize that non-cycling SCN (ConN and IN) corresponds to a T value in the bistable region ($0.5324 \leqslant T \leqslant 1.3647$) when the solution stands at the SS (see Fig. 2). However, by perturbing the system, it would be possible to induce oscillations. This is in agreement with clinical results reported by Haurie et al. (1999a,b), in which occurrence of oscillations in the ANC of CN, ConG and IN patients before and during treatment with G-CSF was observed. In particular, their results showed that the occurrence of significant cycling in the ANC of neutropenics not classified as cyclical was much more prevalent than had been previously thought, and that not all the patients classified as cyclic showed significant ANC periodicity. Moreover, they found that "administration of G-CSF was able to induce significant cycling in neutropenic patients that were not cycling prior to treatment. In patients who had significant cycling before treatment, G-CSF may either decrease the period or obliterate any statistical evidence of cycling" (Haurie et al., 1999a,b, see Fig. 9.). Our hypothesis is that cyclical and non-cyclical (congenital and idiopathic) neutropenia correspond to two possible states (cycling and non-cycling) of the same stem cell disorder. Perturbations in the system, due for instance to G-CSF administration, could potentially destabilize the system and a change of state may occur, so that ANC oscillations could either start or stop. These results can be interpreted as an indication of bistability in the system.

Moreover, the potential existence of a region of bistability could be exploited by perturbing the haematopoietic system to stabilize ANC levels. If this were possible, cyclic neutropenia could be triggered into intermittent phases of low-level oscillatory, or non-oscillatory, behaviour (c.f. Fig. 6 for an example) and only occasional doses of G-CSF would be required to maintain this state. This idea of using a "physiological black hole" to trigger or destroy oscillations was originally put forward by Winfree (1973, 1980) in a different context.

Because of the oscillatory nature of CN and the nonlinear interactions between G-CSF and circulating neutrophils through clearance processes, this disorder is a good candidate for designing dynamic treatment protocols. We have shown, based on a computer simulation model, that phase-dependent protocols should be able to achieve as good as, or better, results than standard protocols, while using less G-CSF for fewer days of treatment. Obviously, this result opens up the possibility of decreasing side-effects as well as increasing the cost-effectiveness of the treatment, thereby improving the quality of life for patients. A test of the concepts developed in this paper is straightforward to carry out, and we are currently exploring this possibility with our clinical collaborators.

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