BIOAUTOMATION, 2009, 12, 53-69

Time Delay and Epo Dose Modulation in a Multilevel Model for Erythropoiesis

Svetoslav Nikolov[1,†], Xin Lai[2], Olaf Wolkenhauer[2], Julio Vera[2, †,*]

[1]*Institute of Mechanics and Biomechanics – Bulgarian Academy of Sciences, 4 Acad. G. Bonchev Str., 1113 Sofia, Bulgaria*

[2]*Systems Biology and Bioinformatics Group, Department of Computer Science University of Rostock, 18051 Rostock, Germany*
E-mail: julio.vera@informatik.uni-rostock.de

[*] *Corresponding author*
[†] *Equal contributors*

Received: December 29, 2008 **Accepted: February 12, 2009**

Published: March 16, 2009

Abstract: *In this paper we have extended and adapted a multi-level model in ordinary differential equations accounting for erythropoiesis. At the subcellular level, the model includes equations for the regulation of red blood differentiation through Epo stimulated JAK2-STAT5 activation, while at the cell population level the model describes the basic physiological features involved in erythropoiesis. Furthermore, we included additional equations describing the exogenous injection of Epo, one of the usual treatments for several haematological diseases. Our analysis indicates that time-delay associated with the proliferation-differentiation process can provoke pathological sustained oscillations in the erythropoiesis, while other (shorter) time-delays in the model accounting for nucleo-cytoplasmic shuttling of STAT5 or hypoxia-mediated control of Epo synthesis cannot. The consequences of time delays on the dynamics of the multi-level model are analysed using Hopf's bifurcation theorem. We also investigated the effects that subcellular-level downregulation of the proteins involved in the JA2-STAT5 pathway have in the dynamics of red blood cells population. We found that downregulation of Epo receptor or STAT5 synthesis can reduce considerably the mean value of red blood cells concentration. Our analysis revealed that a realistic scenario for Epo injection (twice-per-day short pulses) can compensate effects of low-medium downregulation, while for intense down regulation Epo injection seems not able to restore the desired hematocrit levels.*

Keywords: *Systems biology, Signalling pathways, Bifurcation analysis, Epo, Anaemia, Leukaemia.*

Background

Erythropoiesis is the process in which mature red blood cells (erythrocytes) are generated through the differentiation of the hematopoietic stem cells (HSCs) into colony forming units (CFUs), progenitors of red blood cells [11]. The process is controlled through a feedback loop involving the hypoxia level and modulated through the production and subsequent delivery of the hormone erythropoietin (*Epo*) in the bone marrow, where HSC are. This feedback loop ensures the adequate balance between generation of new red blood cells and destruction of old ones in the spleen. Among the signalling pathways involved in erythropoiesis, the activation of JAK2-STAT5 pathway through *Epo* receptor plays a major role. When the hormone erythropoietin (*Epo*) binds the receptor, constitutively associated JAK2 is activated and promote receptor activation by phosphorylation of several tyrosine residues in the receptor [15]. In subsequent steps, the transcription factor STAT5 is recruited to the activated *EpoR*, is phosphorylated, dimerises and gets activated. The activated STAT5 translocates to the

nucleus, where it initiates the transcription of several target genes involved in the control of erythropoiesis [12]. Several authors claim that proper functioning of JAK2-STAT5 signalling is crucial for red blood cells differentiation and maturation and the pathway appears deregulated in several kinds of leukaemia [14]. From a physiological perspective, some experimental works suggest the ability of a deregulation in this system to induce long period oscillations in their circulating reticulocyte counts (*N*) and hemoglobin levels (*Epo*) [23]. In addition, several pathologies have been described inducing oscillations in the red blood cells dynamics, including cyclic neutropenia [8] and chronic myelogenous leukemia [5].

Previous work, based on mathematical modelling of this pathway, studied the relevance of the nucleo-cytoplasmic shuttling of STAT5 [26] and the signal responsiveness and amplification [27]. In addition, physiological aspects of erythropoiesis have been investigated for long time with the help of mathematical models in either ordinary or partial differential equations [1, 2, 4, 6, 7, 16, 17, 24]. In this paper we modified the model presented in [22] to investigate the effects of time delay and the ability of (exogenous) injected *Epo* dose modulation to compensate deregulation in the JAK2-STAT5 pathway.

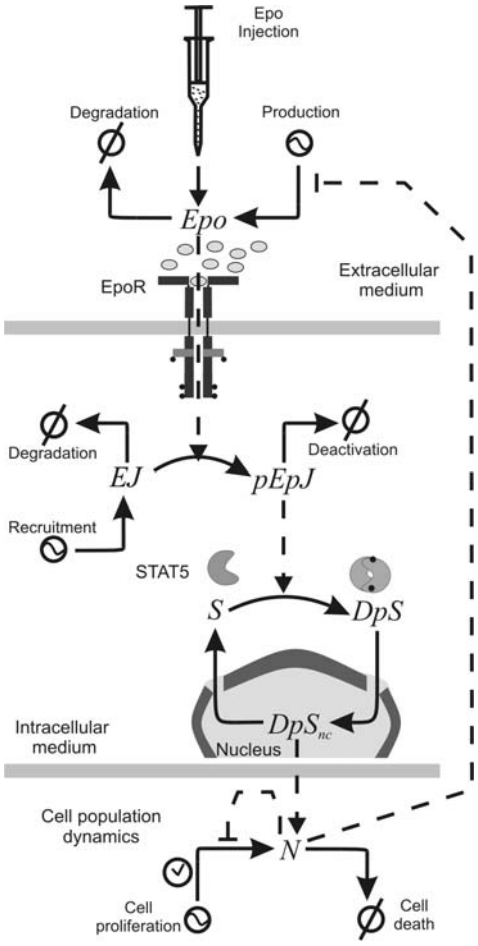

Fig. 1 Structure of the mathematical model proposed to describe *Epo* signalling related to differentiation and proliferation of red blood cells. The model contains a module describing the molecular processes accounting for the activation of JAK2-STAT5 pathway and a second module describing how these processes affect the dynamics of red blood cells. In addition, the dynamics of the hormone *Epo* are also represented.

Results

Mathematical model

A multi-level mathematical model in ordinary differential equations describing the erythropoiesis was proposed in [22]. The model includes two aspects of erythropoiesis: i) cell signalling regulation of red blood differentiation, for which we used and adapted the model in [27] that described the dynamics of *Epo* stimulated JAK2-STAT5 pathway; and ii) simplified description of the physiological features involved in erythropoiesis, for which we modified and expanded the simple model in ODE for the dynamics of erythropoiesis proposed by [1]. In the current work, we modified the model in [22] including additional equations accounting for exogenous injection of *Epo*, the usual treatments in case of anaemia. The model is depicted in Fig. 1.

The model includes six differential equations accounting for the dynamics of the *Epo* receptor complex (Eq. (1) – (2)), the transcription factor STAT5 (Eq. (3) – (4)), the population of red blood cells (5) and the extracellular concentration of *Epo* (6). In case of EpoR/JAK2 complex the model includes two state variables accounting for not bound to *Epo* and therefore non-activated receptor complex, *EJ,* and Epo-bound activated EpoR/JAK2 complex, *pEpJ.* The biochemical processes described are: i) *Epo* mediated receptor activation; ii) receptor deactivation; iii) recruitment of new receptor complex to the plasma membrane; and iv) degradation of non-activated EpoR/JAK2.

In case of STAT5, the two states considered accounts for activated cytosolic STAT5, *DpS*, and activated nuclear STAT5, DpS_n. We have implicitly included mass conservation accounting for the balance between STAT5 synthesis and degradation, in way non-activated cytosolic STAT5, *S*, is $S = S_{TOT} - 2\,DpS - 2\,DpS_{nc}$ with S_{TOT} total amount of STAT5. The model includes the following processes related to STAT5 dynamics: i) activated receptor mediated activation of STAT5; ii) nuclear translocation of cytosolic activated STAT5; and iii) deactivation and subsequent cytosolic translocation of nuclear STAT5.

The equation accounting for the dynamics of extracellular concentration of *Epo*, *Epo*, includes a description of the following processes: i) synthesis on new *Epo* controlled by the erythrocytes concentration through a hypoxia feedback loop; ii) degradation of *Epo*; and iii) exogenous injection of *Epo*. The equation describing the dynamics of red blood cells population includes a term accounting for activated nuclear STAT5 mediated proliferation-differentiation of new erythrocytes and another for their degradation.

$$\frac{d}{dt} EJ = \gamma_0 - \gamma_2\, EJ\, Epo - \gamma_1\, EJ \tag{1}$$

$$\frac{d}{dt} pEpJ = \gamma_2\, EJ\, Epo - \gamma_3\, pEpJ \tag{2}$$

$$\frac{d}{dt} DpS = \gamma_5 \left(S_{TOT} - 2\,DpS - 2\,DpS_{nc}\right) pEpJ - \gamma_6\, DpS \tag{3}$$

$$\frac{d}{dt} DpS_{nc} = \gamma_6\, DpS - \gamma_4 \left(DpS_{nc}\left(t-\tau_1\right)\right) \tag{4}$$

$$\frac{d}{dt} N = N\left(t-\tau_3\right)\left[\gamma_7\, DpS_{nc}^{g_5}\left(t-\tau_3\right)\right] F_1\left(N\right) - \gamma_8\, N \tag{5}$$

$$\frac{d}{dt} Epo = \gamma_9 F_2\left(N\right) - \gamma_{10}\, Epo + \gamma_{inj}\left(t\right) \tag{6}$$

where: $F_1(N)=\frac{\beta k_1^{g_1}}{k_1^{g_1}+N^{g_1}(t-\tau_3)}$, $F_2(N)=\frac{1}{1+k_2 N^{g_2}(t-\tau_2)}$; τ_1 is the elapsed time of activated STAT5 in the nucleus; τ_2 is the time associated to the hypoxia controlled process of *Epo* synthesis, release and transport; τ_3 is the delay associated to the set of processes accounting for the differentiation-proliferation of the red blood cells from progenitors. With γ_1-γ_{10} are denoted the kinetic rate constants; k_1, k_2 and β are positive parameters; g_1, g_2 and g_5 are kinetic orders. τ_1 and τ_2 are in the range of minutes, while τ_3 lasts for several days.

We notice that the simplistic description of some processes provokes the emergence of time-delay in Eq. (4) – (6) [21]. Our previous results [22] indicated that only the time delay associated to the differentiation-proliferation of the red blood cells from progenitors have relevant dynamical consequences (τ_3). Values for the parameters were taken from [22].

In our investigation, we were especially interested in two features of the model. Firstly, the effect that time-delays ($\gamma_{inj}=const$) have in the stability of the system; in order to investigate this, we used qualitative (bifurcation) analysis. Secondly, we analysed the pathological effects that downregulation of the *Epo* receptor and the STAT5 transcription factor have in the dynamics of red blood cells population, phenomena associated to the pathological conditions existing in several kinds of leukaemia and anaemia [14]. In some of our simulations we investigated how tuning of the time-dependent function accounting for exogenous injection of *Epo* ($\gamma_{inj}(t)$) can compensate pathological states with downregulation of *Epo* receptor recruitment (represented by γ_0) and/or STAT5 total available amount (S_{TOT}).

Analysis of time-delay effects on stability through bifurcation analysis

Let $y_1=EJ$, $y_2=pEpJ$, $y_3=DpS$, $y_4=DpS_{nc}$, $y_5=N$, and $y_6=Epo$. Thus, after some algebraic calculations for fixed points of the system (1) - (6) we obtain:

$$\begin{aligned}
&\bar{y}_1^{(1)}=\frac{\gamma_0\gamma_{10}}{\gamma_1\gamma_{10}+\gamma_2(\gamma_9+\gamma_{inj})},\ \bar{y}_2^{(1)}=\frac{\gamma_0\gamma_2(\gamma_9+\gamma_{inj})}{\gamma_3[\gamma_1\gamma_{10}+\gamma_2(\gamma_9+\gamma_{inj})]},\\
&\bar{y}_3^{(1)}=\frac{\gamma_0\gamma_2\gamma_4\gamma_5 S_{TOT}(\gamma_9+\gamma_{inj})}{2\gamma_0\gamma_2\gamma_5(\gamma_4+\gamma_6)(\gamma_9+\gamma_{inj})+\gamma_3\gamma_4\gamma_6[\gamma_1\gamma_{10}+\gamma_2(\gamma_9+\gamma_{inj})]},\\
&\bar{y}_4^{(1)}=\frac{\gamma_0\gamma_2\gamma_5\gamma_6 S_{TOT}(\gamma_9+\gamma_{inj})}{2\gamma_0\gamma_2\gamma_5(\gamma_4+\gamma_6)(\gamma_9+\gamma_{inj})+\gamma_3\gamma_4\gamma_6[\gamma_1\gamma_{10}+\gamma_2(\gamma_9+\gamma_{inj})]},\ \bar{y}_5^{(1)}=0,\ \bar{y}_6^{(1)}=\frac{\gamma_9+\gamma_{inj}}{\gamma_{10}},
\end{aligned} \tag{7}$$

$$\begin{aligned}
&\bar{y}_1^{(2)}=\frac{1}{\gamma_1}\left(\gamma_0-\gamma_3\bar{y}_2^{(2)}\right),\ \bar{y}_2^{(2)}=\frac{\gamma_4\gamma_6\bar{y}_4^{(2)}}{\gamma_5\gamma_6 S_{TOT}-2(\gamma_4+\gamma_6)\bar{y}_4^{(2)}},\ \bar{y}_3^{(2)}=\frac{\gamma_4}{\gamma_6}\bar{y}_4^{(2)},\ \bar{y}_4^{(2)}=\frac{\gamma_6}{2(\gamma_4+\gamma_6)}(S_{TOT}-S),\\
&\bar{y}_5^{(2)}=k_1\sqrt[3]{\frac{\gamma_7\beta\bar{y}_4^{(2)}}{\gamma_8}-1},\ \bar{y}_6^{(2)}=\frac{1}{\gamma_{10}}\left[\frac{\gamma_9}{1+k_2\left(\bar{y}_5^{(2)}\right)^7}+\gamma_{inj}\right],\ where\ \bar{y}_4^{(2)}>\frac{\gamma_8}{\gamma_7\beta}.
\end{aligned} \tag{8}$$

Further, we obtain the characteristic equation for the linearization of system (1) – (6) near the equilibrium $\bar{E}(\bar{y}_i)\quad i=1\div 6$. Thus, we consider a small perturbation about the equilibrium level, i.e. $y_i=\bar{y}_i+x_i$. Substituting these into the differential equations in system (1) – (6), we have

$$\begin{aligned}
\frac{dx_1}{dt}&=-c_1x_1-c_2x_6-\gamma_2x_1x_6,\\
\frac{dx_2}{dt}&=c_3x_1-\gamma_3x_2+c_2x_6+\gamma_2x_1x_6,\\
\frac{dx_3}{dt}&=c_4x_2-c_5x_3-c_6x_4-2x_2x_3-2x_2x_4,\\
\frac{dx_4}{dt}&=\gamma_6x_3-\gamma_4\ell^{-\tau_1\chi}x_4,\\
\frac{dx_5}{dt}&=-\gamma_8x_5+c_7\ell^{-\tau_3\chi}x_4+c_8\ell^{-\tau_3\chi}+c_9\ell^{-2\tau_3\chi}x_4x_5-c_{10}\ell^{-2\tau_3\chi}x_5^2-c_{11}\ell_5^{-3\tau_3\chi}x_5^3-\\
&\quad-c_{12}\ell^{-3\tau_3\chi}x_4x_5^2-c_{13}\ell^{-4\tau_3\chi}x_5^4-c_{14}\ell^{-4\tau_3\chi}x_4x_5^3-c_{15}\ell^{-5\tau_3\chi}x_4x_5^4,\\
\frac{dx_6}{dt}&=-c_{16}\ell^{-\tau_2\chi}-\gamma_{10}x_6-c_{17}\ell^{-2\tau_2\chi}x_5^2-c_{18}\ell^{-3\tau_2\chi}x_5^3-c_{19}\ell^{-4\tau_2\chi}x_5^4-\\
&\quad-c_{20}\ell^{-5\tau_2\chi}x_5^5-c_{21}\ell^{-6\tau_2\chi}x_5^6-c_{22}\ell^{-7\tau_2\chi}x_5^7.
\end{aligned}\tag{9}$$

where:

$$\begin{aligned}
&c_1=\gamma_1+\gamma_2\bar{y}_6,\ c_2=\gamma_2\bar{y}_1,\ c_3=\gamma_2\bar{y}_6,\ c_4=\gamma_5S_{TOT}-2\left(\bar{y}_3+\bar{y}_4\right),\ c_5=2\bar{y}_2+\gamma_6,\\
&c_6=2\bar{y}_2,\ c_7=\gamma_7\beta\bar{y}_4\left(1-\frac{\bar{y}_5^{\,3}}{k_1^3}\right),\ c_8=\gamma_7\beta\bar{y}_4\left(1-3\frac{\bar{y}_5^{\,3}}{k_1^3}-\frac{\bar{y}_5}{k_1^3}\right),\ c_9=\gamma_7\beta\left(1-\frac{4}{k_1^3}\right),\\
&c_{10}=6\frac{\gamma_7\beta\bar{y}_4\bar{y}_5^{\,2}}{k_1^3},\ c_{11}=4\frac{\gamma_7\beta\bar{y}_4\bar{y}_5}{k_1^3},\ c_{12}=6\frac{\gamma_7\beta\bar{y}_5^{\,2}}{k_1^3},\ c_{13}=\frac{\gamma_7\beta\bar{y}_4}{k_1^3},\\
&c_{14}=4\frac{\gamma_7\beta\bar{y}_5}{k_1^3},\ c_{15}=\frac{\gamma_7\beta}{k_1^3},\ c_{16}=7\gamma_9k_2\bar{y}_5^{\,6},\ c_{17}=21\gamma_9k_2\bar{y}_5^{\,5},\ c_{18}=35\gamma_9k_2\bar{y}_5^{\,4},\\
&c_{19}=35\gamma_9k_2\bar{y}_5^{\,3},\ c_{20}=21\gamma_9k_2\bar{y}_5^{\,2},\ c_{21}=7\gamma_9k_2\bar{y}_5,\ c_{22}=\gamma_9k_2.
\end{aligned}\tag{10}$$

Note that functions $F_1(N)$ and $F_2(N)$ are written in Maclaurin series near equilibrium $\bar{E}$, when only linear term is taken. Hence, we obtain the stability matrix in the form

$$\begin{pmatrix} -c_1 & 0 & 0 & 0 & 0 & -c_2 \\ c_3 & -\gamma_3 & 0 & 0 & 0 & c_2 \\ 0 & c_5 & -c_6 & -c_7 & 0 & 0 \\ 0 & 0 & \gamma_6 & -\gamma_4 \ell^{-\tau_1 \chi} & 0 & 0 \\ 0 & 0 & 0 & c_8 \ell^{-\tau_3 \chi} & c_9 \ell^{-\tau_3 \chi} - \gamma_8 & 0 \\ 0 & 0 & 0 & 0 & -c_{17} \ell^{-\tau_2 \chi} & -\gamma_{10} \end{pmatrix} \tag{11}$$

The stability matrix (11) leads to the following characteristic equation:

$$\begin{aligned} &\chi^6 + K_1\chi^5 + K_2\chi^4 + K_3\chi^3 + K_4\chi^2 + K_5\chi + K_6 = \\ &= \ell^{-\tau_1\chi}\left(T_1\chi^5 + T_2\chi^4 + T_3\chi^3 + T_4\chi^2 + T_5\chi + T_6\right) + \ell^{-\tau_2\chi}T_7\chi^2 + \\ &+ \ell^{-\tau_3\chi}\left(T_8\chi^5 + T_9\chi^4 + T_{10}\chi^3 + T_{11}\chi^2 + T_{12}\chi + T_{13}\right) + \ell^{-(\tau_1+\tau_2)\chi}T_{14}\chi^2 + \\ &+ \ell^{-(\tau_1+\tau_3)\chi}\left(T_{15}\chi^4 + T_{16}\chi^3 + T_{17}\chi^2 + T_{18}\chi + T_{19}\right) + \ell^{-(\tau_2+\tau_3)\chi}\left(T_{20}\chi + T_{21}\right) \end{aligned} \tag{12}$$

where

$$\begin{aligned} &K_1 = c_1 + c_6 + \gamma_3 + \gamma_8 + \gamma_{10},\ K_2 = c_1(\gamma_3 + \gamma_8 + \gamma_{10} + c_6) + \gamma_3(\gamma_8 + \gamma_{10} + c_6) + c_6(\gamma_8 + \gamma_{10}) + \gamma_8\gamma_{10}, \\ &K_3 = c_1(\gamma_3c_6 + \gamma_3\gamma_8 + \gamma_3\gamma_{10} + c_6c_8 + \gamma_{10}c_6 + \gamma_8\gamma_{10} + \gamma_6c_7) + \gamma_3(\gamma_6c_7 + \gamma_8c_6 + \gamma_{10}c_6 + \gamma_8\gamma_{10}) + \gamma_6c_7(\gamma_8 + \gamma_{10}) + \\ &+ \gamma_8\gamma_{10}c_6,\ K_4 = \gamma_3(\gamma_4c_1c_7 + \gamma_6\gamma_8c_7 + \gamma_8c_1c_6 + \gamma_{10}c_1c_6),\ K_5 = \gamma_3\gamma_6c_7(\gamma_8c_1 + \gamma_{10}c_1 + \gamma_8\gamma_{10}),\ T_1 = -\gamma_4, \\ &T_2 = -\gamma_4(\gamma_3 + \gamma_8 + \gamma_{10} + c_1 + c_6),\ T_3 = -\gamma_4[c_1(\gamma_3 + \gamma_8 + \gamma_{10} + c_6) + \gamma_3(\gamma_8 + \gamma_{10} + c_6) + (\gamma_8 + \gamma_{10})c_6 + \gamma_8\gamma_{10}], \\ &T_4 = -\gamma_4c_6(\gamma_3c_1 + \gamma_3\gamma_8 + \gamma_3\gamma_{10} + \gamma_8\gamma_{10}),\ T_5 = -\gamma_3\gamma_4c_6(\gamma_8c_1 + \gamma_{10}c_1 + \gamma_8\gamma_{10}),\ T_6 = \gamma_3\gamma_4\gamma_8\gamma_{10}c_1c_6,\ T_8 = c_9, \\ &T_7 = c_2c_7c_{17}(c_1 - c_3),\ T_9 = c_9(\gamma_3 + \gamma_{10} + c_1 + c_6),\ T_{10} = c_9[c_1(\gamma_3 + \gamma_{10} + c_6) + \gamma_3(c_6 + \gamma_{10}) + \gamma_6c_7 + \gamma_{10}(\gamma_4 + c_6)], \\ &T_{11} = -c_9(\gamma_6c_7 - \gamma_3\gamma_6c_7 - \gamma_3c_1c_6),\ T_{12} = \gamma_3\gamma_6c_7c_9(\gamma_{10} + c_1),\ T_{13} = -\gamma_3\gamma_6\gamma_{10}c_1c_7c_9,\ T_{14} = \gamma_4c_2c_5c_{17},\ T_{15} = \gamma_4c_9, \\ &T_{16} = \gamma_4c_9(\gamma_3 + c_1 + c_6),\ T_{17} = \gamma_4c_6c_9(\gamma_3 + \gamma_{10}),\ T_{18} = \gamma_3\gamma_4c_6c_9(\gamma_{10} + c_1),\ T_{19} = -\gamma_3\gamma_4\gamma_{10}c_1c_6c_9, \\ &T_{20} = -\gamma_6c_2c_5c_8c_{17},\ T_{21} = \gamma_6c_2c_5c_8c_{17}(c_3 - c_1). \end{aligned} \tag{13}$$

Because of the presence of more than one delay in (1) – (6), the analysis of the sign of the real parts of the eigenvalues is very complicated, and a direct approach cannot be considered [3], [19]. Thus, in our analysis we will use a method consisting of determining the stability of the steady state when two delays are equal to zero, and using some analytical arguments we will deduce conditions for stability of the steady states when three delays are nonzero.

The case $\tau_1 = \tau_2 = \tau_3 = 0$.

Assume that $\tau_1 = \tau_2 = \tau_3 = 0$. Then the characteristic Eq. (12) is written as a six degree polynomial equation

$$\chi^6 + K_1^*\chi^5 + K_2^*\chi^4 + K_3^*\chi^3 + K_4^*\chi^2 + K_5^*\chi + K_6^* = 0. \tag{14}$$

where:

$$\begin{aligned} &K_1^* = K_1 - T_1 - T_8, \quad K_2^* = K_2 - T_2 - T_9 - T_{15}, \quad K_3^* = K_3 - T_3 - T_{10} - T_{16}, \\ &K_4^* = K_4 - T_4 - T_7 - T_{11} - T_{14} - T_{17}, \quad K_5^* = K_5 - T_5 - T_{12} - T_{18} - T_{20}, \quad K_6^* = K_6 - T_6 - T_{13} - T_{19} - T_{21} \end{aligned} \tag{15}$$

According to Routh-Hurwitz criterion for stability of steady state, defined by (7) and (8), all eigenvalues of (12) have negative real parts if only if

$$K_i^* > 0, \tag{16}$$

Hence, we have the following lemma.

Lemma.
If $K_i^* > 0,\ i = 1 \div 6$, *the equilibriums* (7) *and* (8) *of system* (1) – (6) *are locally asymptotically stable.*

The case $\tau_1 = \tau_2 = 0$ **and** $\tau_3 > 0$.

Here, we consider the case $\tau_1 = \tau_2 = 0$ and $\tau_3 > 0$. Our choice is motivated by the following biological reasons: the time delay τ_1 represents the delay associated to the nuclear shuttling of STAT5, the time delay τ_2 represents the time that takes the hypoxia level to control *Epo* synthesis. Setting $\tau_1 = \tau_2 = 0$ in (12), the characteristic equation becomes

$$\begin{aligned}\chi^6 + B_1\chi^5 + B_2\chi^4 + B_3\chi^3 + B_4\chi^2 + B_5\chi + B_6 = \\ = \ell^{-\tau_3\chi}\left(T_8\chi^5 + M_1\chi^4 + M_2\chi^3 + M_3\chi^2 + M_4\chi + M_5\right)\end{aligned} \tag{17}$$

where

$$B_1 = K_1 - T_1,\quad B_2 = K_2 - T_2,\quad B_3 = K_3 - T_3,\quad B_4 = K_4 - T_4 - T_7 - T_{14},$$
$$B_5 = K_5 - T_5,\quad B_6 = K_6 - T_6,\quad M_1 = T_9 + T_{15},\quad M_2 = T_{10} + T_{16},$$
$$M_3 = T_{11} + T_{17},\quad M_4 = T_{12} + T_{18} + T_{20},\quad M_5 = T_{13} + T_{19} + T_{21}$$

This characteristic Eq. (17) is transcendental and cannot be solved analytically. Moreover, it has an indefinite number of roots [9, 10, 20, 21]. The stability of equilibrium state depends on the sign of the real parts of the roots of (17). We let $\chi = m + in\ \ (m, n \in R)$ and rewrite (17) in terms of its real and imaginary parts as

$$\begin{aligned}
&m^6 - n^6 - 15m^4n^2 + 15m^2n^4 + B_1\left(m^5 - 10m^3n^2 + 5mn^4\right) + B_2\left(m^4 + n^4 - 6m^2n^2\right) + \\
&\quad + B_3\left(m^3 - 3mn^2\right) + B_4\left(m^2 - n^2\right) + B_5m + B_6 = \ell^{-m\tau_3}\left\{T_8\left[\left(m^5 - 10m^3n^2 + 5mn^4\right)\cos n\tau_3 + \right.\right. \\
&\quad \left.\left. + \left(n^5 + 5m^4n - 10m^2n^3\right)\sin n\tau_3\right] + M_1\left[\left(m^4 + n^4 - 6m^2n^2\right)\cos n\tau_3 + \left(4m^3n - 4mn^3\right)\sin n\tau_3\right] + \right. \\
&\quad \left. + M_2\left[\left(m^3 - 3mn^2\right)\cos n\tau_3 + \left(3m^2n - n^3\right)\sin n\tau_3\right] + M_3\left[\left(m^2 - n^2\right)\cos n\tau_3 + 2mn\sin n\tau_3\right] + \right. \\
&\quad \left. + M_4\left(m\cos n\tau_3 + n\sin n\tau_3\right) + M_5\cos n\tau_3\right\}, \\
&6m^5n - 20m^3n^3 + 6mn^5 + B_1\left(n^5 + 5m^4n - 10m^2n^3\right) + 4mnB_2\left(m^2 - n^2\right) + B_3\left(3m^2n - n^3\right) + 2B_4mn + \\
&\quad + B_5n = \ell^{-m\tau_3}\left\{T_8\left[\left(n^5 + 5m^4n - 10m^2n^3\right)\cos n\tau_3 - \left(m^5 - 10m^3n^2 + 5mn^4\right)\sin n\tau_3\right] + \right. \\
&\quad \left. + M_1\left[4mn\left(m^2 - n^2\right)\cos n\tau_3 - \left(m^4 + n^4 - 6m^2n^2\right)\sin n\tau_3\right] + M_2\left[\left(3m^2n - n^3\right)\cos n\tau_3 - m\left(m^2 - 3n^2\right)\sin n\tau_3\right] + \right. \\
&\quad \left. + M_3\left[2mn\cos n\tau_3 - \left(m^2 - n^2\right)\sin n\tau_3\right] + M_4\left(n\cos n\tau - m\sin n\tau_3\right) - M_5\sin n\tau_3\right\}.
\end{aligned} \tag{18}$$

To find the first bifurcation point we look for purely imaginary roots $\chi = \pm in$, $n \in R$, of (17), i.e. we set $m = 0$. Then the above two equations are reduce to

$$\begin{aligned}
&-n^6 + B_2n^4 - B_4n^2 + B_6 = \left(T_8n^5 - M_2n^3 + M_4n\right)\sin n\tau + \left(M_1n^4 - M_3n^2 + M_5\right)\cos n\tau, \\
&B_1n^5 - B_3n^3 + B_5n = \left(T_8n^5 - M_2n^3 + M_4n\right)\cos n\tau + \left(-M_1n^4 + M_3n^2 - M_5\right)\sin n\tau.
\end{aligned} \tag{19}$$

or another one

$$\cos n\tau_3 = \frac{\left(-n^6 + B_2 n^4 - B_4 n^2 + B_6\right)\left(M_1 n^4 - M_3 n^2 + M_5\right) + n^2\left(T_8 n^4 - M_2 n^2 + M_4\right)\left(B_1 n^4 - B_3 n^2 + B_5\right)}{n^2\left(T_8 n^4 - M_2 n^2 + M_4\right)^2 + \left(-M_1 n^4 + M_3 n^2 - M_5\right)^2} =$$
$$= -\frac{\Psi(\tau_3)}{\left|Q(in, \tau_3)\right|^2},$$
$$\sin n\tau_3 = \frac{n\left(B_1 n^4 - B_3 n^2 + B_5\right)\left(-M_1 n^4 + M_3 n^2 - M_5\right) + n\left(T_8 n^4 - M_2 n^2 + M_4\right)\left(-n^6 + B_2 n^4 - B_4 n^2 + B_6\right)}{n^2\left(T_8 n^4 - M_2 n^2 + M_4\right)^2 + \left(-M_1 n^4 + M_3 n^2 - M_5\right)^2} =$$
$$= \frac{\Theta(\tau_3)}{\left|Q(in, \tau_3)\right|^2}. \tag{20}$$

Note that $n = 0$ can be solution of (20) if $B_6 = M_5$. If the first bifurcation point is $\left(n_b^0, \tau_b^0\right)$, then the other bifurcation points $\left(n_b, \tau_b\right)$ satisfy $n_b \tau_b = n_b^0 \tau_b^0 + 2\nu\pi, \quad \nu = 1, 2, ..., \infty$. One can notice that if *n* is a solution of (19) (or (20)), then so is –*n*. Hence, in the following we only investigate for positive solutions *n* of (19), or (20) respectively. By squaring the two equations into system (19) and then adding them, it follows that

$$\begin{aligned} &n^{12} + \left(B_1^2 - T_8^2 - 2B_2\right)n^{10} + \left[B_2^2 - M_1^2 - 2\left(B_1 B_3 - B_4 - T_8 M_2\right)\right]n^8 + \\ &+ \left[B_3^2 - M_2^2 + 2\left(B_1 B_5 - B_2 B_4 - B_6 + M_1 M_3 - T_8 M_4\right)\right]n^6 + \\ &\left[B_4^2 - M_3^2 + 2\left(B_2 B_6 + M_2 M_4 - B_3 B_5 - M_1 M_5\right)\right]n^4 + \\ &+ \left[B_5^2 - M_4^2 + 2\left(M_3 M_5 - B_4 B_6\right)\right]n^2 + B_6^2 - M_5^2 = 0. \end{aligned} \tag{21}$$

Here, we note that this is a hexatic equation about n^2 and that the left side is positive for large values of n^2 and negative for $n = 0$ if and only if $M_5^2 > B_6^2$, i.e. when Eq. (21) has at least one positive real root. Moreover, to apply Hopf bifurcation theorem the following theorem in this situation applies:

Theorem 1.
Suppose that n_b *is the least positive simple root of* (21). *Then,* $in(\tau_b) = in_b$ *is a simple root of* (17) *and* $m(\tau_3) + in(\tau_3)$ *is differentiable with respect to* τ_3 *in the neighbourhood of* $\tau_3 = \tau_b$.

The proof of this theorem in details can be found in [13]. To establish an Andronov-Hopf bifurcation at $\tau_3 = \tau_b$, we need to show that the following transversality condition $\left.\frac{dm}{d\tau_3}\right|_{\tau_3 = \tau_b} \neq 0$ is satisfied. Hence, if we denote

$$\begin{aligned} H(\chi, \tau_3) = &\chi^6 + B_1\chi^5 + B_2\chi^4 + B_3\chi^3 + B_4\chi^2 + B_5\chi + B_6 - \\ &- \ell^{-\tau_3\chi}\left(T_8\chi^5 + M_1\chi^4 + M_2\chi^3 + M_3\chi^2 + M_4\chi + M_5\right), \end{aligned} \tag{22}$$

then

$$\frac{d\chi}{d\tau_3} = -\frac{\frac{\partial H}{\partial \tau_3}}{\frac{\partial H}{\partial \chi}} = \frac{-\chi\ell^{-\tau_3\chi}\left(T_8\chi^5 + M_1\chi^4 + M_2\chi^3 + M_3\chi^2 + M_4\chi + M_5\right)}{6\chi^5 + 5B_1\chi^4 + 4B_2\chi^3 + 3B_3\chi^2 + 2B_4\chi + B_5 + P_1 - P_2} \tag{23}$$

where

$$P_1 = \tau_3 \ell^{-\tau_3 \chi}\left(T_8\chi^5 + M_1\chi^4 + M_2\chi^3 + M_3\chi^2 + M_4\chi + M_5\right),$$
$$P_2 = \ell^{-\tau_3 \chi}\left(5T_8\chi^4 + 4M_1\chi^3 + 3M_2\chi^2 + 2M_3\chi + M_4\right). \quad (24)$$

Denote (without loss of generality) by U_l, $l = 1, ..., 4$, the positive roots of *H*, and set $n_l = \sqrt{U_l}$. Note that the unique solution $\varphi \in [0, 2\pi]$ of (20) is

$$\begin{vmatrix}
\varphi(\tau_3) = \arctan(-\Theta(\tau_3)/\Psi(\tau_3)) \quad if \quad \sin\varphi(\tau_3) > 0, \cos\varphi(\tau_3) > 0; \\
\varphi(\tau_3) = \pi/2 \quad if \quad \sin\varphi(\tau_3) = 1, \cos\varphi(\tau_3) = 0; \\
\varphi(\tau_3) = \pi + \arctan(-\Theta(\tau_3)/\psi(\tau_3)) \quad if \quad \cos\varphi(\tau_3) < 0; \\
\varphi(\tau_3) = 3\pi/2 \quad if \quad \sin\varphi(\tau_3) = -1, \cos\varphi(\tau_3) = 0; \\
\varphi(\tau_3) = 2\pi + \arctan(-\Theta(\tau_3)/\Psi(\tau_3)) \quad if \quad \sin\varphi(\tau_3) < 0, \cos\varphi(\tau_3) > 0.
\end{vmatrix}$$

It is easy to see that function $\varphi(\tau_3)$ defined above is continuous on *I* (assume that $I \subseteq R_{+0}$). Furthermore $\varphi'(\tau_2)$ is well defined for $\varphi(\tau_2) \in [0, 2\pi]$. Observe that if $\varphi(\tau_2) \neq \pi/2; 3\pi/2$, then $\Psi(\tau_2) \neq 0$. When $\varphi(\tau_2) = \pi/2; 3\pi/2$, we have $\Psi(\tau_2) = 0$. Evaluating the real part of Eq. (23) at $\tau_3 = \tau_b$ and setting $\chi = in_b$ yield

$$\left.\frac{dm}{d\tau_3}\right|_{\tau_3=\tau_b} = \mathrm{Re}\left(\frac{d\chi}{d\tau_3}\right)\Bigg|_{\tau_3=\tau_b} =$$
$$= \frac{n_b^2\left\{6n_b^{10} + 5\left(B_1^2 - T_8^2 - 2B_2\right)n_b^8 + 4\left[B_2^2 - M_1^2 - 2\left(B_1B_3 - B_4 - T_8M_2\right)\right]n_b^6\right\}}{L^2 + L_1^2} +$$
$$+ \frac{n_b^2\left\{3\left[B_3^2 - M_2^2 + 2\left(B_1B_5 - B_2B_4 - B_6 + M_1M_3 - T_8M_4\right)\right]n_b^4\right\}}{L^2 + L_1^2} + \quad (25)$$
$$+ \frac{n_b^2\left\{2\left[B_4^2 - M_3^2 + 2\left(B_2B_6 + M_2M_4 - B_3B_5 - M_1M_5\right)\right]n_b^2 + B_5^2 - M_4^2 + 2\left(M_3M_5 - B_4B_6\right)\right\}}{L^2 + L_1^2},$$

where

$$L = 5B_1n_b^4 - 3B_3n_b^2 + B_5 + \tau_b\left(-n_b^6 + B_2n_b^4 - B_4n_b^2 + B_6\right) -$$
$$-\left(5T_8n_b^4 - 3M_2n_b^2 + M_4\right)\cos n_b\tau_b + 2n_b\left(2M_1n_b^2 - M_3\right)\sin n_b\tau_b,$$
$$L_1 = 2n_b\left(3n_b^4 - 2B_2n_b^2 + B_4\right) + \tau_b\left(B_1n_b^5 - B_3n_b^3 + B_5n_b\right) + \quad (26)$$
$$+ 2n_b\left(2M_1n_b^2 - M_3\right)\cos n_b\tau_b + \left(5T_8n_b^4 - 3M_2n_b^2 + M_4\right)\sin n_b\tau_b$$

Let $\theta = n_b^2$; then, (21) reduces to

$$g(\theta)=\theta^6+\left(B_1^2-T_8^2-2B_2\right)\theta^5+\left[B_2^2-M_1^2-2\left(B_1B_3-B_4-T_8M_2\right)\right]\theta^4+$$
$$+\left[B_3^2-M_2^2+2\left(B_1B_5-B_2B_4-B_6+M_1M_3-T_8M_4\right)\right]\theta^3+$$
$$\left[B_4^2-M_3^2+2\left(B_2B_6+M_2M_4-B_3B_5-M_1M_5\right)\right]\theta^2+$$
$$+\left[B_5^2-M_4^2+2\left(M_3M_5-B_4B_6\right)\right]\theta+B_6^2-M_5^2. \tag{27}$$

Then, for $g'(\theta)$ we have

$$g'(\theta)\Big|_{\tau_3=\tau_b}=\frac{dg}{d\theta}\Big|_{\tau_3=\tau_b}=6\theta^5+5\left(B_1^2-T_8^2-2B_2\right)\theta^4+4\left[B_2^2-M_1^2-2\left(B_1B_3-B_4-T_8M_2\right)\right]\theta^3+$$
$$+3\left[B_3^2-M_2^2+2\left(B_1B_5-B_2B_4-B_6+M_1M_3-T_8M_4\right)\right]\theta^2 \tag{28}$$
$$+2\left[B_4^2-M_3^2+2\left(B_2B_6+M_2M_4-B_3B_5-M_1M_5\right)\right]\theta+B_5^2-M_4^2+2\left(M_3M_5-B_4B_6\right).$$

If n_b is the least positive simple root of (21), then

$$\frac{dg}{d\tau_3}\Big|_{\theta=n_b^2}>0. \tag{29}$$

Hence,

$$\frac{dm}{d\tau_3}\Big|_{\tau_3=\tau_b}=\mathrm{Re}\left(\frac{d\chi}{d\tau_3}\right)\Big|_{\tau_3=\tau_b}=\frac{n_b^2g'\left(n_b^2\right)}{L^2+L_1^2}>0. \tag{30}$$

According to the Hopf bifurcation theorem [18], we define the following theorem:

Theorem 2.
If n_b is the least positive root of (21)*, then an Andronov-Hopf bifurcation occurs as τ_3 passes through τ_b.*

Corollary 2.1.

If $\tau_3<\tau_b$, then the equilibrium $\bar{E}$ (Eq. (7) *or* (8)*) of system* (1) – (6) *is locally asymptotically stable.*

The case $\tau_1,\tau_2,\tau_3>0.$ Now, we return to the study of (12), when $\tau_1,\tau_2,\tau_3>0.$ In order to investigate the local stability of the equilibrium state $\bar{E}$ of system (1) – (6), we prove a result regarding the sign of the real parts of the characteristic roots of (12) in the next theorem:

Theorem 3.
If all roots of (17) *are with negative real parts for $\tau_3>0$, then there exists a $\tau_2^{bif}(\tau_3)>0$ (or $\tau_1^{bif}(\tau_3)>0$) such that all roots of the characteristic Eq.* (12) *have negative real parts at $\tau_2<\tau_2^{bif}(\tau_3)$ (or $\tau_1<\tau_1^{bif}(\tau_3)$), i.e. when $\tau_2\in\left[0,\tau_2^{bif}(\tau_3)\right)$ (or $\tau_1\in\left[0,\tau_1^{bif}(\tau_3)\right)$) respectively.*

Here, the proof of Theorem 3 is omitted because she is a private case of Theorem 2.1 proved in [25] for general case, i.e $0 = \tau_0 < \tau_1 < ... < \tau_n$.

Corollary 3.1.
If τ_3^{bif} *is defined as in Theorem 2, then for any* $\tau_3 \in [0, \tau_b)$ *there exists a* $\tau_2^{bif}(\tau_3) > 0$ *(or* $\tau_1^{bif}(\tau_3) > 0$*) such that the steady state* $\bar{E}$ *of system* (1) – (6) *is locally asymptotically stable when* $\tau_2 \in \left[0, \tau_2^{bif}(\tau_3)\right)$ *(or* $\tau_1 \in \left[0, \tau_1^{bif}(\tau_3)\right)$*).*

Simulations of the effects of Epo dose modulation on downregulation of STAT5 and EpoR

We first simulated the effects that the downregulation in S_{tot} (total amount of STAT5) and γ_0 (*EpoR* recruitment) have in the dynamics of the system. In Fig. 2 we represent the effect that modulation of S_{tot} and γ_0 have in the mean value of the cell population, represented in our model by *N*. Downregulation for any of the two parameters reduces the mean value of N, which could be interpreted as a reduction in the hematocrit levels of an individual. Interesting enough, our simulations indicate that the combined strong upregulation of both parameters would provoke a significant increase in the mean value of *N* and the emergence of sustained oscillations with high peak and period of several days [22].

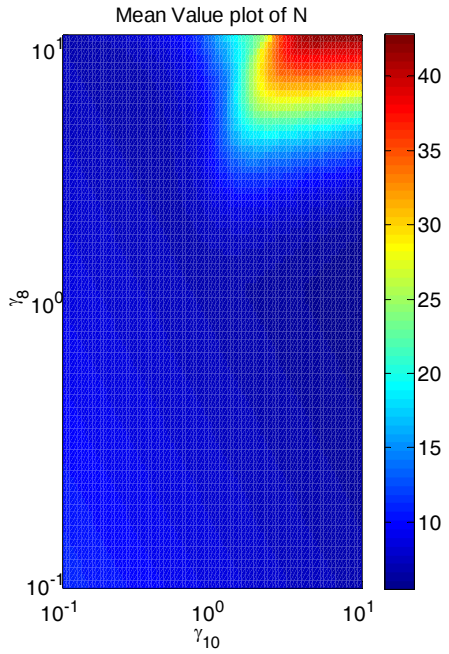

Fig. 2 Simulation of the mean value of the cell population *N* for different values of parameters S_{tot} and γ_0

We further simulated the effect that injection of exogenous *Epo* would have in the dynamics of red blood cells for different levels of downregulation in S_{tot} and γ_0. Towards this end, we first assume continuous injection of exogenous *Epo*, modelled as an extra constant rate γ_{inj} in the differential equation accounting for *Epo* dynamics. We then simulated different scenarios for S_{tot} and γ_0 downregulation ranging from the original value to 10% of it (strong downregulation) for both parameters (Fig. 3). For each set of parameters (S_{tot}, γ_0) we compute

the minimum value of constant *Epo* injection γ_{inj} necessary to recover the original value in the mean of red blood cells population (N_0). We allowed a physiological interval of values in continuous injection of $\gamma_{inj} \in [0,10]$. As we can see in Fig. 3, the intensity of necessary continuous *Epo* injection increases when the downregulation of STAT5 amount or *EpoR* recruitment increases. Both effects are additive and higher amount of *Epo* injection are required to restore the original state when both parameters are downregulated at the same time. Blank area in Fig. 3 represents the range of values in S_{tot} and γ_0 downregulation for which continues injection of *Epo* (in the physiological interval considered) is not enough to recover the initial average blood cell population.

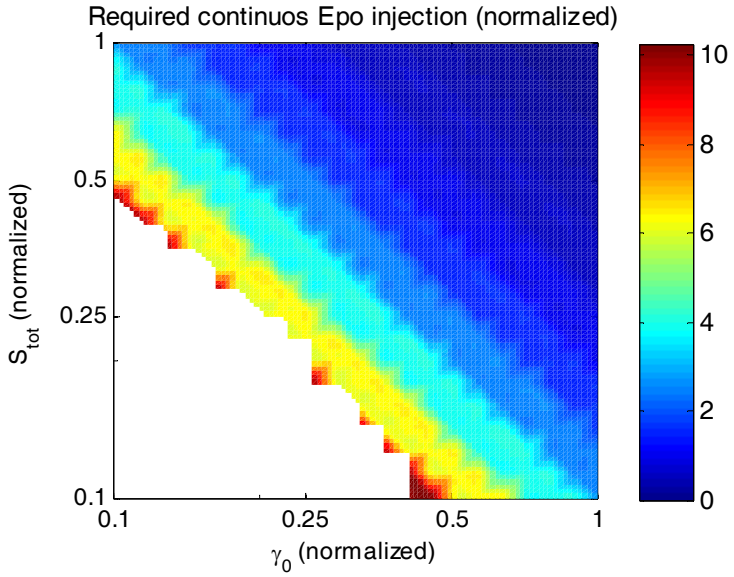

Fig. 3 Minimum value of constant *Epo* injection γ_{inj} necessary to recover the original value in the mean of red blood cells population *N*

We further simulated the effect of short periodic injections of exogenous *Epo* in the system, which corresponds to the way *Epo* is administered to real patients. Fig 4 is a sketch of this procedure where injections are characterised by the elapsed time of every injection (T_{inj}) and the average value of exogenous *Epo* injected (Epo_{inj}).

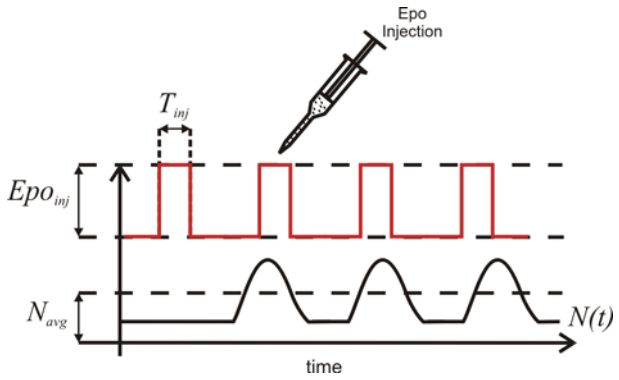

Fig. 4 Scheme representing the periodic pulse injections of exogenous *Epo* in the system

Towards this end, we assumed that *Epo* is injected twice per day in doses lasting for two hours (T_{inj} = 2 hr) and with an average value ranging in the interval of normalized values for

$Epo_{inj} \in [0, 30]$. We further simulated the minimum average *Epo* injection (Epo_{inj}) necessary to recover the 95%, 90% and 80% of the original value in the mean of red blood cells population, N_0 (Fig. 5). Getting the full recovery (represented as 95% of the original value of N) of the initial average of red blood cells is only possible for reduced downregulation of S_{tot} and γ_0; high doses of *Epo* are required for medium downregulation, while the recovery is unfeasible for strong downregulation of both parameters, alone or in combination (Fig. 5 top left). In case of a 90% recovery for N, injections of *Epo* are able to restore the initial state when only one of the parameters is downregulated in the interval considered; however, when both parameters are downregulated at the same time the system can recover 90% of N_0 only for low and medium downregulation, but high doses of *Epo* injection are required (Fig. 5 centre left). Finally, for 80% recovery for N, injections of *Epo* are able to restore the desired state when only one of the parameters is downregulated at even low doses of *Epo* injection; the recovery of 80% of N_0 is still only possible for intermediate levels of concurrent downregulation in S_{tot} and γ_0 in the interval considered, but in most of the cases the values of *Epo* injections required are low (Fig. 5 down left). Interesting enough, low doses of *Epo* pulse injection are required for most of the feasible couples (S_{tot}, γ_0) when the objective is to reach 80% of N_0 and only in the boundary of feasibility high doses or *Epo* are required (Fig. 5 down right); on the other hand, when the aim is to get almost total recovery of the initial population of red blood cells, the intensity of *Epo* injections required increases monotonically, with very low *Epo* dose for very reduced downregulation and the maximum allowed *Epo* in the border of feasibility (Fig. 5 top right).

Discussion and conclusions

In this paper we extended and adapted a multi-level model in ordinary differential equations accounting for erythropoiesis discussed in [22]. The model considers the cell signalling regulation of red blood differentiation through *Epo* stimulated JAK2-STAT5 activation, but also a reduced description of the physiological features involved in erythropoiesis. We have modified and expanded such model by including additional equations describing the exogenous injection of *Epo*.

We have investigated two features of the model. Firstly, we analyse changes in the stability of the system due to the existence of time-delay. By using qualitative bifurcation analysis we have confirmed that changes in the time-delay associated with the proliferation-differentiation process can provoke the emergence of pathological sustained oscillations in the erythropoiesis, in accordance with previous results for simpler versions of the model [1, 2]. However, our analysis suggests that other (shorter) time-delays considered in our model related to nucleo-cytoplasmic shuttling of STAT5 or hypoxia-mediated control of *Epo* synthesis are not able to induce self-oscillations in the system.

We were also concerned about the effects that subcellular-level downregulation of *EpoR* and the STAT5 have in the dynamics of red blood cells population. Some authors claim that these phenomena are associated to the pathological conditions existing in several kinds of leukaemia and anaemia [14]. When we simulated and analysed the direct effect of downregulation in the mean value of the red blood cells population, N, we found that downregulation for any of the two parameters reduces considerably the mean value of N, which could be interpreted as a (pathological) reduction in the hematocrit levels of an individual. In order to compensate that hematocrit reduction, we have simulated the effect of external injection of *Epo* in the system, considering different experimental conditions and designs for such injection.

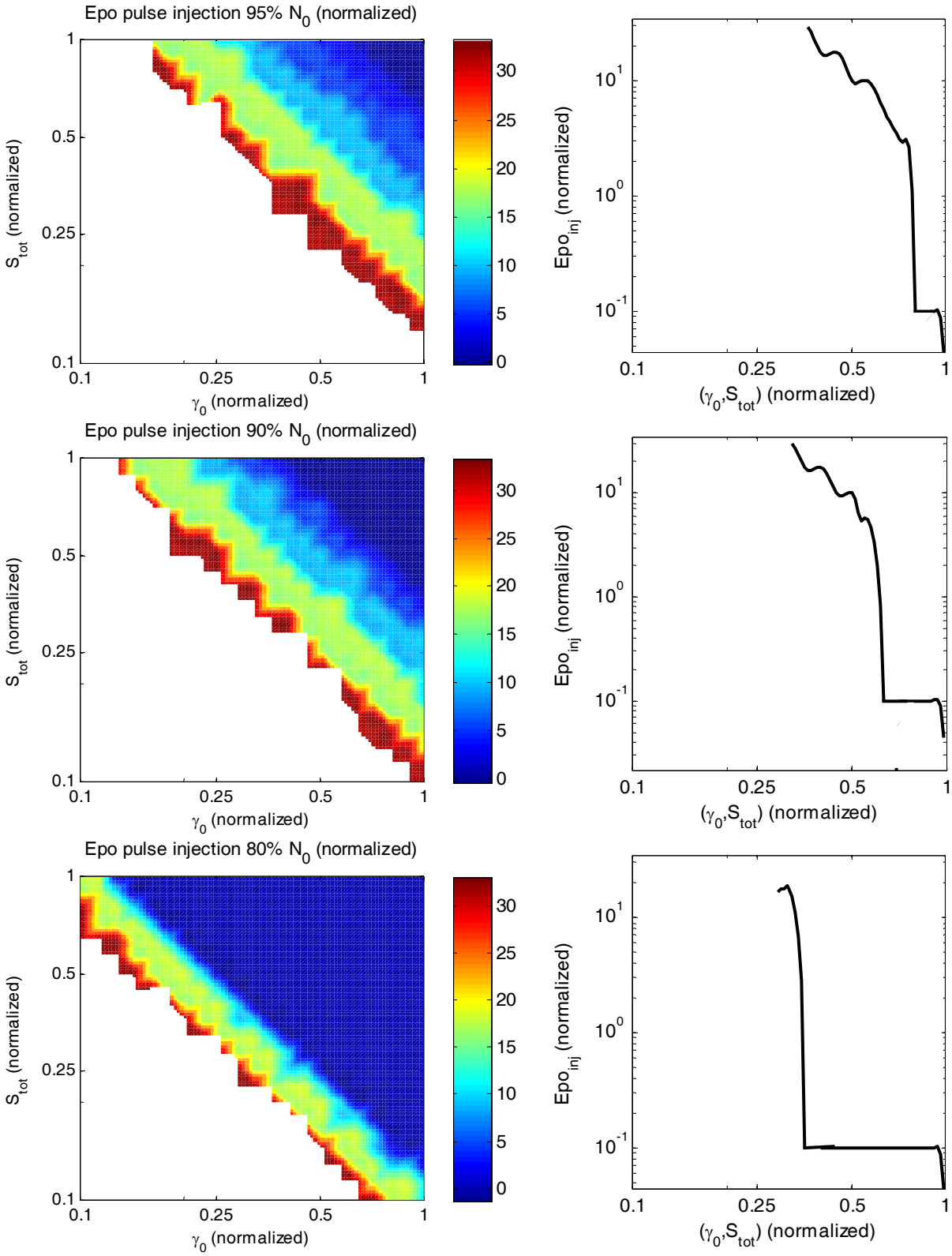

Fig. 5 Computation of the minimum average *Epo* injection (Epo_{inj}) necessary to recover the 95% (top), 90% (centre) and 80% (down) of the original value in the mean of red blood cells population *N* when downregulation of STAT5 and *EpoR* occur (left). The behaviour over the diagonal for equal downregulation of S_{tot}, and γ_0 is represented on the right side for every level of *N*, with logarithmic scale also for *Epo* injection levels.

Among other, we simulated the realistic scenario of twice-per-day short pulses of *Epo* injection and analysed the long-term response of the system to such a design for the treatment, when different intensities in the pulse were considered. We computed the minimum intensity of *Epo* pulse required to stabilise the average amount of erythrocytes around 80-95% the original value N_0. For intense downregulation, *Epo* injection at the levels simulated is not able to restore the desired hematocrit levels, which suggest that acute downregulation cannot be treated with the injection of *Epo* alone and could become critical. In case of low-medium downregulation, our simulations predict low doses of *Epo* pulse injection required if the objective is to reach 80% of N_0, but increasing high levels of *Epo* when the aim is the total recovery of the hematocrit levels. In this latter case the outcome of the treatment is quite sensitive to changes in the *Epo* injection intensity.

In the future we want to expand our model considering the processes involved in the physiological level in higher detail, but also the effect of concurrent signalling pathway that control erythropoiesis.

Acknowledgements

J. V. and S. N. designed the study and set up the mathematical model. X. L. performed the calculations concerning the predictive simulations. S. N. performed the bifurcation analysis. The biological interpretation of the results was conducted by J. V. Finally, all the authors including O. W. drafted the manuscript. This work was supported by the German Federal Ministry of Education and Research (BMBF) as part of the project CALSYS-FORSYS under contract 0315264 (www.sbi.uni-rostock.de/calsys).

References

1. Adimy M., F. Crauste (2004). Stability and Instability Induced by Time Delay in an Erythropoiesis Model, Monografias del Seminario Matematico Garcia de Galdeano, 31, 3-12.
2. Adimy M., F. Crauste, Sh. Ruan (2006). Modelling Hematopoiesis Mediated by Growth Factors with Applications to Periodic Hematological Diseases, Bull. Math. Biology, 68(8), 2321-2351.
3. Bellman R., K. Cooke (1963). Differential-difference Equations, New York, Academic Press.
4. Bernard S., L. Pujo-Menjouet, M. C. Mackey (2003). Analysis of Cell Kinetics Using a Cell Division Marker: Mathematical Modeling of Experimental Data, Biophys J., 84(5), 3414-3424.
5. Chikkappa G., G. Borner, H. Burlington, A. D. Chanana, E. P. Cronkite et al. (1976). Periodic Oscillation of Blood Leukocytes, Platelets and Reticulocytes in a Patient with Chronic Myelocytic Leukemia, Blood, 47, 1023-1030.
6. Colijn C., M. C. Mackey (2005) A Mathematical Model of Hematopoiesis: I. Periodic Chronic Myelogenous Leukemia, J. of Theor. Biology, 237(2), 117-132.
7. Colijn C., M. C. Mackey (2005). A Mathematical Model of Hematopoiesis: II. Cyclical Neutropenia, J. of Theor. Biology, 237(2), 133-146.
8. Dale D. C., W. P. Hammond (1988). Cyclic Neutropenia: A Clinical Review, Blood Reviews, 2, 178-185.
9. Elsgolz L., S. Norkin (1974). Introduction to Time Delay Equations, Nauka, Moscow (in Russian).
10. Erbe L., Q. Kong, B. Zhang (1995). Oscillation Theory for Functional Differential Equations, Marcel Dekker Inc., New York.

11. Fisher J. W. (2003). Erythropoietin: Physiology an Pharmacology Update, Exp. Biol. Med. (Maywood), 228(1), 1-14.
12. Jelkmann L. (2004). Molecular Biology of Erythropoietin, Internal Medicine, 43(8), 649-659.
13. Khan Q., D. Greenhagh (1999). Hopf Bifurcation in Epidemic Models with a Time Delay in Vaccination, IMA J. Math. Appl. Med. Biology, 16, 113-142.
14. Kisseleva T., S. Bhattacharya, J. Braunstein, C. W. Schindler (2002). Signaling through the JAK/STAT Pathway, Recent Advances and Future Challenges, Gene, 285, 1-24.
15. Klingmüller U. (1997). The Role of Tyrosine Phosphorylation in Proliferation and Maturation of Erythroid Progenitor Cells-signals Emanating from the Erythropoietin Receptor, Eur. J. Biochem., 1, 249(3), 637-47.
16. Mackey M. C. (1978). Unified Hypothesis for the Origin of Aplastic Anemia and Periodic Hematopoiesis, Blood, 51(5), 941-956.
17. Mahaffy J. M., J. Belair, M. C. Mackey (1998). Hematopoietic Model with Moving Boundary Condition and State Dependent Delay: Applications in Erythropoiesis, J. of Theor. Biology, 190, 135-146.
18. Marsden J., M. McCracken (1976). The Hopf Bifurcation and Its Applications, Springer-Verlag, New York.
19. Nikolov S. (2008). Stability and Bifurcation Behaviour of Genetic Regulatory Systems with Two Delays, Comptes rendus de l'Academie bulgare des Sciences, 61(5), 585-594.
20. Nikolov S., J. Vera, O. Rath, W. Kolch, O. Wolkenhauer (2008). The Role of Inhibitory Proteins as Modulators of Oscillations in NFkB Signalling, IET Systems Biology, in press.
21. Nikolov S., J. Vera, V. Kotev, O. Wolkenhauer, V. Petrov (2008). Dynamic Properties of a Delayed Protein Cross Talk Model, Biosystems, 91, 51-68.
22. Nikolov S., X. Lai, U. W. Liebal, O. Wolkenhauer, J. Vera (2009). Integration of Sensitivity and Bifurcation Analysis to Detect Critical Processes in a Model Combining Signalling and Cell Population Dynamics, Submitted.
23. Orr J. S., J. Kirk, K. G. Gray, J. R. Anderson (1968). A Study of the Interdependence of Red Cell and Bone Marrow Stem Cell Populations, Br. J. Haemat., 15, 23-34.
24. Pujo-Menjouet L., M. C. Mackey (2004). Contribution to the Study of Periodic Chronic Myelogenous Leukemia, C R Biol., 327(3), 235-244.
25. Ruan Sh., J. Wei (2003). On the Zeros of Transcendental Equations with Applications to Stability of Delay Differential Equations with Two Delays, Dynamics of Continuous and Impulsive Systems Series A: Mathematical Analysis, 10, 863-874.
26. Swameye I., T. G. Muller, J. Timmer, O. Sandra, U. Klingmuller (2003). Identification of Nucleocytoplasmic Cycling as a Remote Sensor in Cellular Signaling by Databased Modeling, Proc Natl Acad Sci USA, 100(3), 1028-1033.
27. Vera J., J. Bachmann, A. C. Pfeifer, V. Becker, J. Hormiga, N. V. Torres-Darias, J. Timmer, U. Klingmuller, O. Wolkenhauer (2008). A Systems Biology Approach to Analyse Amplification in the JAK2-STAT5 Signalling Pathway, BMC Systems Biology, 2, 38.

Assoc. Prof. Svetoslav G. Nikolov, Ph.D.
E-mail: s.nikolov@imbm.bas.bg

Svetoslav Nikolov's research and educational interests are in the fields of mathematical modelling, nonlinear dynamics and bifurcation analysis of systems in cell biology. His M.S. in mechanical engineering he receive from the technical University of Sofia, Bulgaria, in 1994 and Ph.D. degree from the Institute of Mechanics and Biomechanics (IMech) – Bulgarian Academy of Sciences (BAS), in 1999. Since 2005 he has been Associate Professor at IMech and since 2004 a joint position as a lecturer at Faculty of Biology, University of Sofia, Bulgaria.

Xin Lai, M.Sc.
E-mail: xin.lai@informatik.uni-rostock.de

He joins the SBI group of the University Rostock in 2008. He works in the FORSYS Group on Systems Biology for Cancer and Aging (CALSYS). His research is focused on mathematical modelling of signalling pathways, microRNA dynamics and development of computational methodologies for Systems biology.

Prof. Olaf Wolkenhauer, Dr.
E-mail: olaf.wolkenhauer@informatik.uni-rostock.de

His first degree is in control engineering, followed by a Ph.D. at the UMIST in Manchester UK, a joint position with the Department of Biomolecular Sciences. Since 2005 he occupies the Chair in Systems Biology & Bioinformatics at the University of Rostock, Germany (www.sbi.uni-rostock.de). His research interests are in systems biology and bioinformatics, focussing on dynamical inter- and intra-cellular processes.

Julio Vera Gonzalez, Dr.
E-mail: julio.vera@informatik.uni-rostock.de

He holds a degree in Applied Physics from the University of La Laguna (1999, Spain). His Ph.D. was in Biochemistry and Molecular Biology (2005, University of La Laguna). He is currently leading the FORSYS Junior Research Group on Systems Biology for Cancer and Aging (CALSYS, www.sbi.uni-rostock.de/calsys), associated to the group of Systems Biology & Bioinformatics at the University of Rostock, Germany. His expertise is in data-based mathematical modelling of metabolic and cell signalling pathways, predictive simulations and development of mathematical methods and software tools for the analysis of biological systems.