A COMPREHENSIVE DYNAMIC MODEL OF CYCLIC NEUTROPENIA

by

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ABSTRACT

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Neutropenia is a hematological disorder that is defined as having a low level of neutrophils in the bloodstream. Low levels of absolute neutrophil counts leave the body defenseless against and vulnerable to infections. Cyclic neutropenia is a type of neutropenia that is described as the oscillations observed in the level of blood neutrophils. The disorder is mostly treated with a cytokine named recombinant granulocyte colony–stimulating factor, rG–CSF, which is administered via injection. A delicate injection schedule is called for because the treatment procedure is costly. However, treatment experiments on an actual patient require frequent sampling from bone marrow and blood, which simply cannot be allowed as it can be detrimental to the health of the patient. Therefore, modeling is a must to carry out treatment experiments. Accordingly, the main motivation in this thesis is to construct a comprehensive dynamic model of cyclic neutropenia. As human physiology is rich in dynamic complexities, system dynamics is selected as the primary methodology. We first construct a model that represents the regulatory structures of neutrophil production for a healthy person. After validating the model, the neutrophil dynamics of a cyclic neutropenia patient is obtained by simply changing the parameter values, but without changing the model structure. Neutrophil production deficiency is the most mentioned cause of cyclic neutropenia in the literature, which is also confirmed in our study. According to our simulation results, the clearance of the apoptotic neutrophils of CN patients takes longer than normal and apoptotic neutrophils can suppress both the production and effects of G–CSF. As a result of experiments with pathogens, we claim that the oscillatory behavior is a characteristic of the neutrophil–GCSF–pathogen system even for a healthy person. This may shed some light on the periodic symptoms observed in patients with diseases caused by an overactive immune system. We experiment with rG–CSF injections too.

ÖZET

DÖNGÜSEL NÖTROPENİNİN KAPSAMLI BİR DİNAMİK MODELİ

Hematolojik bir hastalık olan nötropeni, kandaki nötrofillerin düşük seviyeli olmasıdır ki bu hastalık vücudu enfeksiyonlara karşı açık ve savunmasız bırakır. Nötropeninin bir türü olan döngüsel nötropeni ise kan nötrofil seviyelerinde gözlemlenen salınımlarla tarif edilir. Döngüsel nötropeni çoğunlukla rG–CSF ile yani rekombinant granülosit koloni uyarıcı faktör olarak bilinen bir sitokinin vücuda enjekte edilmesi ile tedavi edilir. Tedavi prosedürü maliyetli olduğu için kusursuz bir enjeksiyon programı gereklidir. Ancak tedaviyi kusursuzlaştırmak üzere yapılan klinik deneyler, kemik iliği ve kandan sık sık numune alınmasını gerektirir. Hastaya zarar verebileceğinden numunelerin sık alınmasına izin verilemez. Bu nedenle, tedavi deneylerini gerçekleştirmek için modelleme çalışması yapmak bir zorunluluktur. Bu zorunluluğa uygun olarak bu tezde kapsamlı bir dinamik döngüsel nötropeni modeli kurulur. İnsan fizyolojisi dinamik karmaşıklık açısından zengin olduğundan sistem dinamiği temel metodoloji olarak seçilir. İlk olarak sağlıklı bir insanın nötrofil üretiminin düzenleyici yapılarını temsil eden bir model kurulur. Modelin geçerliliği sınandıktan sonra döngüsel nötropeni hastasının nötrofil dinamikleri, model yapısı değiştirilmeden sadece parametre değerleri değiştirilerek elde edilir. Döngüsel nötropeninin literatürde en çok bahsedilen nedeni nötrofil üretim eksikliğidir ve çalışmamız bunu doğrulamaktadır. Simülasyon sonuçlarımıza göre döngüsel nötropeni hastalarında apoptotik nötrofillerin temizlenmesi normalden daha uzun sürer ve apoptotik nötrofiller G–CSF'nin hem üretimini hem de etkilerini baskılarlar. Patojenlerle gerçekleştirdiğimiz deneylere göre salınım davranışı nötrofil–GCSF–patojen sisteminin bir özelliğidir ve bu özellik sağlıklı bir insan için de geçerlidir. Tezin bu sonuçları aşırı aktif bağışıklık sistemine sahip hastalarda gözlenen periyodik semptomlara bir ışık tutabilir. Son olarak tezde rG–CSF enjeksiyonlarıyla deneyler gerçekleştirilir.

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LIST OF ACRONYMS/ABBREVIATIONS

ANC	Absolute Neutrophil Count
CLD	Causal Loop Diagram
CLPs	Common Lymphoid Progenitors
CMPs	Common Myeloid Progenitors
CN	Cyclic Neutropenia
DNA	Deoxyribonucleic Acid
DT	Derivative with respect to t
ELANE	Elastase, Neutrophil Expressed
ESA	Erythropoiesis-Stimulating Agents
G-CSF	Granulocyte Colony-Stimulating Factor
HSCs	Hematopoietic Stem Cells
IL-17	Interleukin 17
IL-23	Interleukin 23
MSC	Myeloid Stem Cells
PAMPs	Pathogen-Associated Molecular Patterns
PMP	Post-Mitotic Pool
rG-CSF	Recombinant Granulocyte Colony-Stimulating Factor
SCN	Severe Congenital Neutropenia
SD	System Dynamics

1. INTRODUCTION

Human body is constantly in contact with its environment. The body absorbs nutritious substances from the environment and excretes toxins to it. By doing so, the body aims to maintain its homeostatic balance. Pathogens such as bacteria and viruses may also enter the body. Pathogens, and the antigens produced by pathogens, disturb the homeostatic balance. Thus, they must be eliminated. Immune system is responsible for this elimination process [1]. White blood cells, such as lymphocytes and neutrophils, is an essential part of the immune system. White blood cells counteract the pathogens by phagocytosis and by producing antibodies [2]. In this thesis, we focus on neutrophils, which corresponds to seventy percent of the white blood cells [3].

Neutrophils are produced in the bone marrow. This production is a multistep process and it starts with the hematopoietic stem cells (HSCs), which have self-renewal capacity. HSCs are the precursors of all kinds of blood cells; they either divide to reproduce new HSCs or they differentiate into common myeloid progenitors (CMPs) or common lymphoid progenitors (CLPs) [4]. Myeloblasts, megakaryoblasts, and proerythroblasts arise from CMPs. Four branches derive from myeloblasts; neutrophils, basophils, eosinophils, and monocytes are formed at the end of each branch. Megakaryoblasts evolve into thrombocytes (i.e., platelets). Proerythroblasts differentiate into erythrocytes (i.e., red blood cells). Myeloblasts go through a couple of phases to become neutrophils. These are promyelocytes, myelocytes, metamyelocytes, band neutrophils, and segmented neutrophils in the given respective order. Finally, when the segmented neutrophils are released into the blood stream, they become blood neutrophils [5–7].

Neutrophils have a short lifespan. The half-life of neutrophils in blood is between 6-8 hours. 10^{11} neutrophils enter and leave the blood on a daily basis. A healthy human has approximately 0.5 x 10^{11} neutrophils in his blood. When pathogens enter the body, the pathogen receptors (i.e., pattern recognition receptors) recognize them. The receptors send signals to the neutrophils. After receiving the signal, neutrophils head towards the infection site and they create higher neutrophil intensity at the infection

site [8–10]. Neutrophils take active role in the fight against both bacterial and viral infections. The number of neutrophils increases more in bacterial infections than in viral infections [11].

Neutrophils use cytotoxic substances to eliminate pathogens. When they become senescent, they are more prone to spread this cytotoxic content into tissues [12]. This is not only harmful to the pathogens, but to the cells of the host as well. Hence, senescent neutrophils must be removed. Removal process is described as programmed cell death, in other words, apoptosis [13]. Macrophages, which are a type of monocytes, participate in this process. Neutrophils transmit signals as they go through apoptosis. Macrophages pick up these signals and phagocytose apoptotic neutrophils. After macrophages ingest the apoptotic neutrophils, they restrain the secretion of interleukin-23 (IL-23) cytokine that eventually effects the neutrophil production; cytokines, which are small proteins, are mainly responsible for signaling among cells and they are produced by the immune and endothelial cells. IL-23 reduction restricts IL-17 release. In turn, this decrease in IL-17 limits granulocyte colony-stimulating factor (G-CSF) production [14]. G-CSF is a regulatory cytokine for neutrophils, which is produced within the body, whereas recombinant G-CSF (rG-CSF) is produced in a laboratory setting and administered to the body via injection. According to our understanding, when G-CSF is high, it has four up-regulatory effects on neutrophils: (1) it enhances the proliferation of myelocytes and promyelocytes [15]; (2) it shortens the transit times of mitotic pool and post mitotic pool [16, 17]; (3) it accelerates the release of stored neutrophils from the bone marrow to the bloodstream [18]; (4) it effects the lifetime of neutrophils in tissues [19]. Consequently, when macrophages ingest neutrophils, G-CSF production is restrained, which, in time, down-regulates the neutrophils.

Neutrophil production is a dynamic process, which involves numerous feedback loops. Disruptions in this process would potentially result in diseases as the body gets susceptible to infections. Inefficient neutrophil production leads to low levels of absolute neutrophil count (ANC), which is defined as neutropenia. The most common early signs of neutropenia are mouth ulcers and fever. If left untreated, neutropenia can be life threatening for the patient [20]. There are several reasons of neutropenia

such as genetic factors, dietary deficiencies, and treatment side effects. Furthermore, some infections may attack the neutrophil production process, resulting in neutropenia. HIV and tuberculosis are the examples of such infections. Insufficient intake of vitamins and minerals such as vitamin B12 and copper can be listed under dietary deficiencies. Treatment of cancer with chemotherapy has destructive effect on bone marrow cells, which reduces ANC levels and leads to neutropenia [21].

Ethnic neutropenia, benign familial neutropenia, and cyclic neutropenia are caused by genetic factors. Thus, these are congenital diseases. In this thesis, we mainly focus on cyclic neutropenia (CN), which is a kind of neutropenia that is defined by periodic low levels of circulating neutrophils. In other words, neutrophil density shows oscillations with a cycle of three weeks on the average [20]. According to the relevant literature, the period of these oscillations ranges between 19 to 40 days [22]. There is a shared belief that CN is caused by mutations in a part of the DNA that is responsible for encoding neutrophil elastase (ELANE), which is a protein that organizes the infection response [23, 24]. Nevertheless, mutations in ELANE does not provide an explanation for the ANC cycling. Mir et al. [23] study pathomechanism of CN and they provide new insights about the properties of the dynamics of the ANC cycles. They claim that there exist a cyclic balance between apoptosis, which is caused by endoplasmic reticulum stress, and proliferation of G-CSF-stimulated neutrophil precursors. Dale and Hammond [20] pose questions about pathophysiology of CN: "Why are these cycles so regular? Are there transitions between CN and other hematological disorders?". Their answers to these questions are not direct, but based on the correlative information. For the first question, they imply that the regular cycles of roughly 21 days may be related to the length of the production process of neutrophils that lasts approximately 20 days. The second question is about the cyles and fluctuations seen in other blood cells. According to their clinical research, in some CN patients cycles are observed in all of the blood cells linking them to neutrophil and monocyte cycles. However, for the other CN patients, it is difficult to link the fluctuations seen other blood cells to neutrophils and monocytes cycles. Likewise, it is also difficult to prove that CN cycles are not the primary cause of at least some of the fluctuations seen in other blood cell types. In this thesis, we do not focus on modeling the fluctuations

seen all blood cell types.

The treatment alternatives for CN are injecting rG-CSF, using antibiotics, taking prednisone pills, swallowing lithium carbonate tablets, and transplanting bone marrow [25, 26]. Injecting rG-CSF is the most common treatment alternative for CN because it is more effective than the other treatment options. Long-term treatment with rG-CSF is appropriate for CN patients. Therapeutic studies show that the adverse effects of rG-CSF use, such as leukemia and myelopoiesis exhaustion, are quite rare in CN patients [27]. However, G-CSF treatment (i.e., administering rG-CSF to the body via injection) only elevates the neutrophil levels and does not obliterate the oscillations. Although there is an improvement to the conditions of the patient by regular rG-CSF injections, cyclic neutropenia is not completely eliminated. Another concern is that injecting rG-CSF is an expensive application and, therefore, demands for delicate scheduling [28]. Accordingly, scientists carry out experiments to discover physiology of the disorder and develop better treatment alternatives. The studies on CN require a lot of bloodwork and sampling from the bone marrow, which are painful processes for the patient. Moreover, frequent sampling can be detrimental to the health of the patient . Therefore, these requirements become an important factor that stall the studies [29]. Consequently, there is a strong need for modeling studies that enable painless and fast experimentation.

There are numerous modeling studies on neutrophil production, neutropenia, and cyclic neutropenia as a result of the need mentioned at the very end of the previous paragraph. Early mathematical modeling studies of hematopoiesis (i.e., blood cell production) date back to 1960s [30]. According to Pujo-Menjouet [30], King Smith and Morley [31] set up one of the first computer simulation models of CN. The model includes two feedback loops. One of the loops affects the production of early neutrophil cells. The other has effect on the release of granulocytes into the bloodstream. They show that the oscillatory dynamics can be derived from a simple model with feedback mechanisms. From the same model, they generate granulocyte dynamics for a healthy person, a neutropenia patient, and someone suffering from cyclic neutropenia by changing the flow of marrow granulocyte production. The main focus of the paper

is to show that the two feedback loops provide an explanation for the cycles observed in granulocyte levels. However, the paper is not comprehensive as the mechanisms creating the two feedback loops are not represented in the model; the intermediate variables between the granulocyte concentration and the flow of marrow granulocyte production and the variables between the granulocyte concentration and mature granulocyte storage time are not in the model. We quote this sentence from the paper, "Since the physical reality for much of the model is uncertain, many of the operating functions used have been chosen somewhat arbitrarily.". Accordingly, the main claim of the paper is that "... granulopoiesis is in reality controlled by two feedback loops at least somewhat similar to those used in the simulation.". Comparatively, our study aims to provide a richer and more detailed explanation about the mechanisms creating CN. Mackey and Glass [32] present the usage of delay differential equations in modeling of physiological systems such as respiratory system and hematopoiesis. Mackey [33] constructs a model that represents different phases of hematopoietic stem cells and hypothesizes that the dynamics of aplastic anemia and periodic hematopoiesis (i.e., cyclic neutropenia) originate from increased death rate of stem cells (i.e., excess loss of HSCs), which is a hypothesis incorporated in our model. Bernard et al. [34] extends Mackey's model by adding a state variable that represents circulating neutrophils. This model has two state variables and delay differential equations. The authors assert that oscillations in CN occur because of increased death rate of stem cells, which is in accordance with the hypothesis of Mackey [33]. Colijn and Mackey [35] model neutrophil production which is derived from a previous model introduced by Bernard et al. [34]. In addition to neutrophils, they include thrombocytes and erythrocytes in the model aiming to observe the effects of CN on thrombocytes and erythrocytes, which is out of the scope of this study. There are modeling studies that focus on analyzing the effect of treatment scenarios with rG-CSF administration [29, 36]. These studies first capture the dynamics of CN and observe the changes in the dynamics under different G-CSF treatment scenarios; they find that G-CSF administration leads to larger amplitude and shorter period of oscillations in neutrophil levels. The models presented in Mackey [33], Bernard et al. [34], Foley et al. [29], Colijn et al. [36], and Foley and Mackey [37] do not explicitly represent G-CSF, the different effects of G-CSF on neu-

trophils and neutrophil production, and the effect of apoptotic neutrophils on G-CSF. In some of those studies, rG-CSF is reflected through parameter calibration. These studies ignore pathogens and the dynamic relationships between pathogens, G-CSF, and neutrophils. Only circulating neutrophils are explicitly represented, but tissue neutrophils, senescent neutrophils, and apoptotic neutrophils are not. These models are not comprehensive; the aim is to generate CN dynamics. As a result, their explanatory power is limited. In this study, the aim is to create a comprehensive model that will explain the structures responsible for creating CN in depth. Therefore, the model that will be created in this study will explicitly include G-CSF, the different effects of G-CSF on neutrophils and neutrophil production, the effect of apoptotic neutrophils on G-CSF, pathogens and the dynamic relationships between pathogens, G-CSF, and neutrophils. The model will explicitly include circulating neutrophils, tissue neutrophils, senescent neutrophils, and apoptotic neutrophils. The comprehensive model that will be constructed in this study will enable us to identify the potential mechanisms behind cyclic neutropenia and it will serve as an experimental platform. Craig et al. [38] extend the model of Foley and Mackey [37] by adding a bone marrow reservoir and a marginated neutrophil compartment. After creating the extended model, they analyze the response of the body to chemotherapy drugs. Recently, Irsoy et al. [39] build a system dynamics model of neutrophil production. They study the trade-offs of G-CSF treatment for chemotherapy induced neutropenia. Studying chemotherapy drugs is not a concern in this study.

According to our understanding, (i) the existing CN models are based on the model presented by Bernard et al. [34], which is not comprehensive as discussed in the previous paragraph; (ii) the existing CN models mainly focus on capturing the dynamics of CN rather than bringing structural explanations for the causes of CN; (iii) there is a conviction in the literature that CN is mainly caused by early apoptosis of neutrophil precursors [24, 40, 41], which is a hypothesis that will be incorporated in our model, however, we believe that this cannot be the only cause behind CN; (iv) the existing studies mainly carry out simulations with different rG-CSF administration regimes by mainly calibrating the parameters of the non-comprehensive model. In this thesis, the main motivation is to create a comprehensive model of CN that is based on

the literature, but not on the existing CN models in the literature. The model that will be constructed will address the critiques mentioned in this paragraph; the mechanisms behind cyclic neutropenia will be identified during the modeling process and the complete model will serve as an experimental platform. Therefore, different than the existing model in the literature, the aim of the model that will be constructed in this study is not only to capture the dynamics of CN, but also to represents the structures responsible for these dynamics. Consequently, this thesis has three objectives. The first objective is to construct a model that represents the regulatory structures of neutrophils for a healthy person. After validating the model, we aim to obtain the neutrophil dynamics of a cyclic neutropenia patient without changing the model structure of the comprehensive model. We will first determine the parameters that will convert the neutrophil dynamics of the model for a healthy person to the neutrophil dynamics of a CN patient; next, we will experiment with the parameters such as G-CSF production rate and clearance time of apoptotic neutrophils and determine the values of these parameters. By calibrating the model for a cyclic neutropenia patient, we aim to identify the potential mechanisms behind cyclic neutropenia, which is the second objective of this thesis. As the third and final objective, we aim to demonstrate that the model can be used as an experimental platform by experimenting with different doses and frequencies of rG-CSF injections and evaluating the effectiveness of G-CSF treatment on managing cyclic neutropenia symptoms.

In Chapter 2, the problem is described in detail and research questions are given. The pathological characteristics of CN are also explained in this chapter together with the physiological background. We develop our model by following the methodology of system dynamics (SD). In Chapter 3, we explain the reasons why we select SD to create a comprehensive model of CN. In Chapter 4, we represent the overview of the model by using a causal loop diagram (CLD). The CLD depicts the key feedback mechanisms captured by the model. We present the compartments of the model, give the associated equations, and describe mathematical formulations in Chapter 5. Model behavior and model validation for a healthy person are given in Chapter 6. In this chapter, we carry out an equilibrium run to show the homeostatic balance of neutrophils, experiment with different levels of pathogen entry so as to qualitatively and quantitatively reflect

different infection cases, and validate the model by using data from the literature. In Chapter 7, we explain the transition process from a healthy person to a CN patient. We also present our insights about the pathophysiology of CN. In Chapter 8, we evaluate the model sensitivity to parameter values such as pathogen entry to the body and clearance time of apoptotic neutrophils. Experiments with rG-CSF injections and their results are given in Chapter 9. In the last chapter, we write down our conclusions about the pathomechanism of CN, summarize the results obtained from the experiments, and list research subjects that require further study.

2. PROBLEM DEFINITION AND RESEARCH OBJECTIVES

Cyclic neutropenia is a type of neutropenia that is distinguished by oscillations in neutrophil levels. These oscillations has approximately a 21-day period; period ranges between 19 to 40 days [34]. Extremely low levels of neutrophils, which are assumed to be levels below 12 percent of the normal level (4.3 x 10^9 cells/L [42]), are observed in each cycle. This severe neutropenic phase lasts three to 10 days [43]. CN was firstly diagnosed in the 1910s [20]. Most of the patients are diagnosed in their childhood. The CN patients show periodic symptoms such as mouth ulcers and fever, which significantly reduces the quality of life. Furthermore, an infection level that may not result in a disease in a normal healthy person, may be life-threatening for CN patients if left untreated. Neutrophil levels are affected by a couple of factors including cytokines, infections, and vitamin deficiencies. Even exercise has an effect on neutrophil levels in blood [44]. The duration of the whole neutrophil production process, approximately 20 days, is much longer compared to its circulating lifetime in the bloodstream, which introduces significant delays in the feedback mechanisms of neutrophils. The delays and other complexity elements creates difficulties in obtaining treatment alternatives that completely eliminate CN.

Current treatment options do not provide entire healing from the disease as we stated in the introduction chapter. Regular drug usage is a must for most of the CN patients except for some mild cases. The application of rG-CSF alleviates the symptoms of CN by increasing the average blood neutrophil levels but it does not eradicate oscillations. It leads to an increase in the amplitude of the oscillations and shortens the period of the oscillations from 21 days to 14 days. Although there is a decrease in the duration of time spent below the 12% of the average neutrophil level of a healthy human, the values of the trenches remain the same. There is an improvement, but as patients with low neutrophil levels are prone to infections, the problem persists. Therefore, even the continuous application of the current best treatment alternative

that is the administration of rG-CSF is not successful in increasing the trenches of the oscillations above the critical 12% level. Moreover, rG-CSF administration is an expensive treatment procedure. As there is no perfect treatment option for CN and as there are many interacting complexity elements related to the disorder, there is a strong need to develop a better understanding about the pathomechanisms of CN.

The neutrophil production process involves multiple phases. It is regulated by several feedback loops. The neutrophils, G-CSF, and pathogens are the main variables of the feedback loops. The modeling studies that address cyclic neutropenia involve stem cells, circulating neutrophils and some intermediate phases of neutrophil production. However, G-CSF is not explicitly represented in those models; Mackey [33], Bernard et al. [34], Foley et al. [29], Colijn et al. [36], and Foley and Mackey [37]. Also, the models in those studies do not include the neutrophils in tissues, senescent neutrophils, and apoptotic neutrophils. Neutrophils operate in tissues and some of the feedback mechanisms are triggered by apoptotic neutrophils. Therefore, they should be represented in a complete model. We aim to address these issues by constructing a comprehensive dynamic model of CN, which is a challenging task as the problem involves a rich set of complexity elements.

In this study, our first objective is to construct a comprehensive model that represents the regulatory structures of neutrophils for a healthy person by referring to relevant literature. We aim to represent the neutrophil lifecycle from the stem cell phase to neutrophil apoptosis and clearance. We intend to explicitly include the pathogens and G-CSF as compartments. The second objective is to identify the potential mechanisms behind CN. For this, we make the transition from the model for a healthy person to a model for a CN patient. We aim to detect the most important parameters and their corresponding values that enables this transition. The structure of the model remains exactly the same during the transition process. We claim that our approach of having a structurally sound model that can generate dynamics for a healthy person and a CN patient will improve our understanding of the physiology of CN. Our final objective is to show the use of the model as an experimental platform. For this purpose, we aim to experiment with different doses and frequencies of rG-CSF injections and evaluate

the effectiveness of G-CSF treatment. An efficient dose and frequency of rG-CSF administrations may reduce the cost. For the CN patient, it may also create a further decrease in the duration of time spent below the 12% of the average neutrophil level of a healthy human.

3. RESEARCH METHODOLOGY

A real-life dynamic system consists of interacting elements that change overtime. These interactions create feedback-loops, some of the causal effects are delayed, and there are many non-linear relationships that cannot be simplified and correctly represented as linear. The behavior of a simplified model that ignores these elements of complexity can analytically be obtained. However, such a simplification sacrifices model validity for mathematical simplicity [45]. When over simplification is avoided, we end up with models that cannot be analyzed analytically. Therefore, a simulation methodology based on numerical analysis is a must for such models.

In this thesis, we use system dynamics (SD) as the main methodology. SD is a collection of conceptual and mathematical tools that are used to model and analyze complex dynamic systems. Causal loop diagrams (CLDs) and stock-flow diagrams are two important tools of SD methodology. CLDs are used to represent a complete picture of causal relationships between the variables and the important feedback loops that are responsible for the potential dynamics that can be created by the interacting elements. Stock-flow diagrams focus on the state variables (i.e., stocks) and the flow variables that are connected to the stocks. Stocks are critical because they are the memory variables; they define the state of a system and they have inertia. Flows explain how stocks change over time. Complexity elements such as delays and non-linear relationships are also represented in SD models. Effect formulations in the form of graphical functions are used to depict non-linear relationships between variables.

Physiological systems involve components of complexity such as delays, non-linear relationships, and feedback loops. Circulatory, respiratory, and excretory systems are examples of such physiological systems; they comprise complexity elements as they are self-regulatory in nature. Thus, SD is an appropriate methodology for analyzing physiological problems. There are many successful SD modeling studies on physiological systems. We selected three studies as examples: Demirezen and Barlas [46] model the cholesterol dynamics in a healthy person and in a hypercholesteremic patient; İrsoy et

al. [39] model the neutrophil production process aiming to experiment with chemotherapy application regimes for cancer patients; Rogers et al. [47] model the red blood cell production and suggest individual use of erythropoiesis-stimulating agents (ESA) protocols. The ESA protocols suggested by Roger et al. [47] are used in Mayo Clinic with actual patients and improvements in their conditions are observed as predicted by that study. We aim to create a comprehensive model of CN without sacrificing the realism of the model for mathematical simplicity [45] and we claim that SD is a suitable simulation methodology for modeling the regulatory structure of neutrophils, because the production process of neutrophils is dynamically complex.

4. MODEL OVERVIEW

The human body controls the homeostasis of neutrophils with a rich set of feedback loops. Neutrophil production process is affected by physiological factors such as cytokines and pathogens. The scope of the model covers neutrophils and the elements interacting with them. Identifying the causal relations between the neutrophil cells and other elements is critical because the behavior of the model is a result of their interactions. We create a causal loop diagram (CLD) to represent causal relationships.

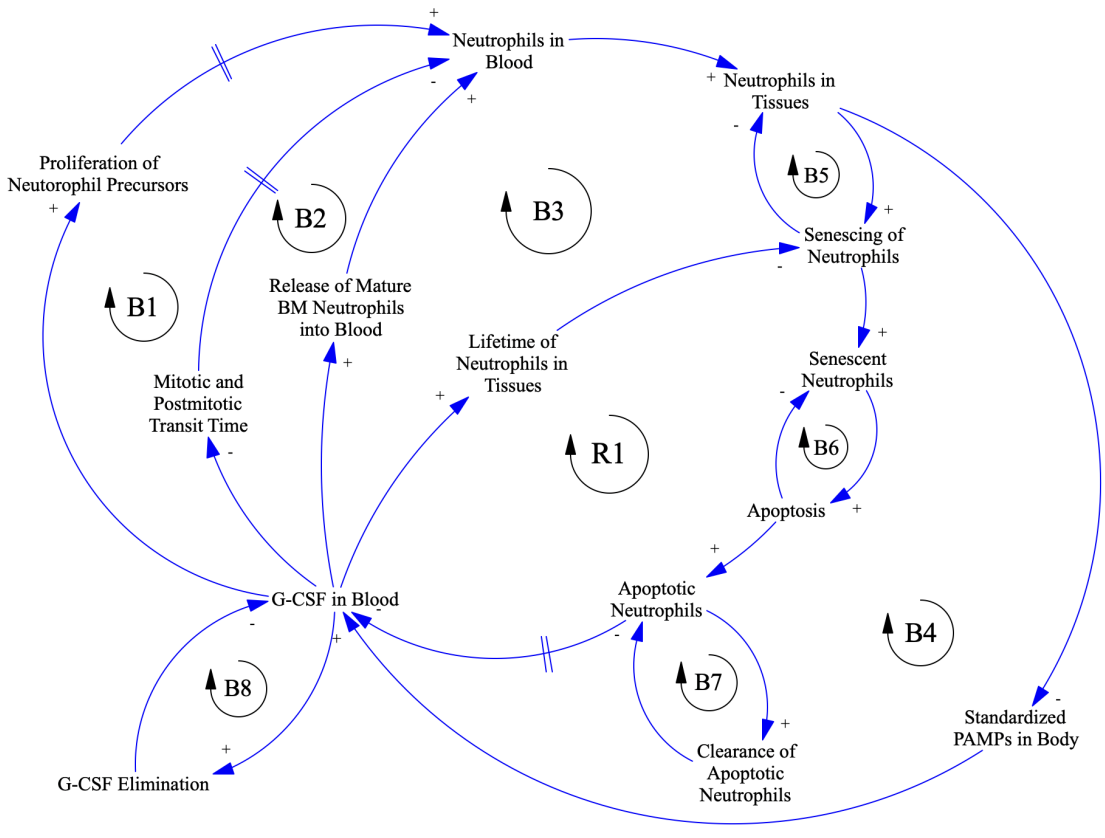


Figure 4.1. Causal loop diagram of the comprehensive model.

We show a causal relationship between two variables by using an arrow: $x \rightarrow y$. Plus (+) sign on the arrow between x and y denotes that there is a positive causal effect of x on y. A positive causal relationship means that a change in the input variable x causes a tendency of change in the output variable y in the same direction. On the

contrary, minus (-) sign on the arrow between x and y denotes that there is a negative causal effect of x on y. A negative causal relationship describes the situation that a change in the input variable x causes a tendency of change in the output variable y in the opposite direction. A mark on an arrow implies that the causal effect is delayed. Two or more causally related variables may form a closed loop known as a feedback loop. A feedback loop can be reinforcing or balancing. A reinforcing feedback loop amplifies a change in a variable on that loop. A balancing feedback loop counteracts a change in a variable on that loop.

Representing all variables in a CLD may cause confusion rather than clarification. For this reason, we select only the most important variables to include in the diagram. For example, we do not show the whole production process here. Originally, neutrophil production is comprised of six phases in the bone marrow. However, we do not show these phases in the CLD. We show neutrophils in blood, neutrophils in tissues, senescent neutrophils, and apoptotic neutrophils in the CLD. Furthermore, apoptotic neutrophils block the effects of G-CSF on neutrophils in the model, but this blocking mechanism is not depicted in the CLD for simplicity. CLD of the model is given in Figure 4.1.

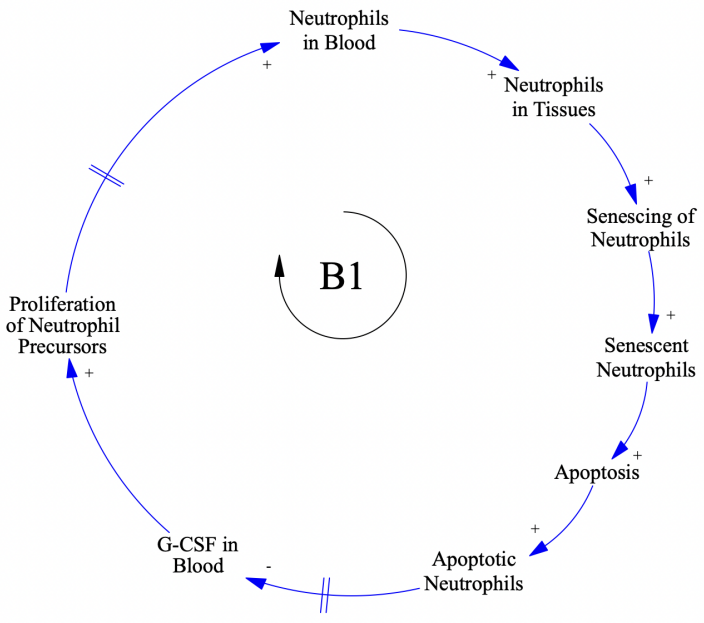


Figure 4.2. B1 feedback loop.

The causal loop diagram of the model is mostly comprised of balancing feedback loops such that only one of the eight feedback loops is a reinforcing feedback loop. For a healthy person, the system is dominated by balancing loops. Neutrophils are regulated because its high and low levels can be harmful to the body.

There are three stimulatory effects of G-CSF on the blood neutrophils. Therefore, there are three paths linking *G-CSF in Blood* and *Neutrophils in Blood*. Each path involves an intermediate variable. These are *Proliferation of Neutrophil Precursors*, *Mitotic and Postmitotic Transit Time*, and *Release of Mature BM Neutrophils into Blood*.

High G-CSF level in the blood enhances the proliferation of neutrophil precursors. We do not show neutrophil precursors in the diagram. We show the effect as a variable. Neutrophil precursors become mature neutrophils by passing through the production phases. Mature neutrophils are stored in the bone marrow for a while and later they enter the blood. Considering all these phases, we add a delay mark to the connection between *Proliferation of Neutrophil Precursors* and *Neutrophils in Blood*. *Neutrophils in Blood* transmigrate into tissues after a short period of time. Therefore, the blood neutrophils have a positive effect on the tissue neutrophils. *Neutrophils in Tissues* becomes senescent. *Senescing of Neutrophils* is the name of process of being senescent. *Senescing of Neutrophils* is the outflow of the *Neutrophils in Tissues*. This means that when *Neutrophils in Tissues* are increased, *Senescing of Neutrophils* is increased too. *Senescing of Neutrophils* is also the inflow of *Senescent Neutrophils*. *Senescing of Neutrophils* positively affects *Senescent Neutrophils*. *Senescent Neutrophils* go into apoptosis after a while and turn into *Apoptotic Neutrophils*. Therefore, we refer the outflow of *Senescent Neutrophils* as *Apoptosis*. When *Senescent Neutrophils* increase, *Apoptosis* increases too. *Apoptosis* positively affects *Apoptotic Neutrophils*. Excess accumulation of *Apoptotic Neutrophils* blocks the production of G-CSF and causes a decrease in *G-CSF in Blood* with a delay. This illustrates a negative causal relationship. The loop is completed with *G-CSF in Blood*. This mechanism is the first balancing loop, B1. It is shown in Figure 4.2.

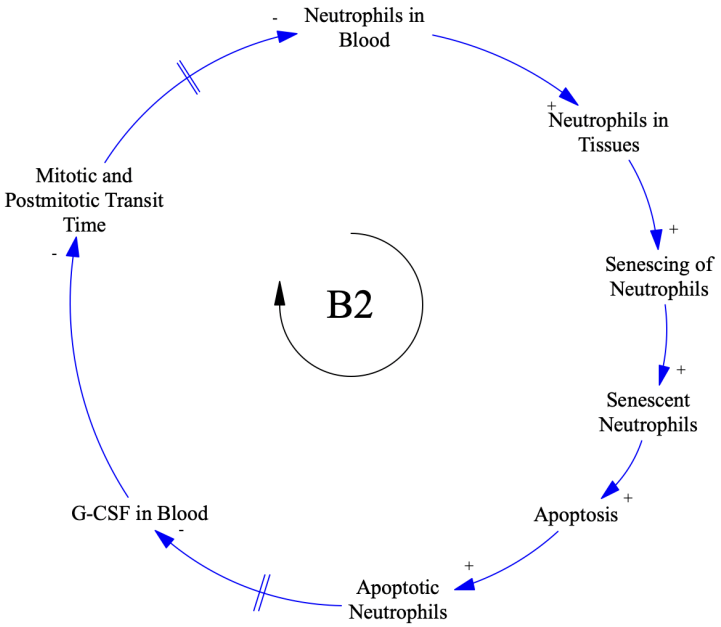


Figure 4.3. B2 feedback loop.

Another effect of high G-CSF on neutrophils is that it shortens the time spent in the mitotic and postmitotic production phases. This leads to an increase in *Neutrophils in Blood*. Apparently, there is a negative relationship between *G-CSF in Blood* and the transit times of mitotic and postmitotic phases. We show this effect in the third balancing feedback loop as a variable named *Mitotic and Postmitotic Transit Time*. The rest of the variables on the B2 feedback loop are the same as in B1. There is a delay between *Mitotic and Postmitotic Transit Time* and *Neutrophils in Blood* because the production process takes time. This feedback loop is represented in Figure 4.3.

The body stores *Mature Neutrophils* in the bone marrow for a while. They are used as a backup in case of emergencies such as a bacterial infection. Rapid release of mature neutrophils into the blood is triggered by the rise of G-CSF level in blood resulting in a quick response to the infection. B2 balancing loop represents this quick acting mechanism (see in Figure 4.4). High levels of *G-CSF in Blood* leads to an increase in the value of the flow named *Release of Mature BM Neutrophils into Blood* and this in turn increases *Neutrophils in Blood*. The rest of the variables in B3 are the same as in B1 and B2.

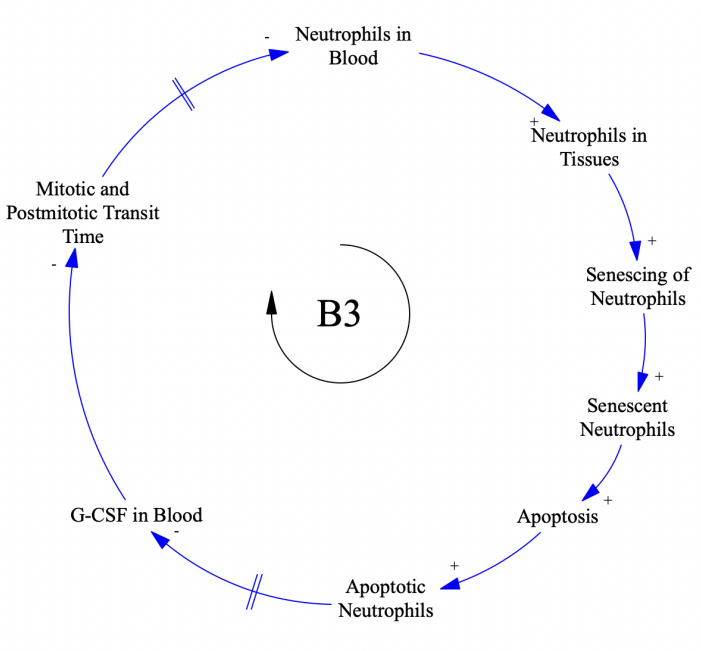


Figure 4.4. B3 feedback loop.

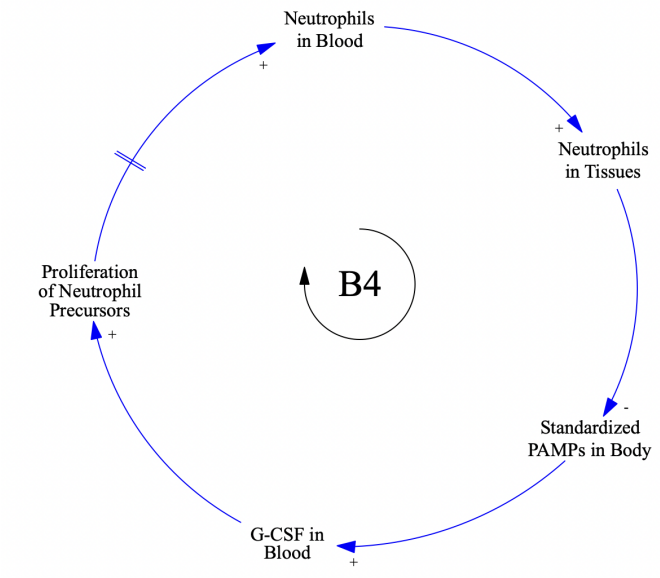


Figure 4.5. B4 feedback loop.

The body detects pathogens when they enter the body. Pathogens are eliminated by *Neutrophils in Tissues*. We represent pathogens as a variable: *Standardized PAMPs in Body*. When pathogens increases, the body produces more G-CSF than it does in the steady state condition [60]. As we mentioned before, G-CSF has three up-regulatory effects on neutrophils. Therefore, there are three balancing loops here. However, we only show one path instead of three paths for simplicity. Figure 4.5 shows the mechanism involving pathogens. We name the loop as B4,which summarizes three balancing loops acting through G-CSF in blood.

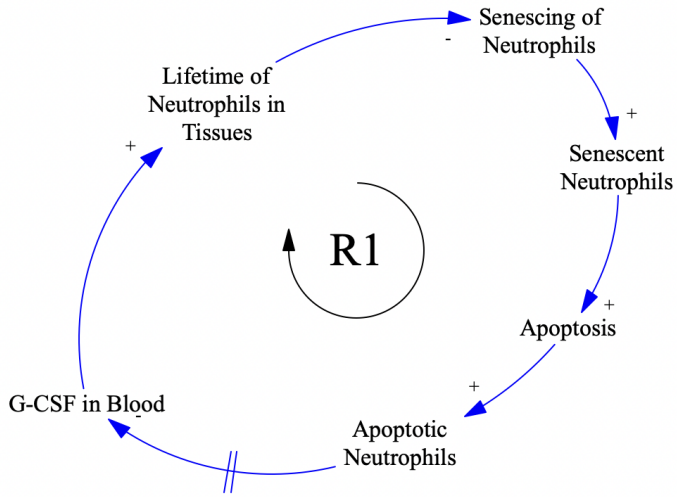


Figure 4.6. R1 feedback loop.

The only reinforcing loop, R1, is presented in Figure 4.6. The feedback loop includes the effect of G-CSF on *Lifetime of Neutrophils in Tissues*. According to the studies, high G-CSF extends the lifetime of *Neutrophils in Tissues* [73, 15]. Longer lifetime leads to a decrease in *Senescing of Neutrophils* which is the outflow of *Neutrophils in Tissues*. This, in turn, results in an accumulation of *Neutrophils in Tissues*. On the other hand, *Senescent Neutrophils* are reduced as a result of the decrease in *Senescing of Neutrophils*. *Senescent Neutrophils* are followed by *Apoptosis* and then *Apoptotic Neutrophils*. Finally, the loop is completed with *G-CSF in Blood*. In summary, a sudden increase in *G-CSF in Blood* is supported by preventing an abrupt increase in *Apoptotic Neutrophils* that stops G-CSF production.

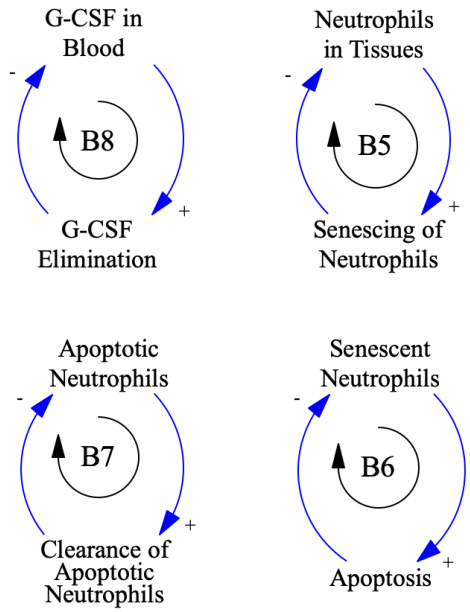


Figure 4.7. B5, B6, B7, B8 feedback loops.

The outflow variables *G-CSF Elimination*, *Senescing of Neutrophils*, *Clearance of Apoptotic Neutrophils*, and *Apoptosis* depend on the stock variables *G-CSF in Blood*, *Neutrophils in Tissues*, *Apoptotic Neutrophils*, and *Senescent Neutrophils*, respectively. They are defined as proportions of the associated stock variables. For example, *G-CSF Elimination* is the outflow of *G-CSF in Blood* and it is defined as *GCSF in Blood / GCSF Elimination Time*. Here, although the stock variable positively affects the outflow, the outflow affects the stock variable negatively. At the end, this mechanism creates a balancing loop, B8. Formulated in the same manner, B5, B6, and B7 are balancing loops too. (See Figure 4.7)

5. MODEL DESCRIPTION

In this study, the model of neutrophil dynamics is constructed based on a normal male person that has a weight of 70 kg and has 5.3 L blood volume. The model consists of five compartments. These are the neutrophil production compartment, compartment for blood and tissue neutrophils, G-CSF compartment, pathogen compartment, and compartment for the effect of apoptotic neutrophils on G-CSF. The neutrophil production compartment describes the production phases of neutrophils in the bone marrow. There are seven stock variables in the production compartment. The production starts with myeloid stem cells (i.e., common myeloid progenitors), which is represented as a stock variable, and ends with the stored neutrophils in PMP (i.e., mature segmented neutrophils), which is another stock variable. There are five other stock variables that follow myeloid stem cells and that come before the stored neutrophils in PMP. These stock variables are described in Section 5.1. The compartment for blood and tissue neutrophils represents the part of neutrophil life cycle after neutrophils are released into the bloodstream. There are five stocks in the compartment for blood and tissue neutrophils. These are circulating neutrophils, marginated neutrophils, neutrophils in tissues, senescent neutrophils, and apoptotic neutrophils. The G-CSF compartment includes a stock variable that stands for the G-CSF amount in the body and the effects of G-CSF on neutrophils. We assume that the human body is constantly in contact with internal and external pathogens. Accordingly, we have a compartment for pathogens. The pathogens serve as a triggering mechanism for G-CSF production and they are eliminated by the tissue neutrophils. Lastly, the compartment for the effect of apoptotic neutrophils on G-CSF illustrates the mechanism that restrains the G-CSF production caused by the excessive amounts of apoptotic neutrophils. This mechanism prevents an unwanted increase in the number of apoptotic neutrophils because, beyond a certain level, apoptotic neutrophils may become harmful to the body.

5.1. Neutrophil Production Compartment

In this compartment, we illustrate the neutrophil production line in the bone marrow. There are seven types of cells in the bone marrow that constitute this production line. In the medical literature, the cell types in the bone marrow are categorized into three main groups. These are the stem cell pool, mitotic pool, and post mitotic pool. The stem cell pool includes HSCs and myeloid stem cells. The mitotic pool includes myeloblasts, promyelocytes, and myelocytes. The post mitotic pool includes metamyelocytes, band neutrophils, and segmented neutrophils. The cells in the stem cell pool and in the mitotic pool are capable of proliferation (i.e., reproduction), but the ones in the post mitotic pool are not [3, 10].

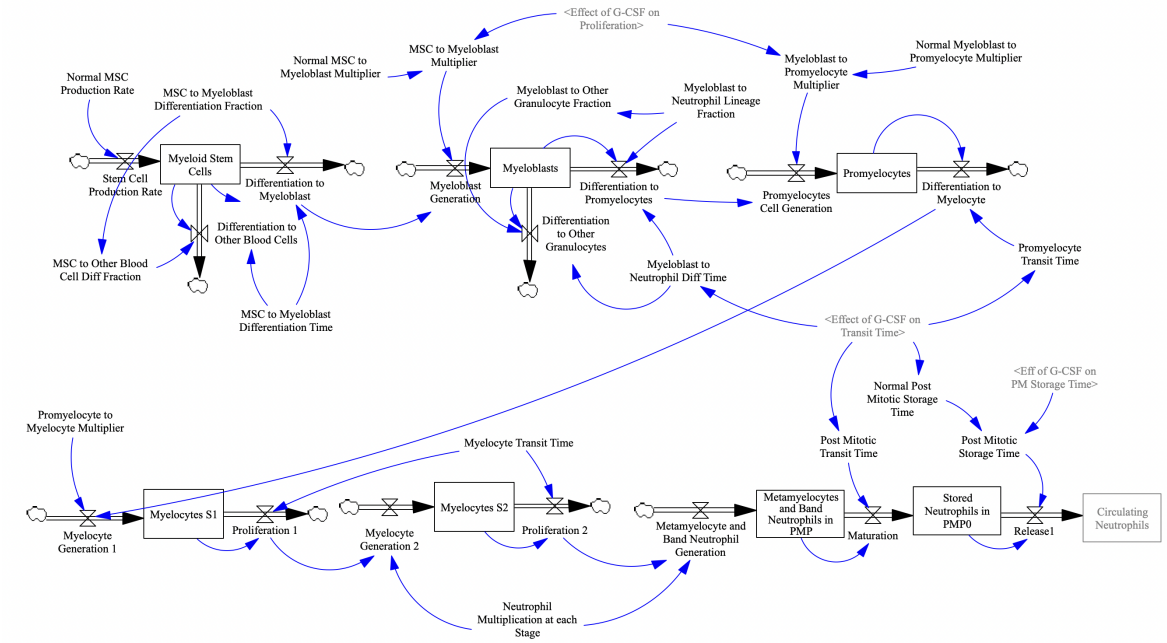


Figure 5.1. Stock-flow diagram of neutrophil production compartment.

According to the literature, a cell can carry out eight cell divisions between myeloblasts and metamyelocytes. However, not all cells undergo eight divisions [9]. Some of them differentiate after the first or the second division, while others continue to divide. After the myelocyte stage, the cells can no longer proliferate. They only mature and differentiate into the next cell type [48]. In our model, neutrophils are pro-

duced in the following line: myeloid stem cells, myeloblasts, promyelocytes, myelocytes, metamyelocytes, band neutrophils, and segmented neutrophils; each one of these seven cell types corresponds to a state variable (i.e., stock variable). Accordingly, there are seven stock variables in this compartment and each stock variable differentiates into the next one. The cells spend specific times in the stages. These durations create delays that are formulated as first-order material delays in the model. The unit of the stock variables is (cells x 10^9) and time unit of the model is hours.

In the bone marrow, the production of neutrophils starts with the hematopoietic stem cells (HSCs). For simplicity, we do not include the HSCs in our model. Our model starts with myeloid stem cells (MSCs) that are the next cell type after HSCs. We take the pool sizes (i.e., the homeostatic values of stock variables) of the latter cell types directly from the literature. Differentiation/proliferation durations (i.e., delay times) are also taken from the literature [5, 7–10]. The values of the rest of the flows are dynamically calculated by the model. The compartment layout is illustrated in Figure 5.1.

Myeloid Stem Cells stock has an inflow and two outflows. The inflow, *Stem Cell Production Rate*, corresponds to a flow that is derived from HSC differentiation. We calculate the value of this flow based on the subsequent stock values. Accordingly, the normal value of the inflow, *Normal MSC Production Rate*, is set to 0.585 cells/hour. *Differentiation to Myeloblasts* and *Differentiation to Other Blood Cells* are the two outflows of the stock variable. D*ifferentiation to Myeloblasts* refers to the flow of *Myeloid Stem Cells* which follow the path of neutrophil production. *Differentiation to Other Blood Cells* indicates the flow of differentiation from myeloid stem cells to erythrocytes (i.e., red blood cells) and platelets (i.e., thrombocytes). *MSC to Myeloblast Differentiation Time* is 25 hours. It is calculated by subtracting the durations of the other cell types from the whole duration of the neutrophil production process [9]. M*SC to Myeloblast Differentiation Fraction* is the fraction of *Stem Cell Differentiation* into *Myeloblasts* which is set to 1/3 [38]. Accordingly, *Stem Cell to Other Blood Cell Differentiation Fraction* is set to (1 - *Stem Cell to Myeloblast Differentiation Fraction*). The homeostatic value of *Myeloid Stem Cells* is 14.65 x 10^9 cells. We determine this

value based on the ratio of stem cells in the bone marrow, which is 1.1 percent of all cells [49]. The equations of the stock variables correspond to approximate integral equations with Euler's method; DT represents the time step of the simulation.

$$\begin{aligned} Myeloid\ Stem\ Cells_t = {} & Myeloid\ Stem\ Cells_{t-DT} \\ & + (Stem\ Cell\ Production\ Rate \\ & + Differentiation\ to\ Myeloblast \\ & - Differentiation\ to\ Other\ Blood\ Cells) \cdot DT \end{aligned} \quad (5.1)$$

$$Stem\ Cell\ Production\ Rate = Normal\ MSC\ Production\ Rate \quad (5.2)$$

$$\begin{aligned} Differentiation\,to\,Myeloblast = {} & (MSC\,to\,Myeloblast\,Differentiation\,Fraction) \\ & \cdot \left(\frac{Myeloid\ Stem\ Cells}{MSC\ to\ Myeloblast\ Differentiation\ Time}\right) \end{aligned} \quad (5.3)$$

Myeloblasts have one inflow and two outflows. The homeostatic amount of Myeloblasts is 13.65 x 10^9 cells. This amount is calibrated from the inflow and the outflow of the stock variable. We have the information that myeloblasts constitute approximately three percent of the neutrophil producing cells in the bone marrow [48]. The inflow, *Myeloblast Generation*, is derived from *Myeloid Stem Cells*. The homeostatic value of *MSC to Myeloblast Multiplier* is *Normal MSC to Myeloblast Multiplier* that is set to two. However, the multiplier value is changed based on *Effect of G-CSF on Proliferation*. The multiplier value represents the process that most myeloid stem cells duplicate themselves and differentiate into myeloblasts. *Differentiation to Promyelocytes* is an outflow of *Myeloblasts* and it represents the differentiation of myeloblasts into promyelocytes. Accordingly, *Myeloblast to Neutrophil Lineage Fraction* is set to 2/3 in line with the proportion of neutrophils in the total number of white blood cells [3]. *Myeloblast to Other Granulocyte Fraction* is (1 – *Myeloblast to Neutrophil Lineage Fraction*). A proportion of myeloblasts produce eosinophils and basophils. The outflow, *Differentiation to Other Granulocytes*, refers to this proportion. *Normal Myeloblast to Neutrophil Diff Time* is set to 35 hours that is in agreement with the flow and percentage of *Myeloblasts* in the bone marrow; this differentiation time shortens as

a result of high G-CSF levels. We use G-CSF as *GCSF* in the following formulations to avoid confusing the dash with the symbol for subtraction.

$$\begin{aligned} Myeloblasts_t = \ & Myeloblasts_{t-DT} + (Myeloblast\ Generation \\ & + Differentiation\ to\ Promyelocytes \\ & - Differentiation\ to\ Other\ Granulocytes) \cdot DT \end{aligned} \tag{5.4}$$

$$\begin{aligned} & Myeloblast\ Generation = \\ & MSC\ to\ Myeloblast\ Multiplier \cdot Normal\ MSC\ Production\ Rate \end{aligned} \tag{5.5}$$

$$\begin{aligned} & MSC\ to\ Myeloblast\ Multiplier = \\ & Effect\ of\ GCSF\ on\ Proliferation \cdot Normal\ MSC\ to\ Myeloblast\ Multiplier \end{aligned} \tag{5.6}$$

$$\begin{aligned} & Differentiation\ to\ Promyelocytes = \\ & \frac{Myeloblast\ to\ Neutrophil\ Lineage\ Fraction \cdot Myeloblasts}{Myeloblast\ to\ Neutrophil\ Diff\ Time} \end{aligned} \tag{5.7}$$

$$\begin{aligned} & Differentiation\ to\ Other\ Granulocytes = \\ & \frac{Myeloblast\ to\ Other\ Granulocyte\ Fraction \cdot Myeloblasts}{Myeloblast\ to\ Neutrophil\ Diff\ Time} \end{aligned} \tag{5.8}$$

$$\begin{aligned} Myeloblast\ to\ Neutrophil\ Diff\ Time = & Effect\ of\ GCSF\ on\ Transit\ Time \\ & \cdot Normal\ Myeloblast\ to\ Neutrophil\ Diff\ Time \end{aligned} \tag{5.9}$$

The amount of promyelocytes has an initial value of 18.2 x 10^9 cells . We formulate the equations of Promyelocytes stock in a similar way as we do for the previous stock variables. The inflow, *Promyelocytes Cell Generation*, is derived from *Myeloblasts* after it is multiplied with *Myeloblast to Promyelocyte Multiplier*. The homeostatic value of the multiplier is *Normal Myeloblast to Promyelocyte Multiplier*, which is set to two. The value of *Myeloblast to Promyelocyte Multiplier* depends on *Effect of G-CSF on Proliferation*. *Normal Promyelocyte Transit Time* is approximately 35 hours and it is shortened by high G-CSF levels.

$$Promyelocytes_t = Promyelocytes_{t-DT} + (Promyelocytes\ Cell\ Generation - Differentiation\ to\ Myelocytes) \cdot DT \quad (5.10)$$

$$Promyelocytes\ Cell\ Generation = Myeloblast\ to\ Promyelocyte\ Multiplier \cdot Normal\ MSC\ Production\ Rate \quad (5.11)$$

$$Myeloblast\ to\ Promyelocyte\ Multiplier = Effect\ of\ GCSF\ on\ Proliferation \cdot Normal\ Myeloblast\ to\ Promyelocyte\ Multiplier \quad (5.12)$$

$$Differentiation\ to\ Myelocyte = \frac{Promyelocytes}{Promyelocte\ Transit\ Time} \quad (5.13)$$

$$Promyelocyte\ Transit\ Time = Effect\ of\ GCSF\ on\ Transit\ Time \cdot Normal\ Promyelocyte\ Transit\ Time \quad (5.14)$$

Promyelocytes differentiate into the *Myelocytes* after doubling their numbers. *Promyelocyte to Myelocyte Multiplier*, which has a value of two, is used in this doubling process. Multiple cell divisions occur in the myelocyte phase. For this reason, we split the myelocytes into two stock variables in our model; *Myelocyte S1* (Myelocyte Stage 1) and *Myelocyte S2* (Myelocyte Stage 2). Their initial values are 20.1 and 80.43 x 10^9 cells respectively. M*yelocyte Generation 1* and *Myelocyte Generation 2* are the inflows of the two stock variables, respectively. Similarly, *Proliferation 1* and *Proliferation 2* are the outflows of the two stock variables. *Neutrophil Multiplication at Each Stage* indicates the proliferation fraction, which is two. *Normal Myelocyte Transit Time* is 58 hours referring to the total time that cells spend in the two stocks and this time decreases with the effect of high G-CSF values.

$$Myelocytes\ S1_t = Myelocytes\ S1_{t-DT} + (Myelocyte\ Generation\ 1 - Prolliferation\ 1) \cdot DT \quad (5.15)$$

$$Myelocytes\ S2_t = Myelocytes\ S2_{t-DT} + (Myelocyte\ Generation\ 2 - Prolliferation\ 2) \cdot DT \quad (5.16)$$

$$Myelocyte\ Generation\ 1 = Promyelocyte\ to\ Myelocyte\ Multiplier \cdot Differentiation\ to\ Myelocyte \quad (5.17)$$

$$Myelocyte\ Generation\ 2 = Neutrophil\ Multiplication\ at\ Each\ Stage \cdot Proliferation\ 1 \quad (5.18)$$

$$Proliferation\ 1 = \frac{Myelocytes\ S1}{Myelocte\ Transit\ Time/3} \quad (5.19)$$

$$Proliferation\ 2 = \frac{Myelocytes\ S2}{Myelocte\ Transit\ Time * 2/3} \quad (5.20)$$

$$Myelocyte\ Transit\ Time = Effect\ of\ GCSF\ on\ Transit\ Time \cdot Normal\ Myelocyte\ Transit\ Time \quad (5.21)$$

Myelocytes differentiate into metamyelocytes and band neutrophils. As we mentioned earlier, Metamyelocytes and band neutrophils are classified under the post-mitotic pool, meaning that mitotic division does not occur at this pool. Accordingly, we name the stock variable as *Metamyelocytes and Band Neutrophils in PMP*. At this stage, the cells mature and take the final form of neutrophils. *Metamyelocyte and Band Neutrophil Generation* is the inflow of the stock variable. The inflow is derived from the outflow of *Myelocytes S2*. The cells spend 60 hours in M*etamyelocytes and Band Neutrophils in PMP* stock and this time gets shorter with high G-CSF levels. The outflow of the stock variable, *Maturation*, is the inflow of the *Stored Neutrophils in PMP*. *Stored Neutrophils in PMP* is the stock variable that represents the mature neutrophils stored in the bone marrow for emergency situations. Post Mitotic Storage Time is affected by the high G-CSF levels. *Normal Post Mitotic Storage Time* is 30 hours. After 30 hours, the neutrophils are released into the blood circulation. This process is represented with the outflow named as Release. In the literature, the size of the post-mitotic pool

is given as 385 x 109 cells (5.5 x 10^9 cells/kg),approximately [9]. We set the size of metamyelocytes as 249.6 x 10^9 cells and the size of stored neutrophils as 129.84 x 10^9 cells. If we sum them up, we find a value of approximately 380 x 10^9 cells, which is in accordance with the literature. The transit time of post-mitotic pool is between four and six days. In our model, the total time spent in the post-mitotic pool is 90 hours. Equations are given below.

$$\begin{aligned} &Metamyelocytes\ and\ Band\ Neutrophils\ in\ PMP_t = \\ &\qquad Metamyelocytes\ and\ Band\ Neutrophils\ in\ PMP_{t-DT} \\ &+ (Metamyelocyte\ and\ Band\ Neutrophil\ Generation - Maturation) \cdot DT \end{aligned} \tag{5.22}$$

$$\begin{aligned} &Metamyelocyte\ and\ Band\ Neutrophil\ Generation = \\ &\qquad Neutrophil\ Multiplication\ at\ Each\ Stage \cdot Proliferation\ 2 \end{aligned} \tag{5.23}$$

$$Maturation = \frac{Metamyelocytes\ and\ Band\ Neutrophils\ in\ PMP}{Post\ Mitotic\ Transit\ Time} \tag{5.24}$$

$$\begin{aligned} &Stored\ Neutrophils\ in\ PMP_t = \\ &\qquad Stored\ Neutrophils\ in\ PMP_{t-DT} + (Maturation - Release) \cdot DT \end{aligned} \tag{5.25}$$

$$Release\ = \frac{Stored\ Neutrophils\ in\ PMP}{Post\ Mitotic\ Storage\ Time} \tag{5.26}$$

$$\begin{aligned} &Post\ Mitotic\ Storage\ Time = \\ &Eff\ of\ GCSF\ on\ PM\ Storage\ Time \cdot Normal\ Post\ Mitotic\ Storage\ Time \end{aligned} \tag{5.27}$$

$$\begin{aligned} &Post\ Mitotic\ Transit\ Time = \\ &Effect\ of\ GCSF\ on\ Transit\ Time \cdot Normal\ Post\ Mitotic\ Transit\ Time \end{aligned} \tag{5.28}$$

5.2. Compartment for Blood and Tissue Neutrophils

In the model, we represent the neutrophils in the bloodstream as *Circulating Neutrophils* stock variable. *Release* is the inflow of the *Circulating Neutrophils* and it is also the outflow of *Stored Neutrophils in PMP*. Approximately half of the neutrophils in the blood vessels are adhered to the inner surface of the vessels and these neutrophils comprise *Marginated Neutrophils* stock [50,51]. The total size of circulating and marginated neutrophils is between 20 x 10^9 cells and 70 x 10^9 cells.

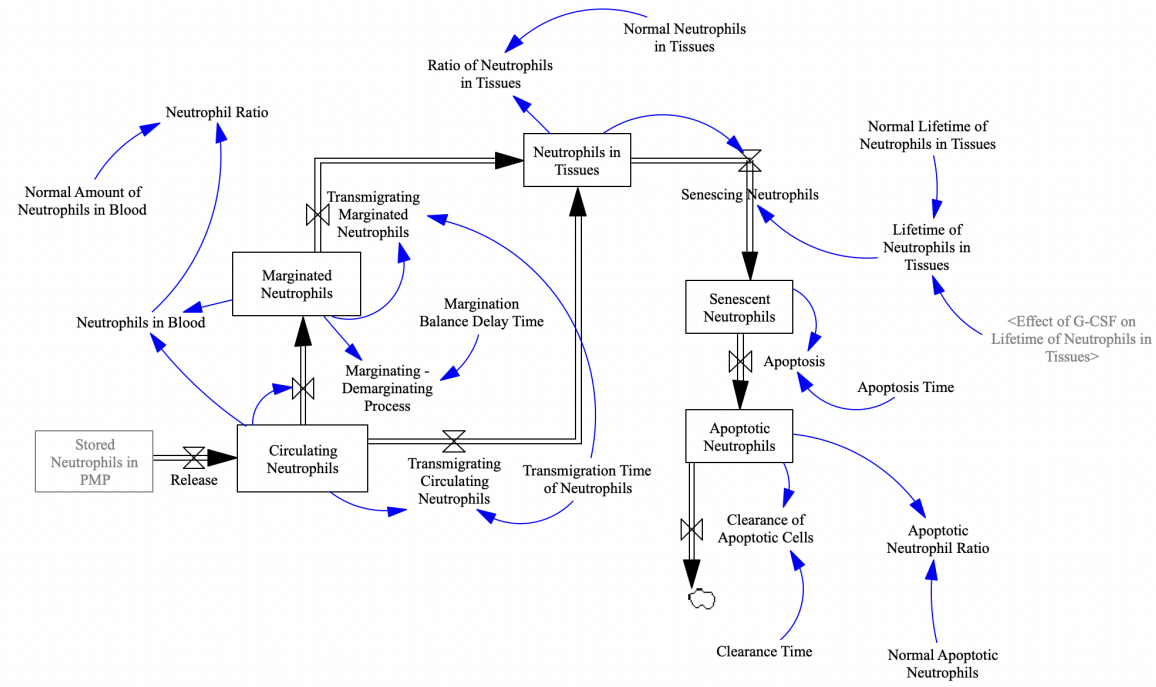


Figure 5.2. Compartment for blood and tissue neutrophils.

The marginated and the circulating neutrophils exchange constantly. This transition between *Circulating Neutrophils* and *Marginated Neutrophils* is represented as *Marginating-Demarginating Process*. We assume that *Margination Balance Delay Time*, is half an hour. We also assume that the time of their transmigration into tissues is equal. Correspondingly, the *Transmigration Time of Neutrophils* is set to eleven hours, which is derived from the circulation half-life, which is between six and eight hours [5,8,51]. Later, the neutrophils transmigrate into the tissues and constitute

Neutrophils in Tissues stock that has an initial value of 70.72 x 10^9 cells according to our calculations, which are based on the lifetime of neutrophils in tissues. Neutrophils operate in tissues and attack infectious pathogens. We set *Normal Lifetime of Neutrophils in Tissues* as 17 hours [6, 52]. *Lifetime of Neutrophils in Tissues* is effected by high G-CSF levels. After approximately 15-20 hours, *Neutrophils in Tissues* become senescent and move into the *Senescent Neutrophils* stock variable. Besides, a small number of neutrophils in the blood can be senescent without visiting the tissues [9]. We could show them as two flows from *Circulating Neutrophils* and *Marginated Neutrophils* to *Senescent Neutrophils*. However, we do not represent these flows because the change in *Senescent Neutrophils*, which is caused by the flows, is compensated with adjusting the delay time of *Neutrophils in Tissues*. The senescent neutrophils start programmed cell death (i.e., apoptosis) after a short period of time. We arbitrarily set *Apoptosis Time* (i.e., the duration of senescent neutrophils to become apoptotic) to an hour and calibrated the model accordingly. In the apoptosis process, neutrophils send eat-me signals to the macrophages and dendric cells. Finally, macrophages clear the apoptotic cells by phagocytosis. *Clearance Time* is set to an hour [53]. We represent the clearance process with the outflow, *Clearance of Apoptotic Cells*.

We use some of the variables in this compartment as inputs to other compartments. For example, we obtain *Apoptotic Neutrophil Ratio* from *Apoptotic Neutrophils*. *Apoptotic Neutrophil Ratio* is used as an input in the *Compartment for the Effect of Apoptotic Neutrophils on G-CSF*. *Normal Apoptotic Neutrophils* is 4.16 x 10^9 cells. The other instance can be given as *Ratio of Neutrophils in Tissues* that is used as an input in pathogen elimination. *Normal Neutrophils in Tissues* is set to 70.72 x 10^9 cells. We also create reporting variables from the existing stocks. Sum of the *Circulating Neutrophils* and *Marginated Neutrophils* is introduced as *Neutrophils in Blood*. *Neutrophils in Blood* is divided to *Normal Amount of Neutrophils in Blood* to generate *Neutrophil Ratio*. *Neutrophil Ratio* is used as an indicator of neutropenia.

$$\begin{aligned} Circulating\ Neutrophils_t = {} & Circulating\ Neutrophils_{t-DT} \\ & + (Release - Marginating\ Demarginating\ Process \\ & - Transmigrating\ Circulating\ Neutrophils) \cdot DT \end{aligned} \tag{5.29}$$

$$\begin{aligned} Marginated\ Neutrophils_t = {} & Marginated\ Neutrophils_{t-DT} \\ & + (Marginating\ Demarginating\ Process \\ & - Transmigrating\ Marginated\ Neutrophils) \cdot DT \end{aligned} \tag{5.30}$$

$$\begin{aligned} Neutrophils\ in\ Tissues_t = {} & Neutrophils\ in\ Tissues_{t-DT} \\ & + (Transmigrating\ Circulating\ Neutrophils \\ & + Transmigrating\ Marginated\ Neutrophils \\ & - Senescing\ Neutrophils) \cdot DT \end{aligned} \tag{5.31}$$

$$\begin{aligned} Senescent\ Neutrophils_t = {} & Senescent\ Neutrophils_{t-DT} \\ & + (Senescing\ Neutrophils - Apoptosis) \cdot DT \end{aligned} \tag{5.32}$$

$$\begin{aligned} Apoptotic\ Neutrophils_t = {} & Apoptotic\ Neutrophils_{t-DT} \\ & + (Apoptosis - Clearance\ of\ Apoptotic\ Cells) \cdot DT \end{aligned} \tag{5.33}$$

$$Marginating\ Demarginating\ Process = \frac{Circulating\ Neutrophils - Marginated\ Neutrophils}{Margination\ Balance\ Delay\ Time} \tag{5.34}$$

$$Transmigrating\ Circulating\ Neutrophils = \frac{Circulating\ Neutrophils}{Transmigration\ Time\ of\ Neutrophils} \tag{5.35}$$

$$Transmigrating\ Marginated\ Neutrophils = \frac{Marginated\ Neutrophils}{Transmigration\ Time\ of\ Neutrophils} \tag{5.36}$$

$$Senescing\ Neutrophils = \frac{Neutrophils\ in\ Tissues}{Lifetime\ of\ Neutrophils\ in\ Tissues} \tag{5.37}$$

$$\begin{aligned} Lifetime\ of\ Neutrophils\ in\ Tissues = \\ Effect\ \ of\ GCSF\ on\ Lifetime\ of\ Neutrophils\ in\ Tissues \\ \cdot\ Normal\ Lifetime\ of\ Neutrophils\ in\ Tissues \end{aligned} \tag{5.38}$$

$$Apoptosis = \frac{Senescent\ Neutrophils}{Apoptosis\ Time} \tag{5.39}$$

$$Clearance\ of\ Apoptotic\ Cells = \frac{Apoptotic\ Neutrophils}{Clearance\ Time} \tag{5.40}$$

$$Ratio\ of\ Neutrophils\ in\ Tissues = \frac{Neutrophils\ in\ Tissues}{Normal\ Neutrophils\ in\ Tissues} \tag{5.41}$$

$$Apoptotic\ Neutrophil\ Ratio = \frac{Apoptotic\ Neutrophils}{Normal\ Apoptotic\ Neutrophils} \tag{5.42}$$

5.3. G-CSF Compartment

G-CSF is a cytokine that has regulatory effects on neutrophil production. In homeostatic conditions, G-CSF serum levels are extremely low. The levels are lower than 50 nanogram/ml [12, 54]. Yet, even this amount has an effect on the basal production amounts of neutrophils. Experiments with mice show that only 30 percent of normal production amount is provided in the absence of G-CSF [55]. Thus, even the normal trace serum level of G-CSF increases the amount from 30 percent to 100 percent compared to zero G-CSF level.

The production of G-CSF can be up to 30 times the normal production in extreme conditions such as infection and inflammation [54]. High levels of G-CSF have four main effects on neutrophils. Firstly, G-CSF increases neutrophil production. It affects the proliferation capacity of myeloblasts and promyelocytes [15,16]. Secondly, high levels of

G-CSF shorten the transit times of neutrophils in each stock variable from *Myeloblasts* to *Metamyelocytes and Band Neutrophils*. Additionally, high G-CSF has a release effect on stored bone marrow neutrophils. This release of neutrophils into the bloodstream enables rapid intervention to the infection. Finally, high G-CSF level has an effect on the lifetime of neutrophils in tissues; it prolongs the lifetime of neutrophils [19].

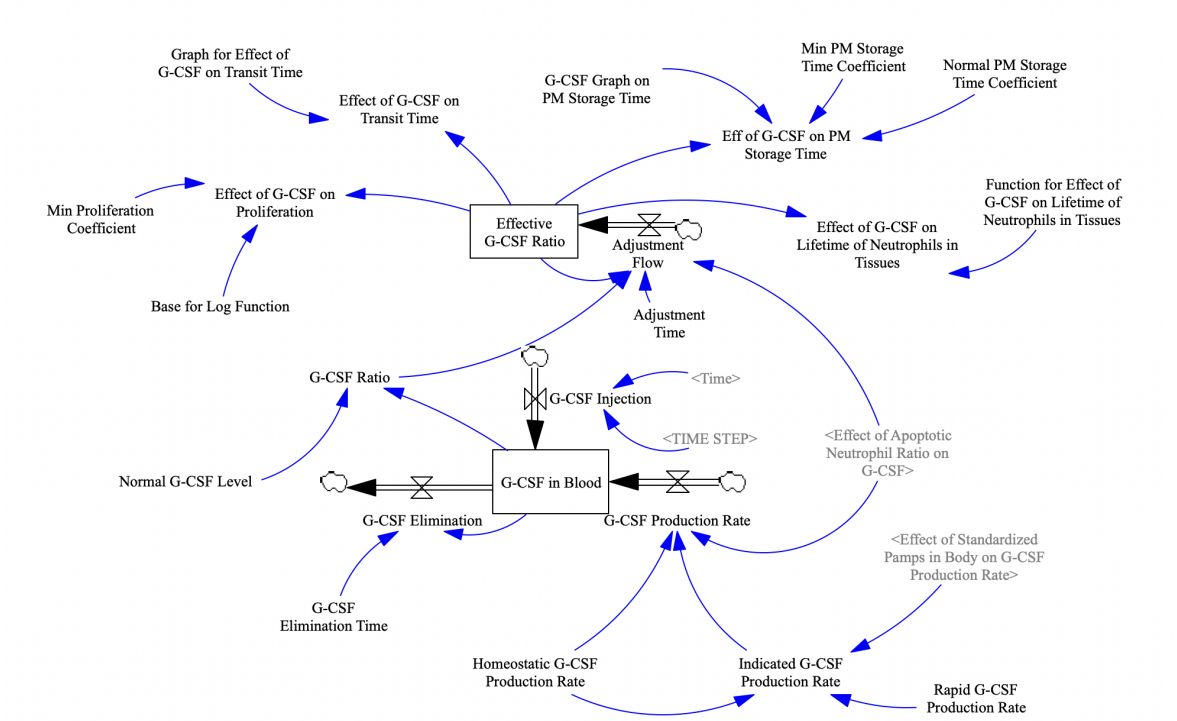


Figure 5.3. G-CSF compartment.

In the model, we represent the G-CSF level with a stock variable, G-CSF in Blood. The unit of the stock variable is nanogram. G-CSF is quickly eliminated with a half-life of 3.5 hours [56]. Accordingly, *G-CSF Elimination Time* is obtained as five hours from the half-life of G-CSF. It is used in *G-CSF Elimination*, which is the outflow. The inflow of the stock variable is *G-CSF Production Rate*. The production flow changes depending on two effects. One effect comes from the pathogens. A rise in the pathogen level creates a stimulatory effect on *G-CSF Production Rate*. Secondly, an accumulation of apoptotic neutrophils neutralizes *G-CSF Production Rate* if it is pumped up. *Homeostatic G-CSF Production Rate* for a healthy human is 26 nanogram/hour [54]. *Rapid G-CSF Production Rate* is set to 780 nanogram/hour,

which is 30 times of *Homeostatic G-CSF Production Rate*. The production of G-CSF ranges between these two values. We show the effect of pathogens on neutrophil production with *Indicated G-CSF Production Rate*. It provides the transition between *Rapid G-CSF Production Rate* and *Homeostatic G-CSF Production Rate* according to *Effect of Standardized Pamps in Body on G-CSF Production Rate*. Lastly, we formulated *G-CSF Production Rate* with *Indicated G-CSF Production Rate* and *Effect of Apoptotic Neutrophil Ratio on G-CSF*. If *Effect of Apoptotic Neutrophil Ratio on G-CSF* is one, the body produces G-CSF at a rate equal to *Indicated G-CSF Production Rate*. If *Effect of Apoptotic Neutrophil Ratio on G-CSF* is zero, the body produces G-CSF at a rate equal to *Homeostatic G-CSF Production Rate* regardless of the value dictated by *Indicated G-CSF Production Rate*.

$$\begin{aligned} GCSF\ in\ Blood_t = {} & GCSF\ in\ Blood_{t-DT} \\ & + (GCSF\ Production\ Rate - GCSF\ Elimination) \cdot DT \end{aligned} \tag{5.43}$$

$$\begin{aligned} & GCSF\ Production\ Rate = \\ & \quad Effect\ of\ Apoptotic\ Neutrophil\ Ratio\ on\ GCSF\ Production\ Rate \\ & \quad \cdot\ Indicated\ GCSF\ Production\ Rate \\ & + (1 - Effect\ of\ Apoptotic\ Neutrophil\ Ratio\ on\ GCSF\ Production\ Rate\ ) \\ & \quad \cdot Homeostatic\ GCSF\ Production\ Rate \end{aligned} \tag{5.44}$$

$$\begin{aligned} & GCSF\ Production\ Rate = \\ & \quad Effect\ of\ Apoptotic\ Neutrophil\ Ratio\ on\ GCSF\ Production\ Rate \\ & \quad \cdot\ Indicated\ GCSF\ Production\ Rate \\ & + (1 - Effect\ of\ Apoptotic\ Neutrophil\ Ratio\ on\ GCSF\ Production\ Rate\ ) \\ & \quad \cdot Homeostatic\ GCSF\ Production\ Rate \end{aligned} \tag{5.45}$$

$$\begin{aligned} &Indicated\ GCSF\ Production\ Rate = \\ &\quad (Effect\ of\ Standardized\ Pamps\ in\ Body\ on\ GCSF\ Production\ Rate \\ &\quad \cdot\ Rapid\ GCSF\ Production\ Rate) \\ &+ (\ 1 -\ Effect\ of\ Standardized\ Pamps\ in\ Body\ on\ GCSF\ Production\ Rate) \\ &\quad \cdot\ Homeostatic\ G-CSF\ Production\ Rate \end{aligned} \tag{5.46}$$

$$GCSF\ Elimination = \frac{GCSF\ in\ Blood}{GCSF\ Elimination\ Time} \tag{5.47}$$

The effects of G-CSF on neutrophils are formulated with effect functions in the model. These effects of G-CSF occur through molecular interactions, therefore, occur with a delay. To express this in our model, we create a stock variable named as *Effective G-CSF Ratio*. Rapid changes in the level of G-CSF Ratio are smoothed with *Effective G-CSF Ratio* with an adjustment time.

$$\begin{aligned} &Effective\ GCSF\ Ratio_t = \\ &\quad Effective\ GCSF\ Ratio_{t-DT} + Adjustment\ Flow \cdot DT \end{aligned} \tag{5.48}$$

$$\begin{aligned} &Adjustment\ Flow = \\ &\quad (Effect\ of\ Apoptotic\ Neutrophil\ Ratio\ on\ GCSF \cdot\ GCSF\ Ratio \\ &\quad -\ Effective\ GCSF\ Ratio)\ /\ Adjustment\ Time \end{aligned} \tag{5.49}$$

Effective G-CSF Ratio is used as a standardized input in the effect formulations. G–CSF has different effects on proliferation at different levels. The lack of G-CSF may lead to a 70 percent decrease in the neutrophil production. On the other hand, high levels of G-CSF may induce elevation of the neutrophil production up to 15-fold. Thus, we need an effect function that represents three points: (i) when *Effective G-CSF Ratio* is zero then effect should be at the minimum level (i.e., *Min Proliferation Coefficient*); (ii) when *Effective G-CSF Ratio* is one then the effect should be one and (iii) when *Effective G-CSF Ratio* is 30 then the effect should be five. After plotting these three points, we figured out that the function must show a logarithmic growth

shape. Therefore, *Effect of G-CSF on Proliferation* is formulated as a logarithmic function. This formulation is capable of representing the minimum proliferation of neutrophils in the absence G-CSF; normal proliferation of neutrophils when G-CSF level is normal; and higher proliferation of neutrophils when the G-CSF level is high. We calibrated the function by changing the base of the logarithm. Accordingly, *Base for Log Function* is determined as 1.82. After this calibration, the function passes through the points: (0, 0.3), (1,1), (30, 5). Note that *Min Proliferation Coefficient* is 0.3. The equation of *Effect of G-CSF on Proliferation* is given in Equation 5.50. The shape of the effect function is illustrated in Figure 5.4.

$$Effect\ of\ GCSF\ on\ Proliferation = log_{1.82}(1.82^{0.3} + (1.82 - 1.82^{0.3}) \cdot GCSF\ Ratio) \quad (5.50)$$

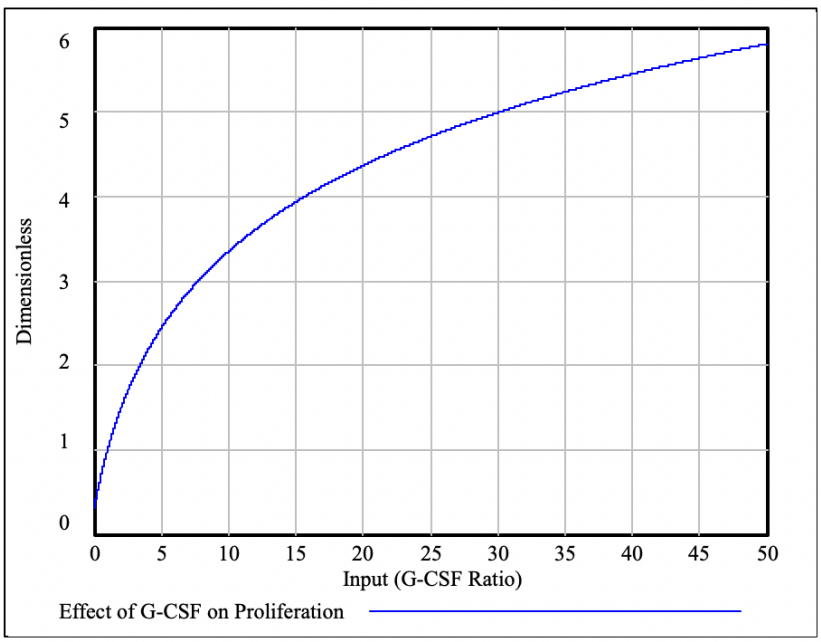


Figure 5.4. Effect of G-CSF on proliferation.

Effect of G-CSF on Transit Time describes the shortening effect of high G-CSF on mitotic and post mitotic phases. When G-CSF level in the circulation increases, the cells in mitotic phase divide in a shorter time period. This effect can decrease the transit times up to 10% of their normal level instantaneously, but on average,

the transit times decrease to approximately 40% of their normal level. The graphical function of the effect is given in Figure 5.5. When the input of the effect function, *Effective G-CSF Ratio*, is zero, the effect function produces one as its output value. The decreasing effect first appears when the input reaches 15. The output sharply decreases from 0.86 to 0.14 while the *Effective G-CSF Ratio* increases from 30 to 70.

$$Effect\ of\ GCSF\ Ratio\ on\ Transit\ Time = \ f(Effective\ GCSF\ Ratio) \quad (5.51)$$

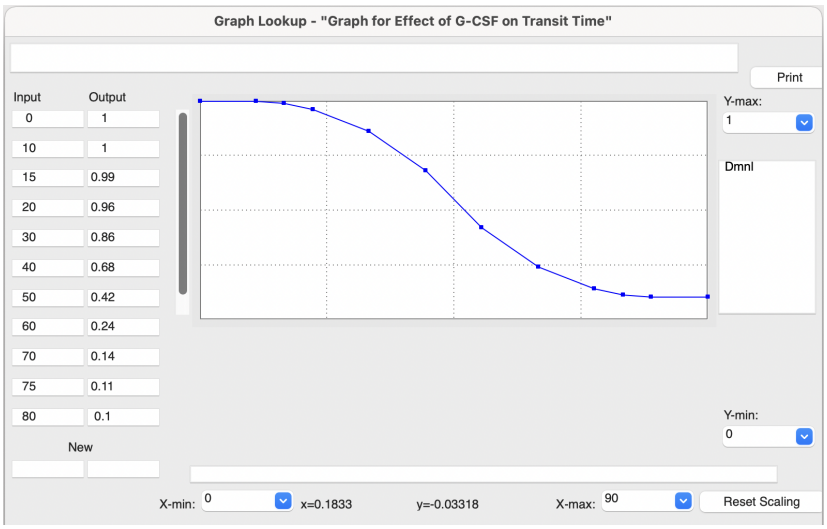


Figure 5.5. Graphical function for effect of G-CSF on transit time.

Effect of G-CSF on PM Storage Time represents the impact of G-CSF on release of neutrophils from the bone marrow. When its input value is high, the effect shortens the storage time and increases the release of neutrophils. This function shows its impact relatively faster compared to other effects. It starts to reduce *PM Storage Time* when the input reaches 1.5 and it shows full impact when the input value reaches 7.5 or above. It is an inverse S-shaped function. The time decreasing effect can shorten the storage time down to 40% of its normal value. Thus, the formulation of the effect function starts with a fixed value of 0.4. This fixed value is called *Min PM Storage Time Coefficient*. The highest value of the effect function is one, in other words, *Normal PM Storage Time Coefficient*. Even though the graphical function ranges between zero and one, the effect function produces values between 0.4 and 1. The equation of the

effect function is shown in Equation 5.52.

$$\begin{aligned} &Effect\ of\ GCSF\ on\ PM\ Storage\ Time\ = \\ &\qquad Min\ PM\ Storage\ Time\ Coefficient \\ &+ (Normal\ PM\ Storage\ Time\ Coefficient - Min\ PM\ Storage\ Time\ Coefficient) \\ &\qquad \cdot\ f(GCSF\ Ratio) \end{aligned} \quad (5.52)$$

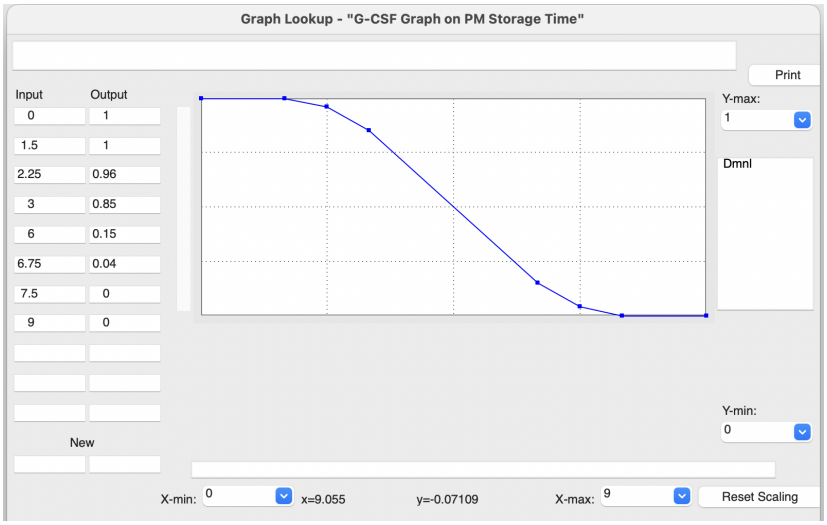


Figure 5.6. Graphical function for effect of G-CSF on PM storage time.

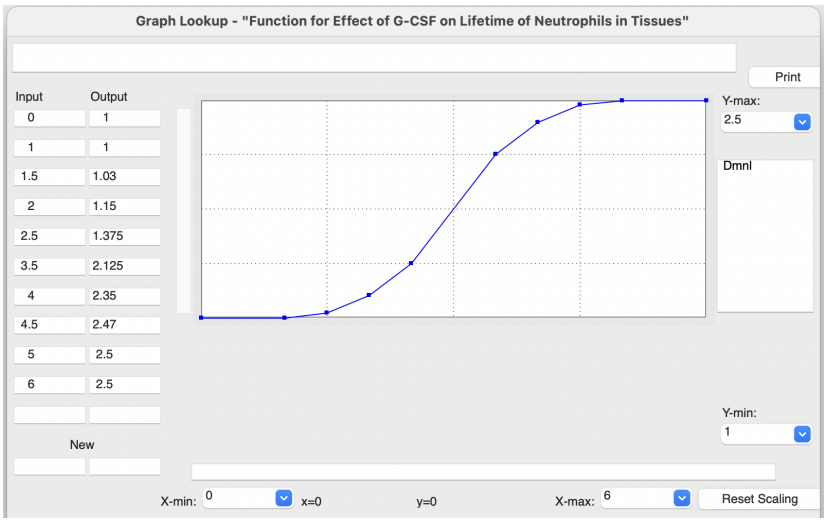


Figure 5.7. Graphical function for G-CSF effect on lifetime of tissue neutrophils.

Lastly, the *Effect of G-CSF on Lifetime of Neutrophils in Tissues* shows the increasing effect of G-CSF on lifetime of neutrophils in tissues. The impact of this effect is rather fast. The input of the function is *Effective G-CSF Ratio*. The function is an S-shaped function. It increases the lifetime of neutrophils in tissues by 2.5 times. As you can see from the graph, the function starts to show its impact when the input exceeds 1. The function shows its full impact when the input reaches 5.

$$Effect\ of\ GCSF\ on\ Lifetime\ of\ Neutrophils\ in\ Tissues\ = f(Effective\ GCSF\ Ratio) \quad (5.53)$$

5.4. Pathogen Compartment

The human body is constantly exposed to pathogens. Up to a threshold, the immune system handles the pathogen entry and eliminates the pathogens without showing a major reaction. The details of the pathogen removal process are not included in our model. We only represent the elimination of pathogens by neutrophils in tissues.

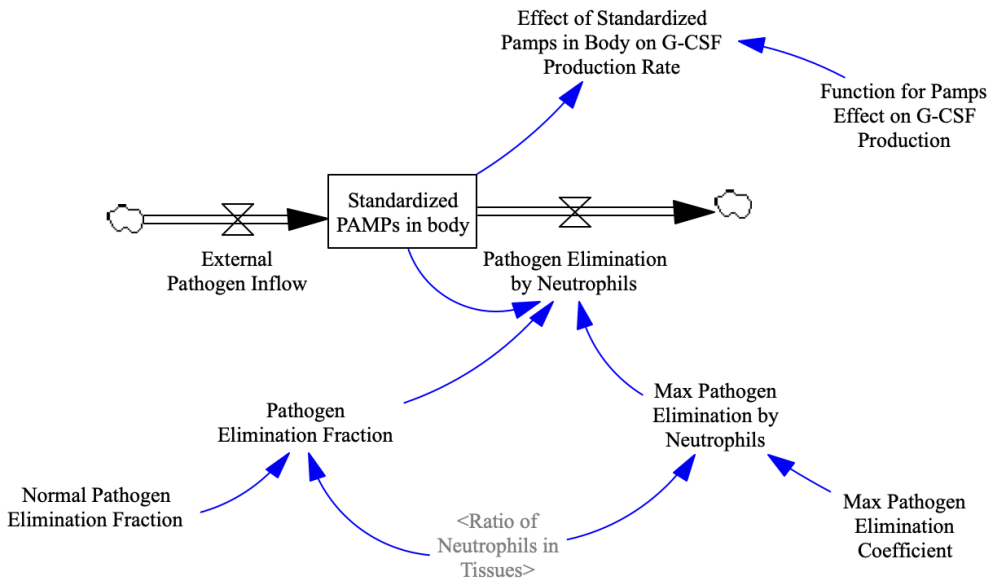


Figure 5.8. Pathogen compartment.

A large entry of pathogens into the body alerts the immune system. The immune system shows a strong response. The neutrophils in the circulation are directed to the infected area. If necessary, the body increases the neutrophil production rate to actively fight against the pathogens.

The production order is given via an increase in G-CSF level [57]. We represent the pathogen amount with a stock variable, *Standardized PAMPs in Body*. PAMPs means pathogen-associated molecular patterns. The inflow of the pathogens, External Pathogen Inflow, is assumed to be constant for a healthy person. It is also assumed that this inflow can be higher or lower depending on environmental conditions. Outflow of the stock variable, *Pathogen Elimination by Neutrophils*, describes the elimination of pathogens. The elimination of pathogens depends on the amount of neutrophils in the tissues. In normal conditions, the elimination is formulated with a pathogen elimination fraction. *Normal Pathogen Elimination Fraction* is 0.1. It denotes that the invading pathogens can be eliminated within 10 hours. However, there is a maximum elimination limit when the amount of pathogens is high. We assume that one unit of neutrophils can remove at most one unit of pathogens per hour. So Maximum Pathogen Elimination Fraction is one. Accordingly, we formulated equations as follows:

$$\begin{aligned} &Pathogen\ Elimination\ by\ Neutrophils\ = \\ &\quad min(Pathogen\ Elimination\ Fraction\ \cdot Standardized\ PAMPs\ in\ Body, \\ &\qquad\qquad Max\ Pathogen\ Elimination\ by\ Neutrophils) \end{aligned} \tag{5.54}$$

$$\begin{aligned} &Pathogen\ Elimination\ Fraction\ = \\ &\quad Ratio\ of\ Neutrophils\ in\ Tissues\ \cdot Normal\ Pathogen\ Elimination \end{aligned} \tag{5.55}$$

$$\begin{aligned} &Max\ Pathogen\ Elimination\ Fraction\ = \\ &Ratio\ of\ Neutrophils\ in\ Tissues \cdot Max\ Pathogen\ Elimination\ Coefficient \end{aligned} \tag{5.56}$$

Existence of pathogens in body affects the production of G-CSF, which has four effects on neutrophils as discussed in the previous section. Hence we add an effect function to

the model: Effect of Std PAMPs in Body on G-CSF Prod. Rate. It works similar to an if-then-else function, but in a smoother way. The graphical function of the effect is given in Figure. 5.9. Formulation is given below;

$$Effect\ of\ Standardized\ PAMPs\ in\ Body\ on\ GCSF\ Production\ Rate\ = f(Standardized\ PAMPs\ in\ Body) \quad (5.57)$$

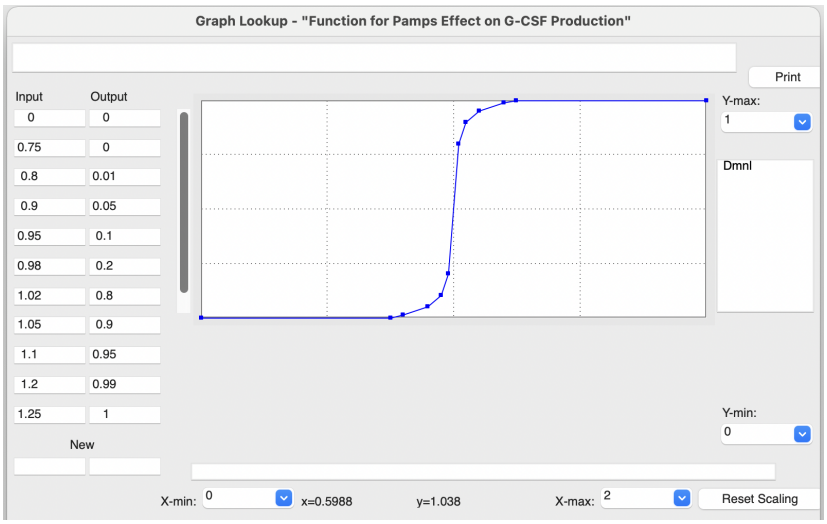


Figure 5.9. Graphical function for PAMPs effect on G-CSF.

5.5. Compartment for the Effect of Apoptotic Neutrophils on G-CSF

In this compartment, we address the feedback mechanism that suppresses G-CSF production. When macrophages clear the apoptotic neutrophils from the tissues, their secretion of interleukin 23 (IL-23) decreases. This reduction of IL-23 blocks the production / activation of IL-17 cytokine, which is necessary for G-CSF production. In short, the clearance of apoptotic neutrophils either puts the G-CSF production on hold (for the CN patient) or returns it to its homeostatic level (for a healthy person) [58]. We use the hysteresis creating structure developed by Dr. Yasarcan to model this effect (i.e., *Effect of Apoptotic Neutrophil Ratio on G-CSF*).

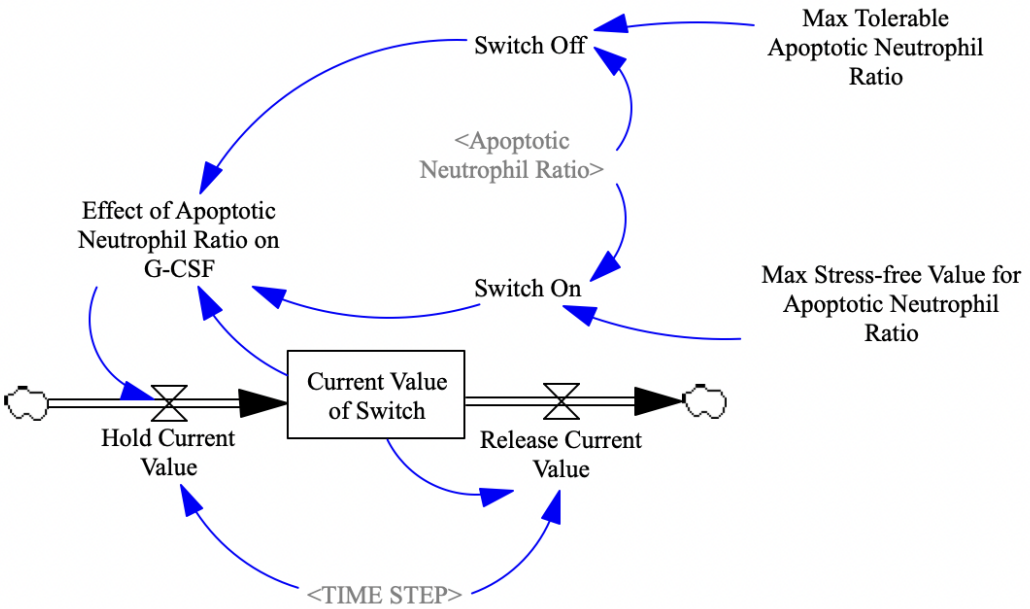


Figure 5.10. Compartment for the effect of apoptotic neutrophils on G-CSF.

In the formulation of *Effect of Apoptotic Neutrophil Ratio on G-CSF* , there are two critical values; *Max Stress-free Value for Apoptotic Neutrophil Ratio* and *Max Tolerable Apoptotic Neutrophil Ratio*. In the model, we assign 1.2 to *Max Stress-free Value for Apoptotic Neutrophil Ratio* and two to *Max Tolerable Apoptotic Neutrophil Ratio*. Consequently, there are three intervals for the input of the effect function. The stress-free interval refers to the situation where the *Apoptotic Neutrophil Ratio* is less than *Max Stress-free Value for Apoptotic Neutrophil Ratio*. Non-tolerable interval refers to the situation where *Apoptotic Neutrophil Ratio* is above *Max Tolerable Apoptotic Neutrophil Ratio*. Tolerable interval refers to the situation where *Apoptotic Neutrophil Ratio* is between *Max Stress-free Value for Apoptotic Neutrophil Ratio* and *Max Tolerable Apoptotic Neutrophil Ratio*. When the *Apoptotic Neutrophil Ratio* is in the non-tolerable interval, *Effect of Apoptotic Neutrophil Ratio on G-CSF*, which is a binary stock variable, becomes zero and returns the G-CSF production to its homeostatic level for a healthy person. When *Apoptotic Neutrophil Ratio* is in the stress-free interval *Effect of Apoptotic Neutrophil Ratio on G-CSF* becomes one and allows the G-CSF production to increase above the homeostatic level when an increase in neutrophils is required. When *Apoptotic Neutrophil Ratio* is in the tolerable interval, *Effect*

of Apoptotic Neutrophil Ratio on G-CSF remains as it is; if it is zero, it remains zero; if it is one, it remains one. In this interval, the value of *Effect of Apoptotic Neutrophil Ratio on G-CSF* depends on its own value an instant ago that is stored in Current Value of G-CSF Switch. As *Effect of Apoptotic Neutrophil Ratio on G-CSF* can assume either zero or one, when the *Apoptotic Neutrophil Ratio* is in the tolerable interval, this can potentially create hysteresis in the production of G-CSF.

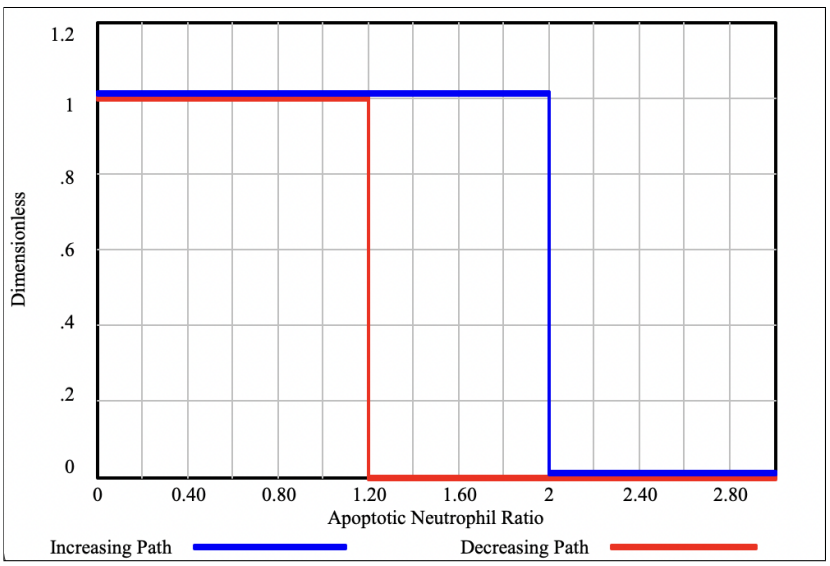


Figure 5.11. The mechanism of the effect function.

We illustrate the mechanism in Figure 5.11. The only effect of apoptotic neutrophils reported in the literature is on G-CSF production rate. However, we claim that apoptotic neutrophils can also deactivate the four effects of G-CSF on neutrophils even though there still is G-CSF in the body. We will discuss this claim in chapter 7.

$$Current\ Value\ of\ Switch_t = Current\ Value\ of\ Switch_{t-DT} + (Hold\ Current\ Value - Release\ Current\ Value) \cdot DT \quad (5.58)$$

$$Hold\ Current\ Value = Effect\ of\ Apoptotic\ Neutrophil\ Ratio\ on\ GCSF\ Production\ Rate \quad (5.59)$$

$$Release\ Current\ Value = \ Current\ Value\ of\ Switch \tag{5.60}$$

$$Switch\ On = \begin{cases} 1, & Apoptotic\ Neutrophil\ Ratio \leq 1.2 \\ 0, & Otherwise \end{cases} \tag{5.61}$$

$$Switch\ Off = \begin{cases} 1, & Apoptotic\ Neutrophil\ Ratio \leq 2 \\ 0, & Otherwise \end{cases} \tag{5.62}$$

$$\begin{matrix} Effect\ of\ Apoptotic \\ Neutrophil\ Ratio \\ on\ GCSF \end{matrix} = \begin{cases} Switch\ Off, & Current\ Value\ of\ Switch = 1 \\ Switch\ On, & Otherwise \end{cases} \tag{5.63}$$

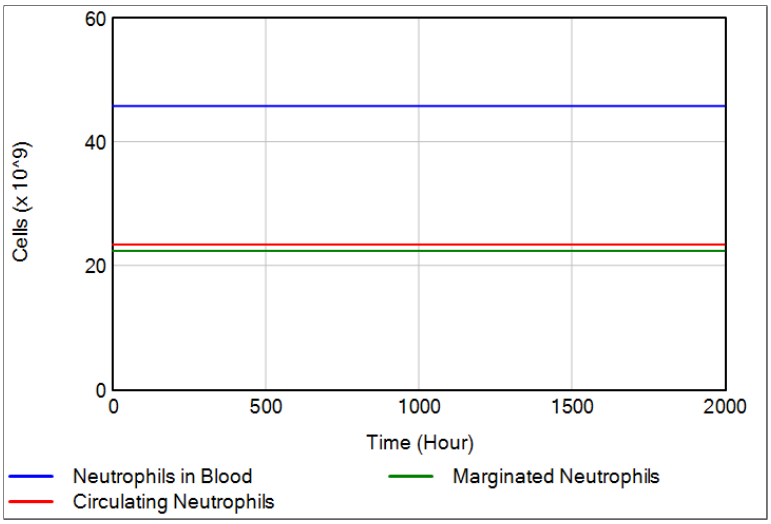
6. MODEL BEHAVIOR AND VALIDATION

Vensim PLE software is used to simulate the model. The time unit of the model is hour and the length of the simulation runs is 2000 hours for most of the experiments. The time step is set to 1/16. The model is initiated with the parameter values for a healthy person. We checked for unit consistency and our model is dimensionally consistent.

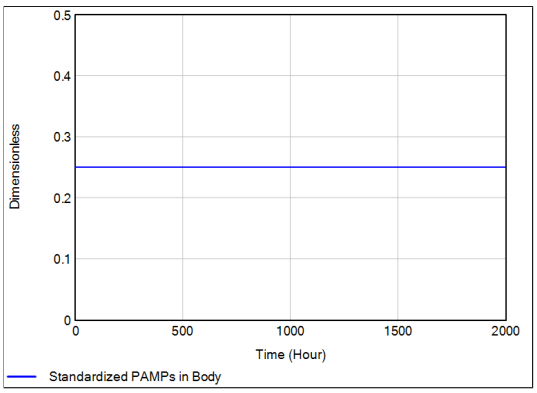
To ensure structural validation, the stock and flow values in the model are taken and/or derived from the existing data in the literature. Furthermore, some of the critical values of the effect functions are also taken from the literature and the shapes of the functions are determined such that they satisfy these critical points. We also carry out behavioral validation runs. First, one-shot pathogen entry is applied, which represents an intense pathogen exposure that is experienced in an instant. Later, we experiment with a high-level continuous pathogen entry. In the third setting with pathogens, we assumed a constant pathogen level. In this third setting, pathogens cannot be eliminated by neutrophils, although they are produced, stimulated, and activated for the elimination of pathogens. Furthermore, we also experiment with the model assuming radiation exposure and compare our results to data taken from a study that represents the ANC (absolute neutrophil count; neutrophil level) values of non-human primates after a radiation exposure. Lastly, we obtain the neutrophil dynamics of CN patients by calibrating model parameter values, but without changing the model structure.

6.1. Equilibrium Run

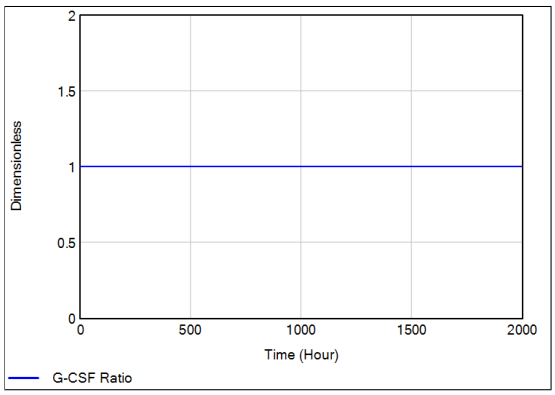
The dynamics of a healthy person is expected to illustrate the homeostatic balance of neutrophil production. Figure 6.1 shows the behavior of the main variables in the steady state condition. Most importantly, all of the state variables remain within their corresponding ranges given by the literature; these values are given in detail in Chapter 5.



(a) Levels of circulating, marginated, and blood neutrophils.



(b) Standardized PAMPs.



(c) G-CSF Ratio.

Figure 6.1. Variable dynamics in equilibrium run.

We show that Circulating Neutrophils, Marginated Neutrophils, and Neutrophils in Blood remain constant when the body is at its homeostatic balance. Note that, Neutrophils in Blood refers to sum of Circulating Neutrophils and Marginated Neutrophils. We also show the graphs of G-CSF Ratio and Std PAMPs in Body because they are important variables for neutrophil dynamics. Circulating Neutrophils are at the level of 23 x 10^9 cells. The level of Marginated Neutrophils is almost at the same level as Circulating Neutrophils. G-CSF Ratio and Std PAMPs in Body also stay at their initial levels at homeostasis.

6.2. Experiments with High Levels of Pathogens

Pathogen entry into the body leads to alterations in neutrophil levels [12]. The pathogen entry may differ in quantity and frequency. In this section, we initially simulate one-shot pathogen entry into the body. This simulation resembles an acute bacterial infection. Later, we experiment with continuous pathogen entry into the body. High levels of continuous pathogen entry into the body is expected when the pathogen density is high in the environment. Finally, we experiment with constant pathogen amount in the body that cannot be eliminated by the neutrophils.

6.2.1. One-Shot Pathogen Entry

In this set of experiments, we enter high levels of pathogens at the beginning of the experiment; we assume an intense pathogen exposure that is experienced in an instant. In real life, "one-shot pathogen entry" may correspond to an acute bacterial infection. The aim of this experiment is not to create the exact numerical values reported in the literature. In fact, the neutrophil dynamics of patients are quantitatively different from each other. However, we observe a common pattern that has an initial increase and then a decline in the neutrophil levels during a bacterial infection.

Ishimine et al. [59] show the neutrophil dynamics of patients with different bacterial infections (see Figure 6.2). In that study, neutrophil levels (i.e., absolute neutrophil count) decrease in the early phases of the infections. Then sharp increases are observed. Neutrophil levels reach approximately twice the normal level for three of the patients. For the other three patients, the neutrophil levels reach up to six times the normal level. Finally, for all of the patients, the neutrophil levels decrease and stay in their normal range. The patients in that study take medications, which is different than our setting. Also, different types of bacteria are used in that study. Furthermore, the physiology of patients is not the same. For these reasons, we are not expecting to get qualitatively similar results. We only expect to see a similarity in the dynamic behaviors.

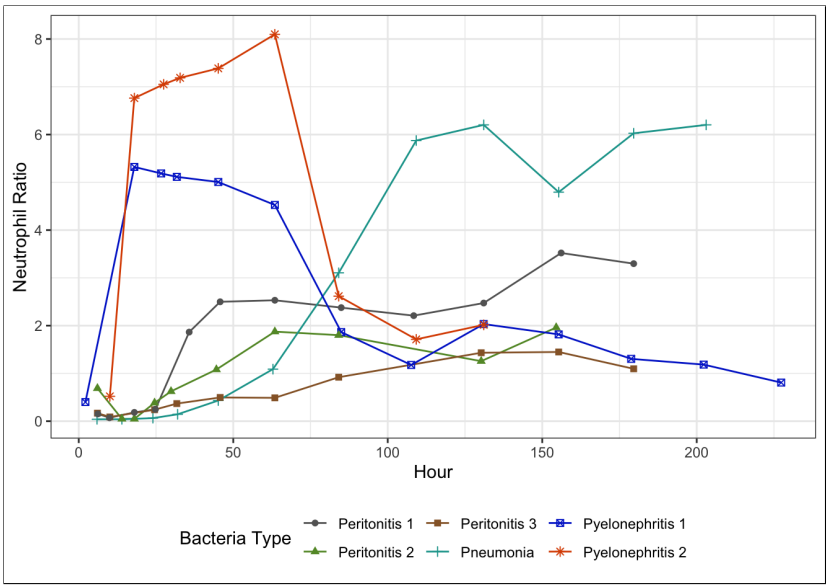


Figure 6.2. Neutrophil ratio of the patients with different bacterial infections (reproduced from [59]).

To be able to simulate the case with an intense pathogen exposure that is experienced in an instant, we experiment with the initial value of the state variable representing pathogens (i.e., *Standardized PAMPs in Body*). At the beginning of the experiment, we enter the initial value of the stock variable as 10-fold, 50-fold, and 250-fold of the assumed standardized normal pathogen level. As a result of our experiment, we observe oscillations in the neutrophil levels in all cases. First, we observe an increase of the neutrophil levels to 1.5-fold of the normal level. Then we observe a decrease in the neutrophil levels down to 0.8-fold of the normal level at around simulated time equals 50 hours. Afterwards, another wave appears with a comparatively higher amplitude in our experiments. The peaks of the second waves are affected by the initial value of pathogens. If the initial pathogen amount is high, the peak of the second wave reaches higher neutrophil level. For example, the neutrophil ratio rises to 4.4 at the second peak when the initial pathogen amount is set to 250-fold of the normal level.

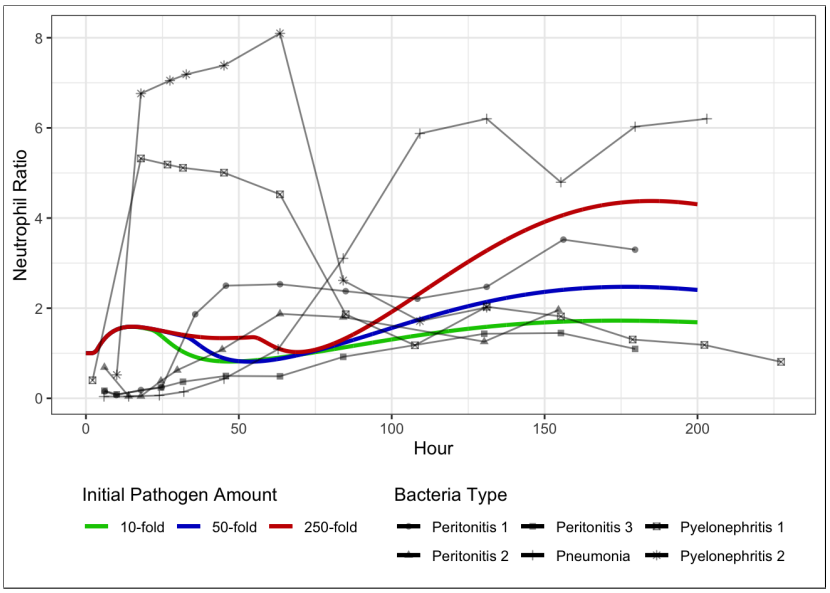


Figure 6.3. Neutrophil ratio with different pathogen entry.

6.2.2. A High-level Continuous Pathogen Entry

In real life, people may be in an environment that has a high pathogen density. In that case, they are under a high risk of infection. Newborn babies may face such an environment. They may show infection episodes if they are in an environment that has a high pathogen density. Figure 6.4 shows the neutrophil counts of a newborn baby during three episodes of urinary infection [60]. At the peaks, neutrophil levels rise to nearly 5-fold of their normal values. Neutrophil levels return to their normal values after a while. These episodes have approximately 20 day periods.

In this experiment, we aim to simulate such a case by increasing the pathogen entry to 5-fold of its normal level. The normal pathogen entry is assumed to be 0.025 standardized pathogens per hour in our model. The flow stays at the 5-fold level (i.e., 0.125) during the experiment. According to our simulation results, we observe oscillations in the neutrophil production line. The body first increases the secretion of G-CSF, but apoptotic neutrophils accumulate in the tissues after a while and slow down G-CSF production. Before the body can re-increase G-CSF production, it needs to wait the elimination of the apoptotic neutrophils, which results in a disruption in

the neutrophil production process. When apoptotic neutrophils are largely eliminated, the production increases again. Therefore, the accumulation and elimination of the apoptotic neutrophils create oscillations. At the peaks, neutrophil levels exceed 5-fold of their normal values. Neutrophil levels do not enter the neutropenic phase at the trenches. These oscillations have approximately 500-hour-period, which is equal to 20.8 days. Note that the 500-hour period observed from a healthy person with urinary infections is the same as the average period observed in the dynamics of neutrophils of a CN patient.

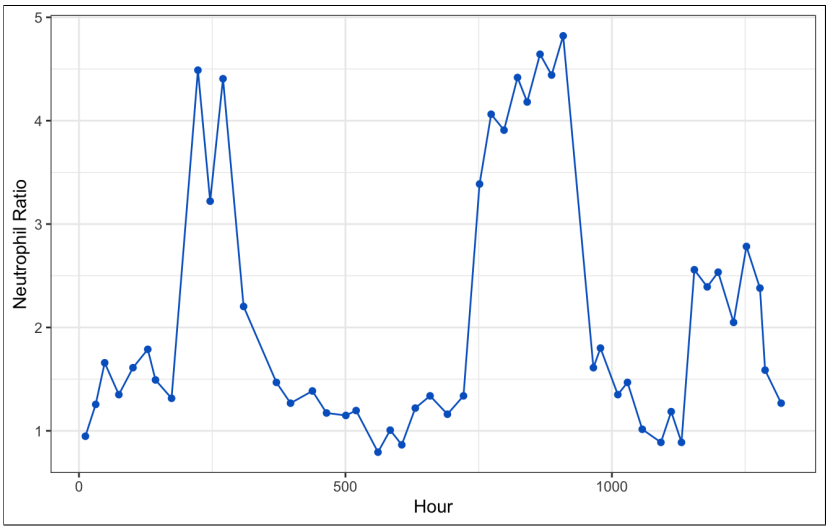


Figure 6.4. Neutrophil ratio of a newborn baby that has urinary infections (reproduced from [60]).

It is important to note that our model is constructed based on an adult male human. Thus, there are differences between the settings of the newborn baby study and our assumptions in the simulation experiment. As we have limited data sources in the literature and it is difficult to find real life experiments including the data from both pathogens and neutrophils, we decide to carry-out this comparison. The similarity between the data presented in Figure 6.4 and the results of the simulation experiment given in Figure 6.5 is that there are high amplitude oscillations with a period of approximately 20 days in both figures.

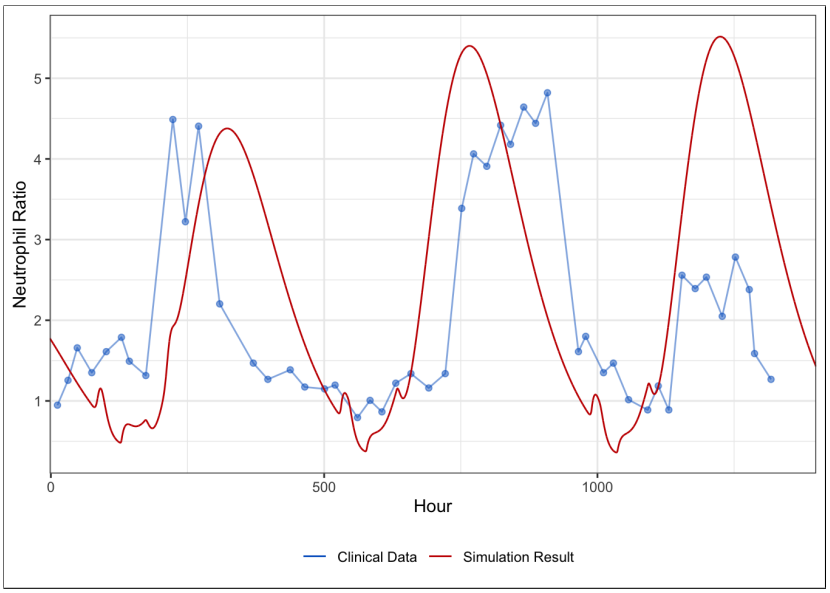


Figure 6.5. Neutrophil ratio with high-level continuous pathogen entry.

6.2.3. Constant Pathogen Level

Common substances such as dust and pollens may be allergenic for people whose immune system is overactive and constantly stimulated. For these people, excess stimulation of the immune system causes disorders like asthma and eczema [61]. Neutrophil levels are increased during severe asthma attacks [62]. The patients with chronic immune stimulation have constantly alarmed receptors. We assume that the body of these patients cannot eliminate the pathogens. We hypothetically simulate this case by setting a high and constant level of PAMPs (*Standardized PAMPs in Body*: 1.75) that cannot be eliminated by the neutrophils. In this setting, neutrophils are produced, stimulated, and activated for the elimination of pathogens, but they simple have no effect on the level pathogens.

According to the simulation results, the model creates similar dynamics to the case with a high-level continuous pathogen entry. We observe the dynamics in Figure 6.6. We observe oscillations with a 500-hour period. Neutrophil levels increase to ten times of the normal values. The levels do not decrease to the low levels observed in the neutropenic phase. These oscillations probably can shed some light on the periodic

symptoms observed in patients with diseases caused by an overactive immune system.

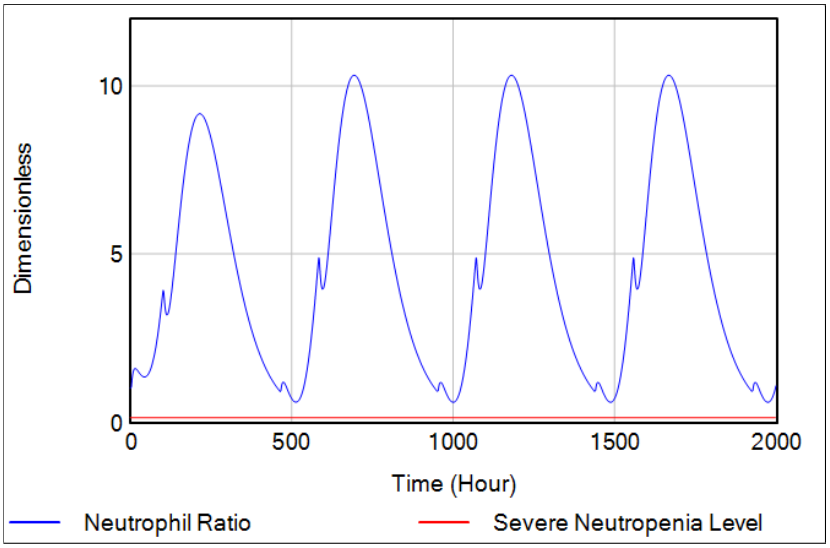


Figure 6.6. Neutrophil ratio with constant pathogen level.

6.3. Irradiated Non-human Primates

Radiation leads to stem cell loss and neutrophil precursor cell loss in the bone marrow [63, 64]. In their study, Harrold et al. [65] use data of non-human primates (rhesus macaques) that are exposed to acute radiation. The primates are divided into two groups. One group is administered with G-CSF injections. The other group is the control group and they receive placebo. We use the neutrophil data from the control group in our comparisons after creating a setting similar to the acute radiation injury. First we set the proliferation parameters of the mitotic pool to 0 for 380 hours reflecting the loss of the stem cells in the presence of radiation. We also create early deaths of neutrophil precursors. Finally, we increase the G-CSF amount so that we can reflect the initial response of the body to radiation. Note that, the first peak observed in the data cannot be observed without reflecting the initial increase in the G-CSF level.

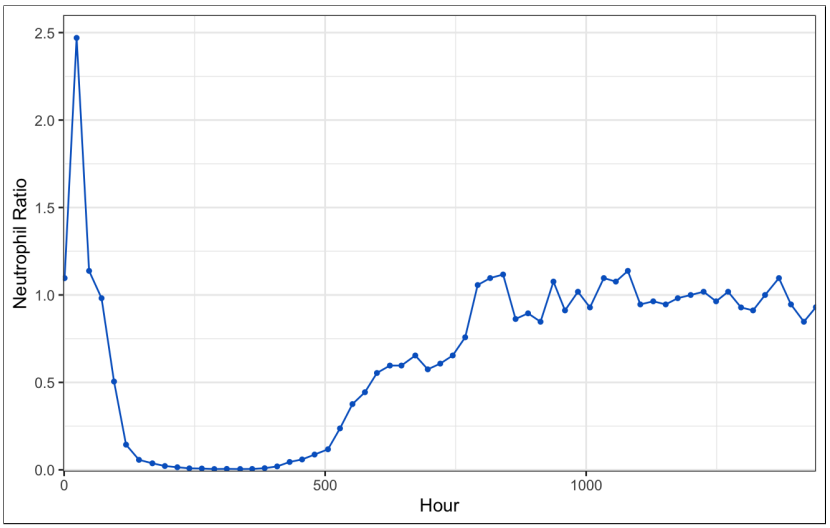


Figure 6.7. Neutrophil dynamics of irradiated non-human primates. The data is taken from [65].

Accordingly, we assume that the body shows a reaction to the radiation shock and secrete G-CSF at the initial phase. As a result of reflecting these changes in the model, we obtain a similar behavior to the irradiated primates. Neutrophil levels first increases to the 2.5-fold of the normal level. We suppose that this increase occurs because of the release effect of G-CSF on stored neutrophils. After the initial phase, we observe a sharp decrease due to the loss of stem cell and progenitor cells. Low levels continue approximately for 400 hours. The neutrophil levels start increasing 500 hours after the radiation injury. The neutrophil levels reach the normal level between the hours 600 and 800; see figures 6.7 and 6.8. The average normal neutrophil levels of the primates are almost the same as the average human neutrophil levels. The normal neutrophil level of the primate used in the clinical experiments was lower than the average. To be able to compare the simulation results with the data, we standardized the neutrophil levels of the primate based on the normal level of that primate.

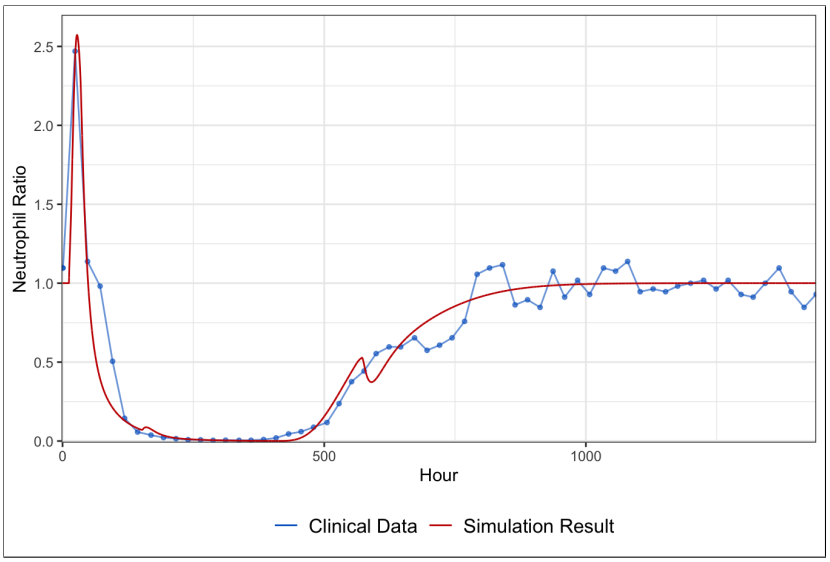


Figure 6.8. Neutrophil dynamics in our model after a radiation injury.

7. CYCLIC NEUTROPENIA PATIENT

CN patients show oscillations in the neutrophil levels. At the peaks of the oscillations, neutrophil levels can reach only 40% of the normal level of a healthy person [43]. At the trenches of the oscillations, neutrophil levels stay under the 12% of the normal level of a healthy person for more than 3 days. The main motivation of our study is to obtain the neutrophil dynamics of a CN patient from the model for a healthy person. In other words, we aim to obtain a structurally sound and comprehensive neutrophil model that is capable of generating both the neutrophil dynamics for a healthy person and for patients with CN. Therefore, we only change some parameter values, but use the same exact model structure. In this chapter, we explain the transition process from a healthy person to a CN patient. In the first section, we describe the parameters that are changed to obtain the neutrophil dynamics for a CN patient. In the second section, we show the neutrophil dynamics of a CN patient produced by our model. In the third section, we give our insights about the pathophysiology of cyclic neutropenia, which we develop during the transition from a healthy person to a CN patient. In the third section, we also discuss an important assumption our model, which is required in producing dynamics for different cases.

7.1. Parameters Used in Transition from a Healthy Person to a CN Patient

7.1.1. Proliferation Multipliers

Production deficiency (i.e. early apoptosis of neutrophil precursors) is the most mentioned cause of CN in the literature. For this reason, we aim to represent a production deficiency by decreasing the multiplier values in the early phases of neutrophil production. According to the literature, the production deficiency occurs at neutrophil precursors [12]. In our model, *Myeloblasts* and *Promyelocytes* refer to the neutrophil precursors. The multiplier values, *Normal MSC to Myeloblast Multiplier* and *Normal Myeloblast to Promyelocyte Multiplier* affect the inflows of these stock variables. Thus,

we select these multiplier values to create the production deficiency effect (see Figure 7.1). Multiplier values are set to 2 in the model for healthy person. We are unable to obtain the percentage of production deficiency. Thus, we calibrate the multiplier values and decrease the values to 0.5. Then the average neutrophil levels for CN patients is obtained. The neutrophil dynamics do not exactly match with clinical data at this stage. In fact, the neutrophil dynamics are highly sensitive to the production deficiency, which is discussed in Section 8.2.

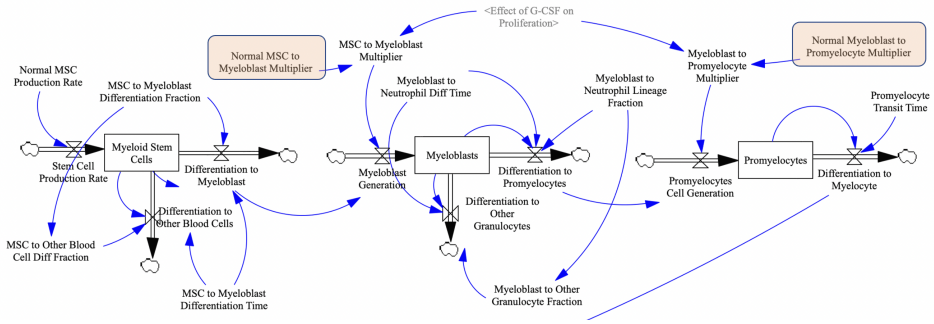


Figure 7.1. Proliferation multipliers.

7.1.2. G-CSF Production Rates

Applying the production deficiency on its own does not produce the CN dynamics. We observe a decrease in the neutrophil levels, but we cannot observe the extremely low neutrophil levels. However, the extremely low neutrophil level at the trenches is a characteristic of the disorder. According to the literature, the G-CSF levels of CN patients cannot increase as much as a heathy persons' levels even when the CN patient is in a neutropenic phase [66]. For example, when a healthy person (i.e., non-CN patient) gets infected, the G-CSF levels reaches to 780 nanogram/liter at the peaks on the average. However, for a CN patient, we assume that the G-CSF levels reach roughly 156 nanogram/liter at the peaks on the average. This assumed value is consistent with the literature. Table 7.1 shows the G-CSF levels of a CN patient during an oscillation period. Therefore, we decreased the homeostatic and rapid G-CSF production rates (see Figure 7.2), which is in accordance with the literature [66, 67]. We decreased

Homeostatic G-CSF Production Rate from 26 nanogram/hour to 0 nanogram/hour. We also decreased Rapid G-CSF Production Rate from 780 nanogram/hour to 156 nanogram/hour. This change is necessary because CN patients tend to have lower G-CSF levels compared to a healthy person.

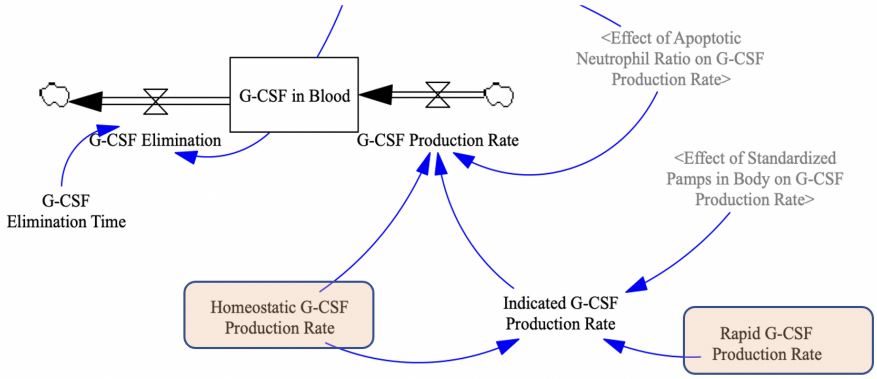


Figure 7.2. G-CSF production rates.

Table 7.1. Neutrophil and G-CSF Levels of a CN Patient During an Oscillation Period [67].

Day	**ANC (x 10^9/L)**	**G-CSF (pg/ml)**
1	1.0	<30
3	0.3	48
7	0.05	165
10	0.05	100
14	1.0	41
17	1.7	35
21	2.5	45

7.1.3. Minimum Proliferation Coefficient

In a healthy person, the lowest proliferation without G-CSF is 30% of its normal level. Neutrophil levels of a CN patient show extremely low values in each cycle.

This shows that the base neutrophil production is either completely absent or in trace amounts in CN patients. Thus, in addition to proliferation deficiency and low G-CSF production rates, we change the Min Proliferation Coefficient parameter to create the severe neutropenic phases at the trenches of the oscillations. Accordingly, we change the Min Proliferation Coefficient from 0.3 to zero.

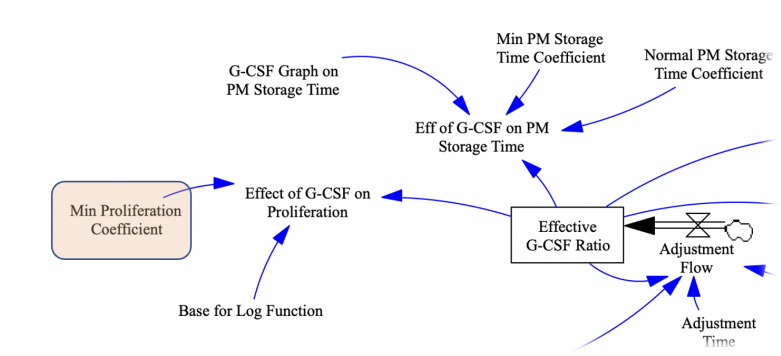


Figure 7.3. Min proliferation coefficient in G-CSF compartment.

7.1.4. Clearance Time Parameter

According to the literature, the monocyte levels also show cycles in CN patients [68]. Monocytes become macrophages after they transmigrate into tissues. Therefore, the levels of macrophages in tissues also show cycles. Since the apoptotic neutrophils are cleared by macrophages, the clearance of apoptotic neutrophils may take longer when the macrophage numbers are low. Depending on this information, we conclude that the clearance time of apoptotic neutrophils should be longer for CN patients. Accordingly, we set the Clearance Time parameter to 12 hours for a CN patient. As a result of this change, it takes longer time to clear apoptotic neutrophils in our model.

Using our model, we verify that longer clearance time is necessary to obtain the neutrophil dynamics for a CN pateint. For this, we carry out experiments with Clearance Time in Section 8.3. Also, when we use shorter Clearance Time for CN patients, we cannot observe neutropenic phases under rG-CSF injections. However, according to the clinical data, severe neutropenic phases are still observed in CN patients under

rG-CSF injections. The dynamics obtained from our model is consistent with the clinical data only when we set Clearance Time to 12 hours. The details of this experiment is provided in Section 8.4.

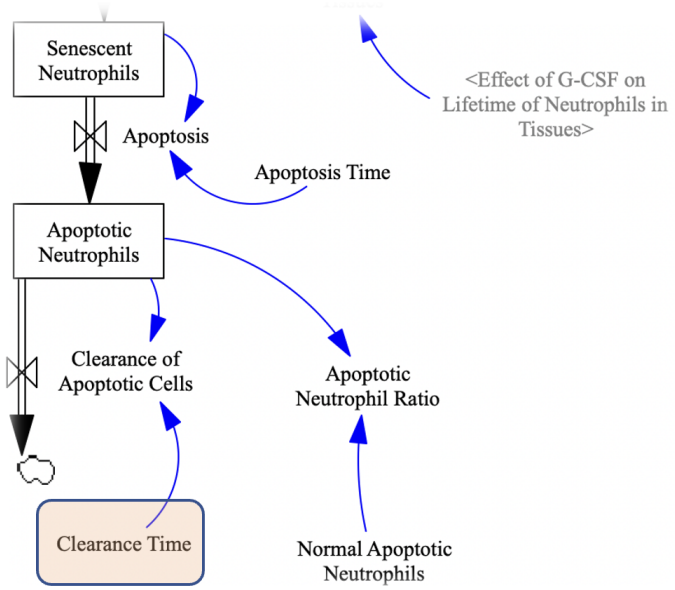


Figure 7.4. Clearance time parameter in compartment for blood and tissue neutrophils.

7.2. Neutrophil Dynamics and Pathophysiology of Cyclic Neutropenia

In summary, we make the following changes in the model for the transition from a healthy person to a CN patient.

- The multiplier values in the early phases of neutrophil production are decreased to represent the production deficiency; the values of *Normal MSC to Myeloblast Multiplier* and *Normal Myeloblast to Myelocyte Multiplier* are changed from 2 to 0.5.
- *Min Proliferation Coefficient*, which is 0.3 for a healthy person, is set to zero.
- *Rapid G-CSF Production Rate* and *Homeostatic G-CSF Production Rate* are set to 156 nanogram/hour and 0 nanogram/hour, respectively. They are originally

780 nanogram/hour and 26 nanogram/hour, respectively, for a healthy person.

- *Clearance Time* for apoptotic neutrophils is increased from one hour to 12 hours.

After these changes, we obtain the neutrophil dynamics of a CN patient with a 21-day period and a lower neutrophil level than a healthy person. According to the results, the severe neutropenia phase lasts approximately eight days in our simulation. The dynamics that we obtain after changing the parameter values is in accordance with the dynamics presented in the literature; for example, see the dynamics given in Figure 7.6 [43].

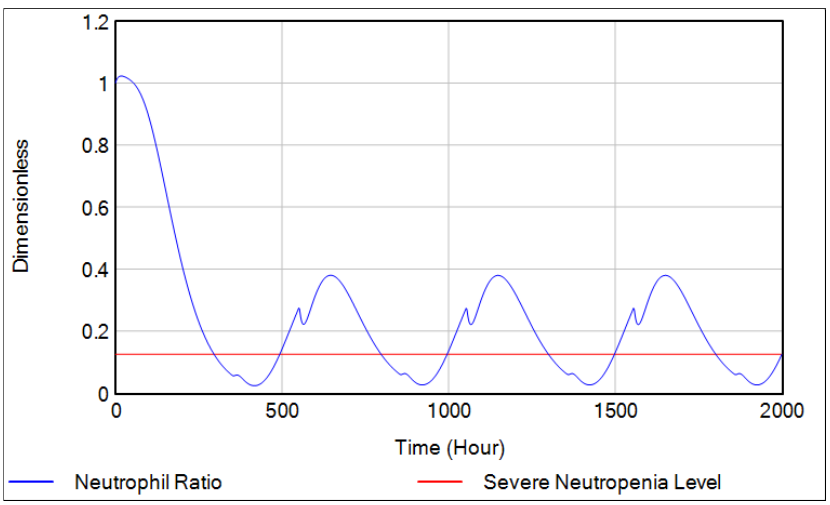


Figure 7.5. Neutrophil ratio of CN patients in our model.

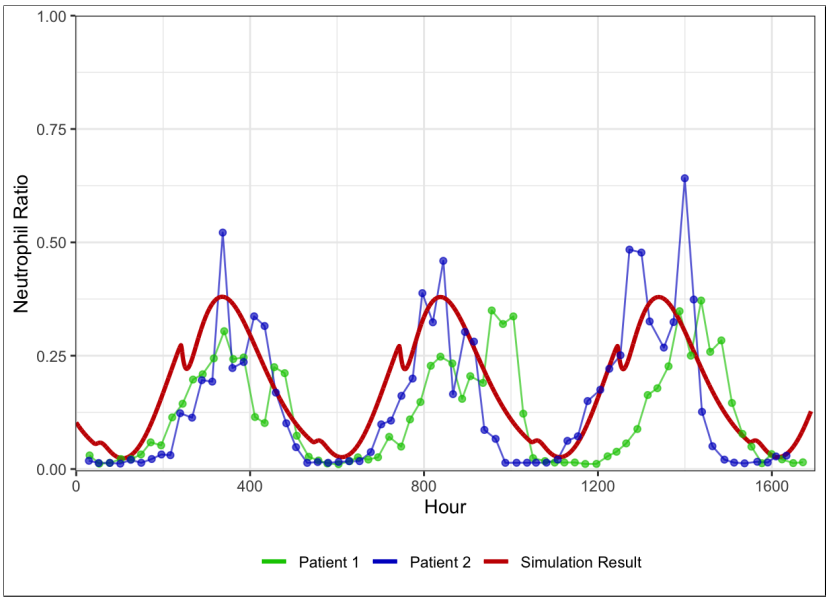


Figure 7.6. Neutrophil ratio of two CN patients that are reproduced from [43] and plotted together with the simulation result of the comprehensive model .

7.3. Our Insights About the Pathophysiology of Cyclic Neutropenia and an Important Assumption of Our Model

The transition process that we follow gives us some clues about the pathophysiology of the disorder. Supported with the results of our model, we claim that production deficiency (i.e., early apoptosis of the neutrophil precursors), by itself, cannot explain the dynamics seen in a CN patient. In addition to production deficiency, the low G-CSF levels and longer clearance time also play a role in creating the severe neutropenic phases in a CN patient.

We have a single structure that represents the neutrophil dynamics for a healthy person, a CN patient, and a CN patient with rG-CSF injections. Moreover, when we are experimenting with pathogen levels in Section 6.2 and when we create the case with radiation in Section 6.3, we still continue to use the same model structure. Before we can achieve this single model structure that is capable of generating dynamics for different cases, we introduced an assumption that is not reported by the literature. According to the literature, apoptotic neutrophils create a feedback mechanism that

suppress the G-CSF production. We used this assumption in our model. However, we assumed that it is not only the G-CSF production that is suppressed by the apoptotic neutrophils, but apoptotic neutrophils also deactivates the effects of available G-CSF in the body. In other words, according to our assumption, the feedback mechanism caused by high levels of apoptotic neutrophils also blocks the response of the body to G-CSF. We reach this claim as a result of our experiments with the model.

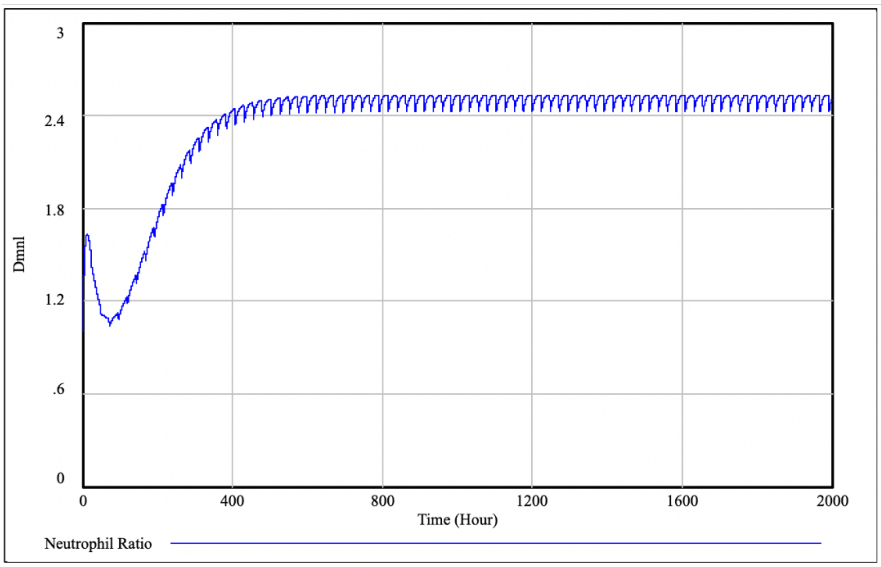


Figure 7.7. Neutrophil ratio for a CN patient with G-CSF injections.

In our simulation experiments, we first assume that the apoptotic neutrophils only restraint G-CSF production. Then we apply rG-CSF injections to CN patients in the model and we obtain a behavior shown in Figure 7.7. The dynamics in the experiment suggest a complete healing from the neutropenic phases. However, we know that the rG-CSF injections do not eliminate the oscillations and severe neutropenic phases [25] (see Figure 7.8). There is a contradiction between the result of our simulation experiment and the clinical results. Then we seek an explanation for the contradiction. In the process, we discover that obtaining consistent results with the clinical data is only possible when the effects of G-CSF are also suppressed by the high levels of apoptotic neutrophils. Accordingly, we reach the claim that high levels of apoptotic neutrophils do not only suppress the production of G-CSF, but they also deactivate the effects of G-CSF mentioned in Section 5.3. When we apply the assumption, the

model produces the behaviour that is shown in Figure 7.9. Note that this behavior is obtained under daily G-CSF injections. We set the dose of injections higher than normal. We aim to obtain dynamics similar to clinical data.

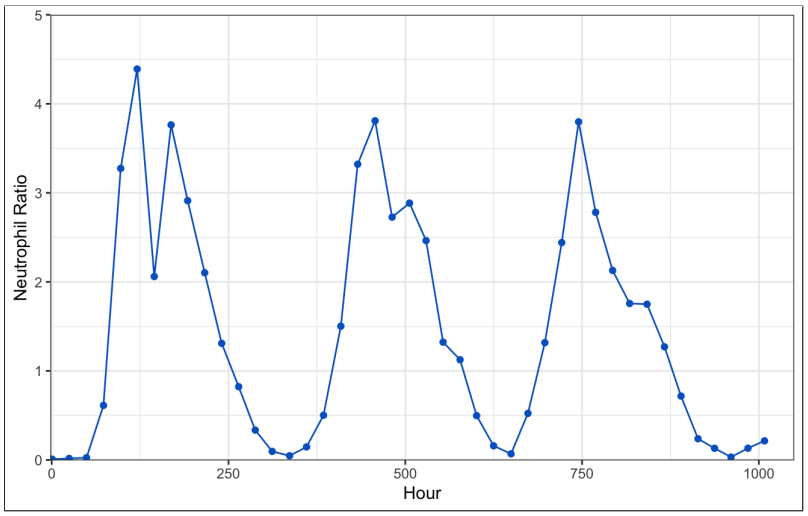


Figure 7.8. Neutrophil ratio of a CN patient under daily G-CSF treatment (reproduced from [25]).

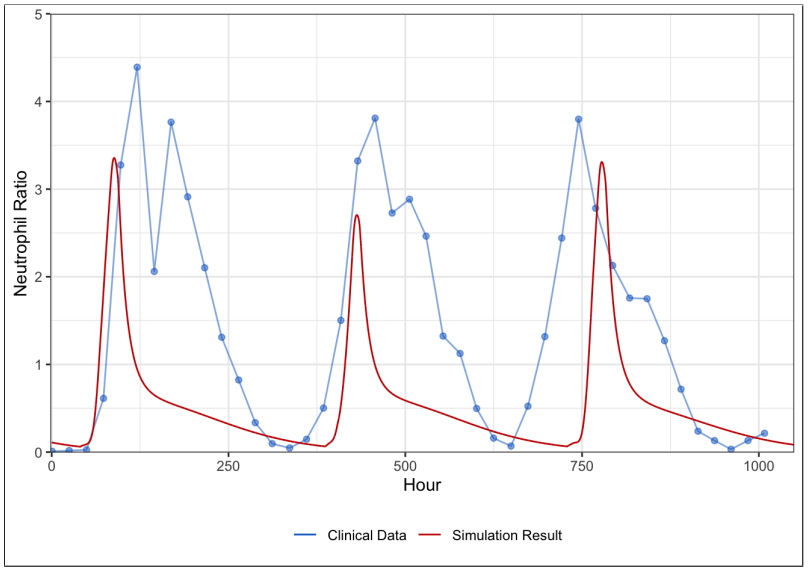


Figure 7.9. Neutrophil ratio of a CN patient under daily G-CSF treatment after our assumption.

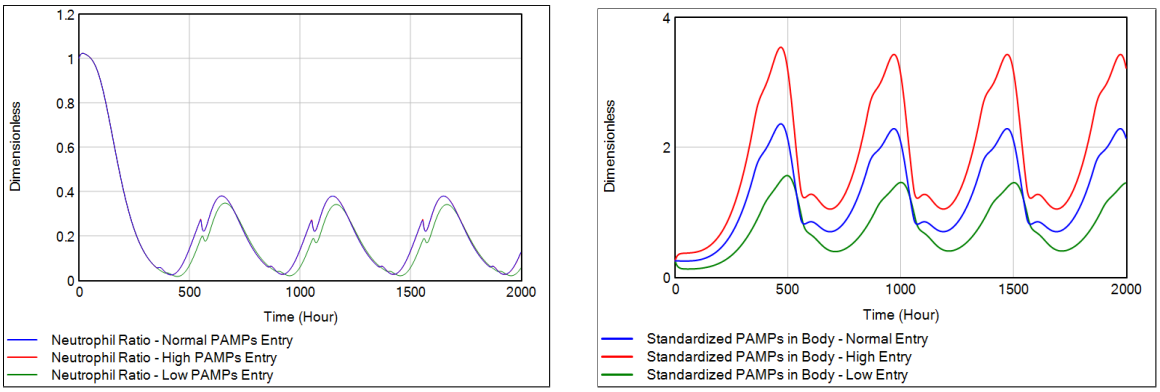
8. SENSITIVITY ANALYSIS FOR A CYCLIC NEUTROPENIA PATIENT

We are not able to obtain data for a few of the parameter values. For such cases, we calibrate the corresponding parameter values. In this chapter, we analyze the sensitivity of the dynamics to the changes in the parameter values for three cases; pathogen entry rate, the production deficiency in CN patients, and clearance time of apoptotic neutrophils. For the sensitivity analysis, we increase and decrease the current values of the calibrated parameter values. Therefore, we present three runs in the graphs; one with the low value, one with the value calibrated for the CN patient, and one with the high value. We also experiment with the shapes of the graphical effect functions. However, we observe no significant changes in the dynamics. Thus, we will not report those experiments.

8.1. Pathogen Sensitivity

As we mentioned earlier, we assume that pathogen entry into the body is constant under normal conditions and their elimination is performed by neutrophils in the tissues. We include a stock variable in the model for pathogens (*Standardized PAMPs in Body*). We calibrated the values of the pathogen related parameters so that the model mimics the desired dynamics for a healthy human. Note that, we are not interested in the corresponding values of the real counterpart of these parameters. After calibrating the pathogen compartment for a healthy person, we use the same exact pathogen compartment structure and pathogen compartment parameter values for the CN patient too. In this section, we report the sensitivity of the model to the pathogen entry rate for a CN patient. We experiment with the value of *External Pathogen Inflow*. We increase the entry of the pathogens by 50%. In this case, we do not observe any difference in neutrophil dynamics for CN patients. However, when we decrease the pathogen entry 50%, the period of oscillations gets longer (Figure 8.1). We also observe a slight decrease in the peak Neutrophil Ratio values.One can say that the

dynamics for CN is not that sensitive to the value of the pathogen entry at around its normal levels.



(a) Neutrophil Ratio.

(b) Standardized PAMPs.

Figure 8.1. Variable dynamics of a CN patient with 50% higher and 50% lower PAMPs entry.

8.2. Neutrophil Production Deficiency Sensitivity

According to the literature, cyclic neutropenia is caused by early apoptosis of the neutrophil precursors (i.e., production deficiency). However, there is no specific information about the percentage of the cells that are lost. We illustrate the production deficiency in our model. by decreasing the multiplier values, which are *Normal MSC to Myeloblast Multiplier* and *Normal Myeloblast to Myelocyte Multiplier*. These values are set to 2 in the model that represents healthy person dynamics. Later, these multiplier values are set to 0.5 in the adjusted model for CN patients. In this section, we experiment with these multiplier values to see the sensitivity of the dynamics. We carry out a set of experiments with the multiplier values. We first set the multiplier values to 0.25, which is half of the value for a CN patient that is 0.5. We also experiment by setting the multiplier values to 1, which is twice of the value for a CN patient.

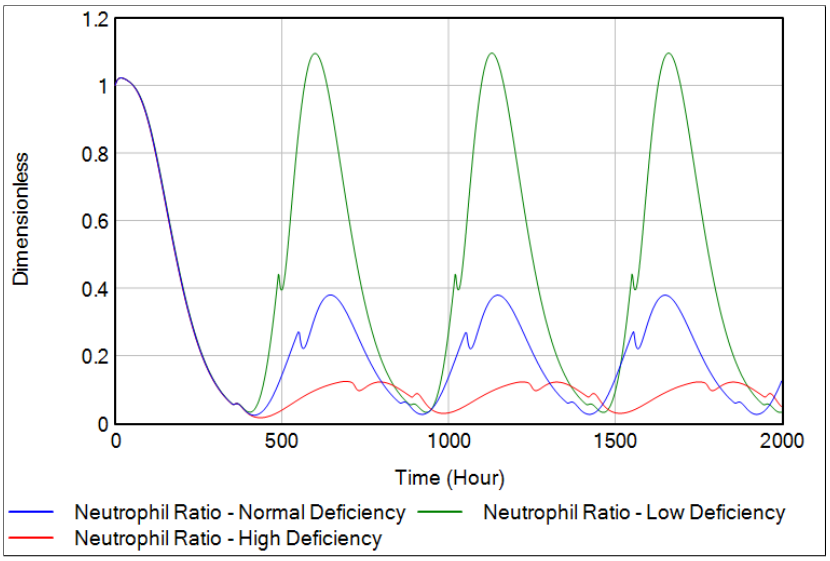


Figure 8.2. Neutrophil ratio with different production deficiency levels.

According to the results of the experiments, neutrophil dynamics is highly sensitive to production deficiency. Decreasing the multiplier values to 0.25 causes the neutrophil level to stay in the severe neutropenic phase and the period of the oscillations gets longer. When the multipliers are set to one, we observe oscillations with larger amplitudes. *Neutrophil Ratio* exceeds even one at the peaks and the period of the oscillations becomes slightly longer. The model is extremely sensitive to neutrophil production deficiency. We suggest that clinical studies focus on production deficiency for CN patients to have a better understanding of these multiplier values.

8.3. Clearance Time Sensitivity

We claim that the clearance of the apoptotic neutrophils of CN patients takes longer than normal. However, we could not find a specific value about clearance time of the apoptotic neutrophils. Clearance Time is set to one hour for a healthy person. We also calibrate the clearance time for CN patients and we set it to 12 hours, which is assumed to be 12 times more than the value for a healthy person. Future clinical studies may help us understand if clearance time really gets longer for a CN patient, which is identified as one of the potential causes of CN in this study. Clearance Time affects the neutrophil dynamics through the feedback mechanism that can suppress

neutrophil production. We analyzed the sensitivity of the model to different values of Clearance Time. Figure 8.3 shows the neutrophil dynamics with different Clearance Time values.

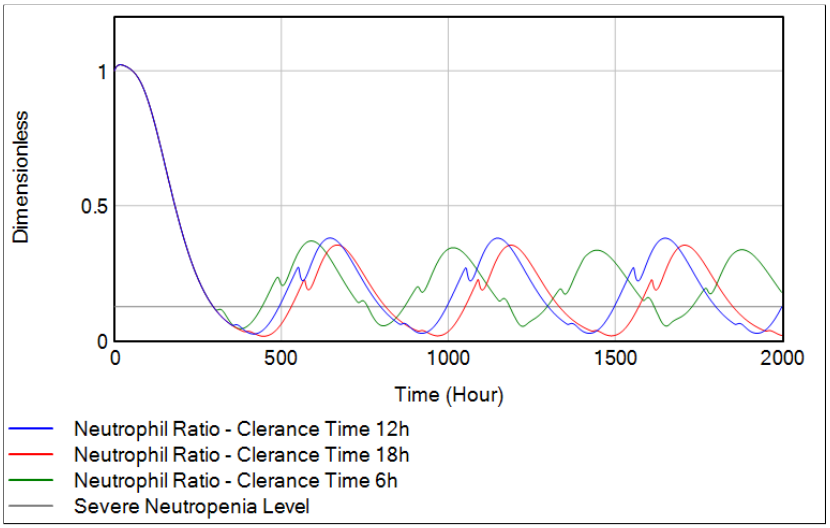


Figure 8.3. Neutrophil ratio with different clearance time values.

For a CN patient, the major effect of clearance time is on the period of the oscillations. The longer clearance time leads to a longer period whereas a shorter clearance time results in a shorter period. The amplitudes of the oscillations are shorter when Clearance Time is set to 6 or 18 hours. We can say that the neutrophil dynamics of the modeled CN patient is highly sensitive to clearance time. Therefore, we confirm that clearance time for CN patients must clinically be studied.

8.4. Clearance Time Sensitivity in the Presence of rG-CSF Injections

To investigate our claim that the clearance time is longer for a CN patient than for a healthy person, we experiment with a shorter clearance time as we also apply rG-CSF injections to the patient. The Figure 8.4 shows the dynamics of Neutrophil Ratio for shorter clearance time. According to the results, when we set the clearance time shorter, neutropenic phases disappear under rG-CSF injections. This shows that if clearance time was shorter than 12 hours, it would prevent the severe neutropenic phases that

are observed in the neutropenic cycles. However, according to the literature, extremely low level trenches are observed under rG-CSF injections. For this reason, we claim that longer clearance time could really be another characteristic of cyclic neutropenia.

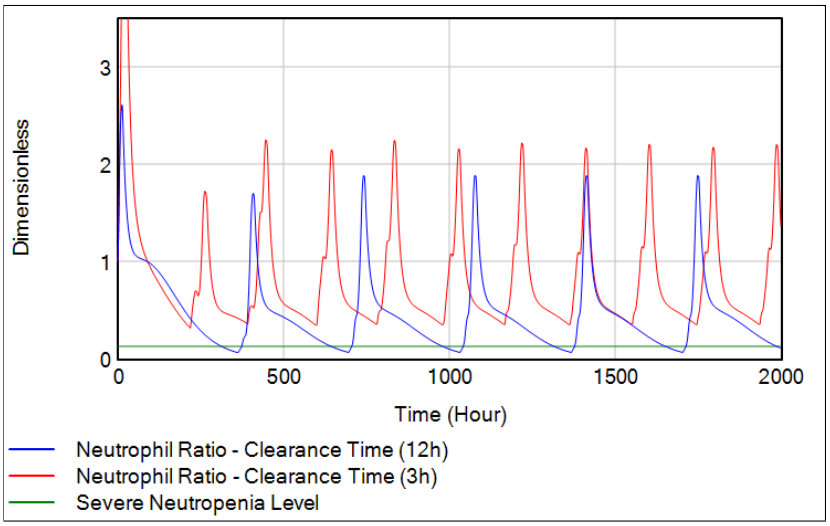


Figure 8.4. Neutrophil ratio with different clearance time values under rG-CSF injections.

9. SCENARIO ANALYSIS WITH rG-CSF INJECTIONS

One of the objectives of this thesis is to show the use of the model as an experimental platform. To serve that objective, we develop a scenario analysis for a CN patient. We carry out rG-CSF injection experiments with different doses and frequencies. According to the literature, the daily sufficient rG-CSF dose for CN patients is 1.5 microgram/kg body weight [23]. As our model is constructed based on a 70-kg human, the daily required amount is 105 microgram of rG-CSF. We use the bioavailability of subcutaneous rG-CSF injection as 70 percent [69]. Accordingly, we obtain a dose of 73.5 microgram / day for the model. We accept this dose as the default (i.e., normal dose) application of rG-CSF injections. rG-CSF injections are usually applied daily. According to the literature, the neutrophil levels of CN patients who are treated with rG-CSF injections still show oscillations and their neutrophil levels still go through the neutropenic phases (i.e., their neutrophil levels go below the severe neutropenia level, which is 12% of the value for a healthy person). According to clinical data, the period of the oscillations is shortened to 14 days from 21 days in the presence of rG-CSF treatment. Note that, the period is 21 days for non-treated CN patients. Our model produces similar results that are reported in clinical studies when we apply daily injections of rG-CSF with the calculated normal dose.

9.1. rG-CSF Doses

In this section, we experiment with different doses of rG-CSF injections. As far as we know, rG-CSF injections cannot eliminate the oscillations. Accordingly, we monitor the peak values of the oscillations to measure the effectiveness of the injections. We also monitor the periods of the oscillations and the durations of the neutropenic phases. By experimenting with different doses of rG-CSF, we want to see if we can obtain even more elevated neutrophil levels and even shorter durations of neutropenic phases. We prefer the total period of the cycle to be shorter because that will potentially result in a shorter neutropenic phase too.

The following figure shows the dynamics of neutrophils with different doses of rG-CSF administration. The peak values change significantly depending on the doses. The period also changes with different doses.

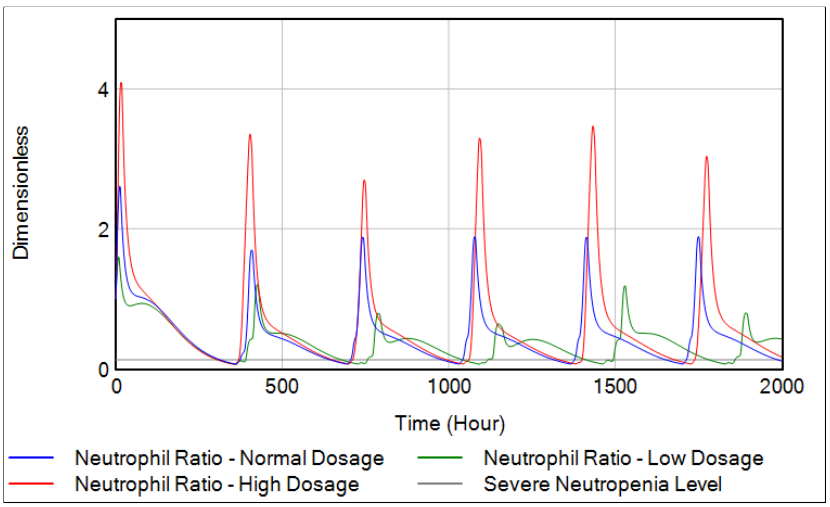


Figure 9.1. Neutrophil ratio with different injection doses.

First, 73.5 microgram of rG-CSF is administered as the normal dose in the experiment. According to the results of the experiment, the period of the oscillations is 14 days. *Neutrophil Ratio* approaches two at the peaks. The neutrophil levels stay in the neutropenic phase for approximately 60 hours. These values that we obtain from our model are consistent with the data reported by the relevant clinical studies [22,23].

We use 24.5 microgram of rG-CSF for the low dose experiment. The dose is lower than the sufficient dose used in clinical experiments [23]. The low dose of rG-CSF administration does not provide significant and consistent elevation in the neutrophil levels. The period of the oscillations becomes 15 days which is longer than the period with the normal dose. The neutrophil ratio does not reach 1 at some of the peaks and there is a significant variation in the peak values. The neutrophil levels stay in the neutropenic phase for approximately 75 hours in each cycle.

For high dose experiment, 245 microgram of rG-CSF is administered daily. High doses of rG-CSF increase the peaks of the oscillations. The peak values of neutrophil

ratio exceed three. The period is 14.41 and the highest peak of the neutrophil ratio is 3.47. The neutrophil levels stay in the neutropenic phase for approximately 65 hours. Even though the high dose administration provides an elevation in the neutrophil levels at the peaks, it fails to shorten the duration of neutropenic phase.

9.2. rG-CSF Injection Frequency

A dose of 73.5 microgram rG-CSF is administered for each experiment in this section. We first administer rG-CSF injections every 12 hours. Then we administer rG-CSF injections every 24 hours. Lastly, we administer rG-CSF injections every 48 hours. Figure 9.2 shows the results of experiments. The period of the oscillations is approximately 14 days. Interestingly, it stays the same for all cases. The peak values of neutrophil levels are affected the most as the injection frequency changes. The duration of the neutropenic phases, which is 102 hours, is the longest when the injections are administered every 48 hours. The neutropenic phases last 60 hours when the injections are administered every 24 hours. The neutropenic phases last 62 hours when the injections are administered every 12 hours. 48-hour injection regime is not able to provide a significant increase in the neutrophil levels. Neutrophil ratio can only reach 0.8 at the peaks. Frequent injections (every 12 hours) provide a significant increase in the neutrophil levels. Neutrophil levels exceed 2.5 at the peaks.

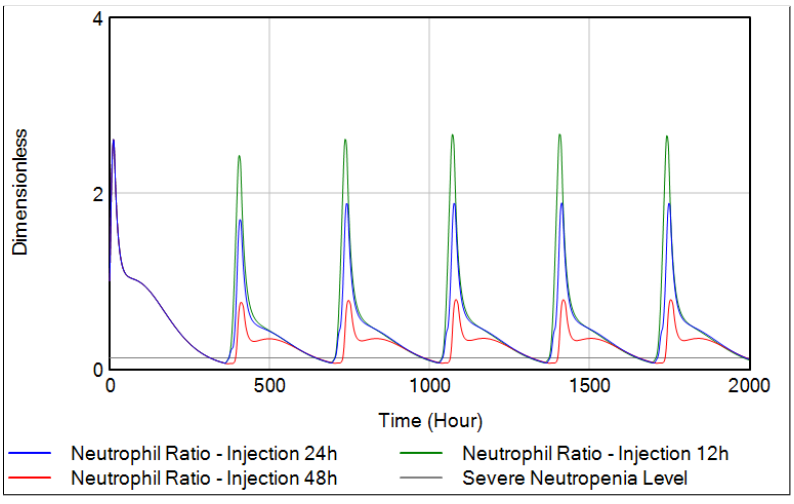


Figure 9.2. Neutrophil ratio with different injection frequencies.

9.3. Combined Experiments with Different Doses and Frequencies

In this section, we carry out experiments combining different frequencies and doses of injections. First, we administer half dose of rG-CSF injections (i.e., 36.75 microgram) every 12 hours. Then we administer double dose of rG-CSF injections (i.e., 147 microgram) every 48 hours. The experiment results are compared with the daily normal dose (i.e., 73.5 microgram).

9.3.1. Injections with Half Dose and 12h Period

In this experiment, we administer half dose injections every 12 hours. As a result, we observe that the period of the oscillations are shortened to 13.25 days. Neutrophil Ratio reaches 2 at the peaks. The neutrophil levels stay in the neutropenic phase for approximately 60 hours. According to our simulation results, we observe a minor improvement in the neutrophil levels.

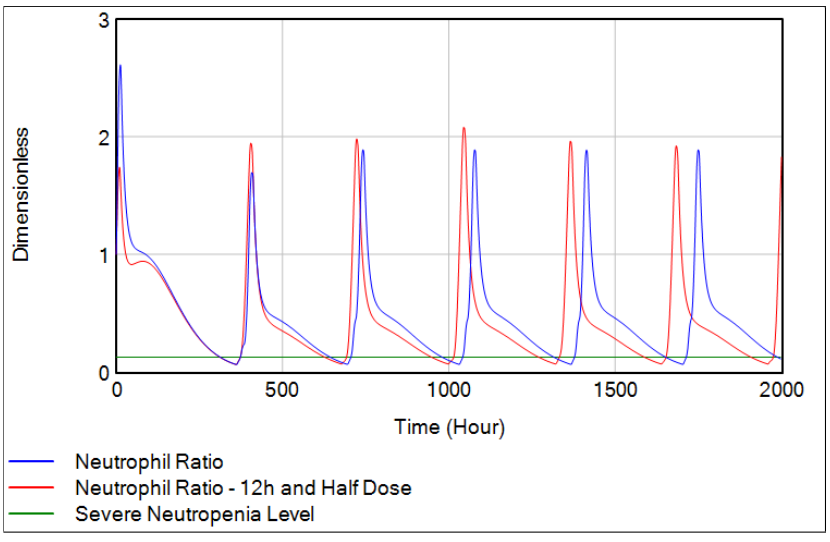


Figure 9.3. Neutrophil ratio with daily normal dose and twice half dose G-CSF injections .

9.3.2. Injections with Double Dose and 48h Period

In this experiment, we inject a double dose of rG-CSF every other day. These infrequent injections do not provide an increase in the neutrophil levels as high as the daily normal dose injections. The period of the oscillations is 14 days. Note that, the severe neutropenic phase becomes longer with double dose injections every 48 hours. The length of the neutropenic phase is 78 hours. It is easier for the patients to apply less frequent injections. However, this situation increases the risk of infection. In their study, Foley et al. [29] claim that alternate day injections may be as effective as daily injections. However, our result contradicts with that claim.

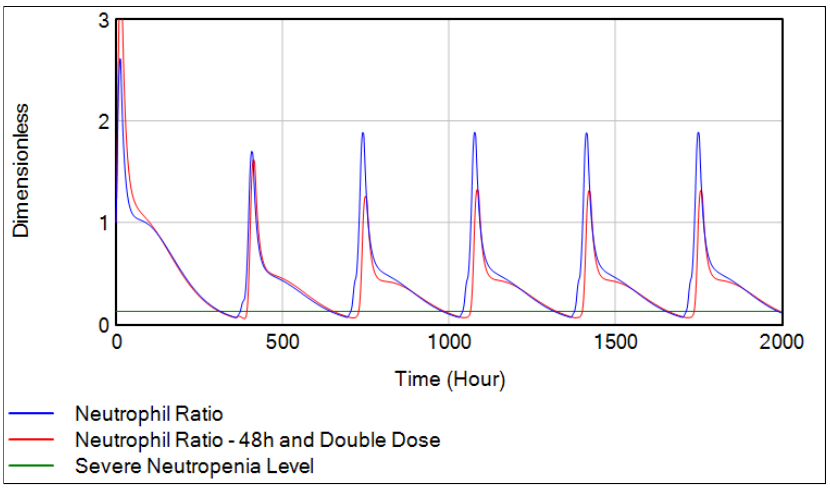


Figure 9.4. Neutrophil ratio with daily normal dose and double dose G-CSF injections on alternate days.

10. CONCLUSION

In this study, a comprehensive model of neutrophil production is constructed based on the information available in the literature. There are 14 stock variables in the model. The model includes feedback loops, nonlinear relationships, and delays as a result of the physiological complexity involved in neutrophil dynamics. In addition to neutrophil production compartment, the model also has G-CSF and pathogen compartments. Furthermore, the tissue neutrophils, apoptotic neutrophils, and the effect of apoptotic neutrophils on G-CSF are also explicitly represented in our comprehensive model. We use the same exact model structure and only experiment with parameter values while illustrating different cases. As the structure of our model is grounded in the literature and as we create dynamics for different cases by only changing parameter values, we develop confidence in the validity of our model.

Our model is capable of representing the neutrophil dynamics of a healthy person, a CN patient, and a CN patient under rG-CSF injections. We obtain the homeostatic neutrophil dynamics of a healthy person in the equilibrium run. The values of the variables stay in the ranges that are given in the literature. Later, we change some parameters in the model creating a CN patient from a healthy person. By doing so, we aim to address the potential causes that constitute the CN pathophysiology. We capture the main characteristics of the neutrophil dynamics for a CN patient: (1) The neutrophil levels of a CN patient are lower than a healthy person; (2) the neutrophil levels oscillate in a CN patient; (3) the oscillations show extremely low neutrophil levels at the trenches; (4) the period of the oscillations approximately is 21 days. The CN-patient dynamics that we obtain by running a simulation of the model is consistent with the clinical data. Furthermore, our model can also create the dynamics of CN patients that have rG-CSF injections. According to our experiment results, the amplitude of the oscillations is increased during rG-CSF injections. Also, rG-CSF injections shorten the period of oscillations in CN patients from 21 days to 14 days. These results are also consistent with the clinical data reported by other studies.

We obtain insights about the pathophysiology of cyclic neutropenia by using the same model structure in the three cases. Firstly, we confirm that there is a production deficiency, early apoptosis of neutrophil precursors, in a CN patient. In addition to neutrophil production deficiency, which is the most mentioned cause of CN in the literature, we claim that the clearance of the apoptotic neutrophils of CN patients takes longer than normal. Note that, we could not find a specific value about clearance time of the apoptotic neutrophils. Therefore, we experiment with the clearance time and calibrate the model for a healthy person by assigning one hour to the parameter named Clearance Time. We also calibrate the model for a CN patient by assigning 12 hours to Clearance Time, which is a value that is 12 times more than the value for a healthy person. Future clinical studies may help us understand if clearance time really gets longer for a CN patient, which is identified as one of the potential causes of CN in this study. Additionally, we conclude that when the apoptotic neutrophils exceed a critical level (i.e., Max Tolerable Apoptotic Neutrophil Ratio), not only the production of G-CSF, but the effects of G-CSF are also suppressed. In other words, according to our assumption, the feedback mechanism caused by high levels of apoptotic neutrophils blocks the response of the body to G-CSF. This blocking effect must also be clinically studied. According to our model, rG-CSF treatment can be more effective if apoptotic neutrophils are eliminated faster. Thus, if in reality the clearance time is higher for a CN patient, by lowering it using a medication or any other means, one can potentially improve the effectiveness of rG-CSF treatment for CN patients.

We calibrate the values of the pathogen related parameters so that the model mimics the desired dynamics for a healthy human. After calibrating the pathogen compartment for a healthy person, we use the same exact pathogen compartment structure and pathogen compartment parameter values for the CN patient too. We also carry out experiments with pathogens in the model for a healthy person. First, we apply one-shot pathogen entry to the body of the healthy person. Secondly, we give a high level continuous pathogen entry to the body. Lastly, we keep the pathogen level constant to see the response of the body. We observe oscillations during these experiments. Consequently, we claim that the oscillatory behavior (i.e., cycles with a period of approximately 21 days and relatively high amplitude) is a characteristic of

the neutrophil-GCSF-pathogen system even for a healthy person. These oscillations probably can shed some light on the periodic symptoms observed in patients with diseases caused by an overactive immune system, which is another research question we pose for clinical studies.

We analyze the sensitivity of the dynamics to the changes in some parameter values as we are not able to obtain data for them. For such cases, we calibrate the model for those parameter values. According to our analysis, the model is extremely sensitive to neutrophil production deficiency (i.e., early apoptosis of neutrophil precursors). We suggest that clinical studies focus on production deficiency for CN patients to have a better understanding of the disorder. Furthermore, we can say that the neutrophil dynamics of the modeled CN patient is highly sensitive to clearance time. Therefore, we confirm that clearance time for CN patients must clinically be studied.

We want to show the use of the model as an experimental platform. For this reason, we carry out experiments with different frequencies and doses of rG-CSF by using the model for CN patients. In these experiments, we try to see if there can be an improvement to the effectiveness of rG-CSF administration. According to our simulation experiments with different doses, we can say that the dose of the injections has a strong effect on the amplitude of the neutrophil oscillations. They also have a significant effect on the period of the oscillations. For instance, the low dose rG-CSF injections are insufficient to increase the neutrophil levels and they lead to a longer period. According to our simulation experiments with the frequency of the injections, the frequency has an effect on the peak values of the oscillations. If we give more frequent rG-CSF injections, the elevation observed in neutrophil levels becomes higher. Lastly, we carry out experiments combining the dose and frequency of rG-CSF injections. We first try frequent injections with low doses. In this case, the period of the oscillations becomes slightly shorter. On the other hand, less frequent injections with high dose are less effective than the daily injections. In the end of our rG-CSF injection experiments, we could not obtain a significant improvement. By these experiments, we only demonstrated that our model can serve as an experimental platform.

For a future study, we plan to use our model for other disorders related to neutrophil production. For example, the neutrophil disorders such as cyclic neutropenia and severe congenital neutropenia (SCN) may have similarities in their pathology mechanisms according to the literature. We also agree with the literature as we manage to create different healthy person and patient profiles using the same exact model structure.

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APPENDIX A: MODEL EQUATIONS

Adjustment Inflow= ("Effect of Apoptotic Neutrophil Ratio on G-CSF" * "G-CSF Ratio" - "Effective G-CSF Ratio") / Adjustment Time

UNITS: 1/Hour

Adjustment Time= 4

UNITS: Hour

Apoptosis= Senescent Neutrophils / Apoptosis Time

UNITS: Cells/Hour

Apoptosis Time= 1

UNITS: Hour

Apoptotic Neutrophil Ratio= Apoptotic Neutrophils / Normal Apoptotic Neutrophils

UNITS: Dmnl

Apoptotic Neutrophils= INTEG (Apoptosis-Clearance of Apoptotic Cells, 4.16)

UNITS: Cells

Base for log function= 1.82

UNITS: Dmnl

Circulating Neutrophils= INTEG (Release-"Marginating - Demarginating Process"-Transmigrating Circulating Neutrophils , 23.3884)

UNITS: Cells

Clearance of Apoptotic Cells= Apoptotic Neutrophils / Clearance Time

UNITS: Cells/Hour

Clearance Time= 1 * 12

UNITS: Hour Assumption: Notlar

Current Value of Switch= INTEG (Hold Current Value-Release Current Value, 1)

UNITS: Dmnl

Differentiation to Myeloblast= MSC to Myeloblast Differentiation Fraction * (Myeloid Stem Cells / MSC to Myeloblast Differentiation Time)

UNITS: Cells/Hour

Differentiation to Myelocyte= Promyelocytes / Promyelocyte Transit Time

UNITS: Cells/Hour

Differentiation to Other Blood Cells= MSC to Other Blood Cell Diff Fraction * (Myeloid Stem Cells / MSC to Myeloblast Differentiation Time)

UNITS: Cells/Hour

Differentiation to Other Granulocytes= Myeloblast to Other Granulocyte Fraction * (Myeloblasts / Myeloblast to Neutrophil Diff Time)

UNITS: Cells/Hour

Differentiation to Promyelocytes = Myeloblast to Neutrophil Lineage Fraction * (Myeloblasts / Myeloblast to Neutrophil Diff Time)

UNITS: Cells/Hour

"Eff of G-CSF on PM Storage Time"= Min PM Storage Time Coefficient + (Normal PM Storage Time Coefficient - Min PM Storage Time Coefficient) * (LOOKUP EXTRAPOLATE ("G-CSF Graph on PM Storage Time" ,"Effective G-CSF Ratio"))

UNITS: Dmnl

"Effect of Apoptotic Neutrophil Ratio on G-CSF"= IF THEN ELSE(Current Value of Switch = 1 , Switch Off , Switch On)

UNITS: Dmnl

"Effect of G-CSF on Lifetime of Neutrophils in Tissues"= LOOKUP EXTRAPOLATE("Function for Effect of G-CSF on Lifetime of Neutrophils in Tissues" , "Effective G-CSF Ratio")

UNITS: Dmnl

"Effect of G-CSF on Proliferation"= LOG(Base for log functionˆMin Proliferation Coefficient + (Base for log function - Base for log functionˆMin Proliferation Coefficient) * "Effective G-CSF Ratio" , Base for log function)

UNITS: Dmnl

"Effect of G-CSF on Transit Time"= LOOKUP EXTRAPOLATE("Graph for Effect of G-CSF on Transit Time" , "Effective G-CSF Ratio")

UNITS: Dmnl

"Effect of Standardized Pamps in Body on G-CSF Production Rate"= LOOKUP EXTRAPOLATE("Function for Pamps Effect on G-CSF Production" , Standardized PAMPs in body)

UNITS: Dmnl

"Effective G-CSF Ratio"= INTEG (Adjustment Inflow, 1)

UNITS: Dmnl

External Pathogen Inflow= 0.025

UNITS: 1/Hour

FINAL TIME = 2000

UNITS: Hour The final time for the simulation.

"Function for Effect of G-CSF on Lifetime of Neutrophils in Tissues"([(0,1)-(6,2.5)], (0,1), (1,1),(1.5,1.03),(2,1.15),(2.5,1.375),(3.5,2.125),(4,2.35),(4.5,2.47),(5,2.5),(6,2.5))

UNITS: Dmnl

"Function for Pamps Effect on G-CSF Production"([(0,0)-(2,1)],(0,0),(0.75,0),(0.8, 0.01), (0.9,0.05), (0.95,0.1),(0.98,0.2), (1.02,0.8),(1.05,0.9),(1.1,0.95),(1.2,0.99), (1.25,1), (2,1))

UNITS: Dmnl

"G-CSF Elimination Time"= 5

UNITS: Hour

"G-CSF Elimination"= "G-CSF in Blood" / "G-CSF Elimination Time"

UNITS: nanogram/Hour

"G-CSF Graph on PM Storage Time"([(0,0)-(9,1)],(0,1),(1.5,1),(2.25,0.96),(3,0.85), (6,0.15),(6.75,0.04),(7.5 ,0),(9,0))

UNITS: Dmnl

"G-CSF in Blood"= INTEG ("G-CSF Injections"+"G-CSF Production Rate"-"G-CSF Elimination", 130)

UNITS: nanogram

"G-CSF Injections"= IF THEN ELSE(MODULO(Time , 24) = 0, 73500 / TIME STEP, 0)*"Injected G-CSF" *0

UNITS: nanogram/Hour

"G-CSF Production Rate"= "Effect of Apoptotic Neutrophil Ratio on G-CSF" * "Indicated G-CSF Production Rate" + (1-"Effect of Apoptotic Neutrophil Ratio on G-CSF") * "Homeostatic G-CSF Production Rate"

UNITS: nanogram/Hour

"G-CSF Ratio"= "G-CSF in Blood"/ "Normal G-CSF Level"

UNITS: Dmnl

"Graph for Effect of G-CSF on Transit Time"([(0,0)-(90,1)],(0,1),(10,1),(15,0.99),(20,0.96),(30,0.86),(40,0.68),(50,0.42),(60,0.24),(70,0.14),(75,0.11),(80,0.1),(90,0.1))

UNITS: Dmnl

Hold Current Value= "Effect of Apoptotic Neutrophil Ratio on G-CSF" / TIME STEP

UNITS: Dmnl/Hour

"Homeostatic G-CSF Production Rate"= 0

UNITS: nanogram/Hour

"Indicated G-CSF Production Rate"= ("Effect of Standardized Pamps in Body on G-CSF Production Rate" * "Rapid G-CSF Production Rate") + (1 - "Effect of Standardized Pamps in Body on G-CSF Production Rate") * "Homeostatic G-CSF Production Rate"

UNITS: nanogram/Hour

INITIAL TIME = 0

UNITS: Hour The initial time for the simulation.

"Injected G-CSF"= 1

UNITS: nanogram

Lifetime of Neutrophils in Tissues= "Effect of G-CSF on Lifetime of Neutrophils in Tissues" * Normal Lifetime of Neutrophils in Tissues

UNITS: Hour

Marginated Neutrophils= INTEG ("Marginating - Demarginating Process" - Transmigrating Marginated Neutrophils , 22.3716)

UNITS: Cells

"Marginating - Demarginating Process"= (Circulating Neutrophils - Marginated Neutrophils) / Margination Balance Delay Time

UNITS: Cells/Hour

Margination Balance Delay Time= 0.5

UNITS: Hour

Maturation= Metamyelocytes and Band Neutrophils in PMP / Post Mitotic Transit

Time

UNITS: Cells/Hour

Max Pathogen Elimination by Neutrophils= Ratio of Neutrophils in Tissues * Max Pathogen Elimination Coefficient

UNITS: 1/Hour

Max Pathogen Elimination Coefficient= 1

UNITS: 1/Hour

"Max Stress-free Value for Apoptotic Neutrophil Ratio"= 1.2

UNITS: Dmnl

Max Tolerable Apoptotic Neutrophil Ratio= 1.8

UNITS: Dmnl

Metamyelocyte and Band Neutrophil Generation= Neutrophil Multiplication at each Stage * Proliferation 2

UNITS: Cells/Hour

Metamyelocytes and Band Neutrophils in PMP= INTEG (Metamyelocyte and Band Neutrophil Generation-Maturation, 249.6)

UNITS: Cells

Min PM Storage Time Coefficient= 0.4

UNITS: Dmnl

Min Proliferation Coefficient= 0

UNITS: Dmnl

MSC to Myeloblast Differentiation Fraction= 1/3

UNITS: Dmnl

MSC to Myeloblast Differentiation Time= 25

UNITS: Hour

MSC to Myeloblast Multiplier= "Effect of G-CSF on Proliferation" * Normal MSC to Myeloblast Multiplier

UNITS: Dmnl

MSC to Other Blood Cell Diff Fraction= 1 - MSC to Myeloblast Differentiation Fraction

UNITS: Dmnl

Myeloblast Generation= MSC to Myeloblast Multiplier * Differentiation to Myeloblast

UNITS: Cells/Hour

Myeloblast to Neutrophil Diff Time= "Effect of G-CSF on Transit Time" * Normal Myeloblast to Neutrophil Diff Time

UNITS: Hour

Myeloblast to Neutrophil Lineage Fraction= 2/3

UNITS: Dmnl

Myeloblast to Other Granulocyte Fraction= 1 - Myeloblast to Neutrophil Lineage Fraction

UNITS: Dmnl

Myeloblast to Promyelocyte Multiplier= "Effect of G-CSF on Proliferation" * Normal Myeloblast to Promyelocyte Multiplier

UNITS: Dmnl

Myeloblasts= INTEG (Myeloblast Generation - Differentiation to Promyelocytes - Differentiation to Other Granulocytes , 13.65)

UNITS: Cells

Myelocyte Generation 1= Neutrophil Multiplication at each Stage * Differentiation to Myelocyte

UNITS: Cells/Hour

Myelocyte Generation 2= Neutrophil Multiplication at each Stage * Proliferation 1

UNITS: Cells/Hour

Myelocyte Transit Time= "Effect of G-CSF on Transit Time" * Normal Myelocyte Transit Time

UNITS: Hour

Myelocytes S1= INTEG (Myelocyte Generation 1-Proliferation 1, 20.1)

UNITS: Cells

Myelocytes S2= INTEG (Myelocyte Generation 2-Proliferation 2, 80.4266)

UNITS: Cells

Myeloid Stem Cells= INTEG (- Differentiation to Other Blood Cells+StemCell Production Rate-Differentiation to Myeloblast , 14.625)

UNITS: Cells

Neutropenia Scalee= (Marginated Neutrophils + Circulating Neutrophils) / Normal Blood Volume

UNITS: Cells / Liter

Neutrophil Multiplication at each Stage= 2

UNITS: Dmnl 7 division after myeloblast division

Neutrophil Ratio= (Circulating Neutrophils + Marginated Neutrophils) / Normal Amount of Neutrophils in Blood

UNITS: Dmnl

Neutrophils in Blood= Circulating Neutrophils + Marginated Neutrophils

UNITS: Cells

Neutrophils in Tissues= INTEG (Transmigrating Circulating Neutrophils + Transmigrating Marginated Neutrophils -Senescing Neutrophils, 70.72)

UNITS: Cells

Normal Amount of Neutrophils in Blood= 45.75

UNITS: Cells

Normal Apoptotic Neutrophils= 4.16

UNITS: Cells x 10^9 cells / hour

Normal Blood Volume= 5.3

UNITS: Liter

"Normal G-CSF Level"= 130

UNITS: nanogram

Normal Lifetime of Neutrophils in Tissues= 17

UNITS: Hour

Normal MSC Production Rate= 0.585

UNITS: Cells/Hour

Normal MSC to Myeloblast Multiplier= 2 / 4

UNITS: Dmnl

Normal Myeloblast to Neutrophil Diff Time= 35

UNITS: Hour

Normal Myeloblast to Promyelocyte Multiplier= 2 / 4

UNITS: Dmnl

Normal Myelocyte Transit Time= 58

UNITS: Hour

Normal Neutrophils in Tissues= 70.72

UNITS: Cells

Normal Pathogen Elimination Fraction= 1/10

UNITS: 1/Hour

Normal PM Storage Time Coefficient= 1

UNITS: Dmnl

Normal Post Mitotic Storage Time= 30

UNITS: Hour

Normal Post Mitotic Transit Time= 60

UNITS: Hour

Normal Promyelocyte Transit Time= 35

UNITS: Hour

Pathogen Elimination by Neutrophils= MIN(Pathogen Elimination Fraction * Standardized PAMPs in body , Max Pathogen Elimination by Neutrophils)

UNITS: 1/Hour

Pathogen Elimination Fraction= Ratio of Neutrophils in Tissues * Normal Pathogen Elimination Fraction

UNITS: 1/Hour

Post Mitotic Storage Time= "Eff of G-CSF on PM Storage Time" * Normal Post Mitotic Storage Time

UNITS: Hour

Post Mitotic Transit Time= "Effect of G-CSF on Transit Time" * Normal Post Mitotic Transit Time

UNITS: Hour

Proliferation 1= Myelocytes S1 / (Myelocyte Transit Time / 3)

UNITS: Cells/Hour

Proliferation 2= Myelocytes S2 / (Myelocyte Transit Time * 2 / 3)

UNITS: Cells/Hour

Promyelocyte Transit Time= "Effect of G-CSF on Transit Time" * Normal Promye-

locyte Transit Time

UNITS: Hour

Promyelocytes= INTEG (Promyelocytes Cell Generation-Differentiation to Myelocyte, 18.2)

UNITS: Cells

Promyelocytes Cell Generation= Myeloblast to Promyelocyte Multiplier * Differentiation to Promyelocytes

UNITS: Cells/Hour

"Rapid G-CSF Production Rate"= 156

UNITS: nanogram/Hour

Ratio of Neutrophils in Tissues= Neutrophils in Tissues / Normal Neutrophils in Tissues

UNITS: Dmnl

Release= Stored Neutrophils in PMP / Post Mitotic Storage Time

UNITS: Cells/Hour

Release Current Value= Current Value of Switch / TIME STEP

UNITS: Dmnl/Hour

SAVEPER = TIME STEP

UNITS: Hour [0,?] The frequency with which output is stored.

Senescent Neutrophils= INTEG (Senescing Neutrophils-Apoptosis, 4.16)

UNITS: Cells

Senescing Neutrophils= Neutrophils in Tissues / Lifetime of Neutrophils in Tissues

UNITS: Cells/Hour

Severe Neutropenia Point= 0.125

UNITS: Dmnl

Standardized PAMPs in body= INTEG (External Pathogen Inflow-Pathogen Elimination by Neutrophils, 0.25)

UNITS: Dmnl

StemCell Production Rate= Normal MSC Production Rate

UNITS: Cells/Hour

Stored Neutrophils in PMP= INTEG (Maturation-Release, 129.842)

UNITS: Cells x 10^9 cells

Switch Off= IF THEN ELSE(Apoptotic Neutrophil Ratio ¡ Max Tolerable Apoptotic Neutrophil Ratio , 1 , 0)

UNITS: Dmnl

Switch On= IF THEN ELSE(Apoptotic Neutrophil Ratio ¡ "Max Stress-free Value for Apoptotic Neutrophil Ratio" , 1 , 0)

UNITS: Dmnl

TIME STEP = 0.0625

UNITS: Hour [0,?] The time step for the simulation.

Transmigrating Circulating Neutrophils= (Circulating Neutrophils / Transmigration Time of Neutrophils)

UNITS: Cells/ Hour

Transmigrating Marginated Neutrophils= Marginated Neutrophils / Transmigration Time of Neutrophils

UNITS: Cells/Hour

Transmigration Time of Neutrophils= 11

UNITS: Hour