Evaluating Treament of Hepatitis C for Hemolytic Anemia Management

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Abstract

The combination therapy of antiviral peg-interferon and ribavirin (RBV) has evolved as one of the better treatments for Hepatitis-C (HCV). In spite of its success in controlling HCV infection, it has also been associated with treatment related adverse side-effects. The most common among them is hemolytic anemia. More than 67 percent of treated chronic HCV patients show signs of acute anemia leading to dose reduction or even therapy cessation. A drug that contains erythropoietin (EPO) is often administered to stimulate the production of RBC in the bone marrow. This paper extends the basic mathematical model of Dahari et al. and studies the effect of combination therapy in light of anemia. In order to achieve this we introduce RBC concentration and drug amount in the model. Analysis of this model provides a quantification of the drug amount that is tolerable by the body without succumbing to hemolytic anemia. This will provide an estimate of the increment in RBC production necessary to keep from hemolytic anemia and settle on a balanced HCV treatment.

A Introduction

At present one hundred and seventy million people live with Hepatitis C virus (HCV) infection world-wide. Currently, there is no vaccine for HCV. The major mode of transmission of HCV is by exposure to infected blood. Sexual and vertical transmission of HCV has been reported; however, it is rare [17]. Hepatitis C causes chronic diseases of the liver like cirrhosis and hepatocellular carcinoma [18]. The Hepatitis C virus infects hepatocytes which form a major portion of the cytoplasmic mass of the liver. Although HCV predominantly replicates in hepatocytes, traces of it have been detected in other cell types [5, 27].

Some patients with Hepatitis C infection will naturally clear the virus without medical intervention. However, a major portion of HCV infected individuals will develop chronic HCV infection in which their body's immune system does not naturally clear the virus. About 70 to 80 percent of HCV patients have chronic hepatitis C infection [17]. The progression to chronic stage HCV infection is a result of weak immune response against HCV (reviewed in [13]). Currently, the standard protocol for the treatment of Hepatitis C involves two antiviral drug treatments given in combination. Patients receive injections of peg-interferon and take ribavirin pills for 48 weeks [10]. Peg-interferon works by binding to specific cell membrane receptors initiating a complex sequence of intracellular events that lead to suppression of cell proliferation [33]. The goal of this treatment is to lower the viral load and eventually achieve Sustained Virological Response (SVR). However, there are negative side effects of the treatment that range from flu-like symptoms and anemia to temporary disability and depression.

The most common and alarming side-effect is anemia. Often, the complete blood count (CBC) drops to unsafe levels in patients. About 67 percent of people treated with combination therapy (peg-interferon and ribavirin) develop negative blood-related side effects [30]. More specifically, patients experience neutropenia (depressed neutrophil levels, a type of white blood cell) and ribavirin-related hemolytic anemia, which is the loss of red blood cells due to ribavirin. Recent studies by Antonini et al. [2], have suggested that neutropenia is not correlated with opportunistic secondary infections and thus, is not great cause for concern. However, the

dose-dependent hemolytic anemia causes dose reduction of ribavirin dosage or complete cessation of ribavirin [25]. Ribavirin induces excessive hemolysis, that is the breakdown of red blood cells (RBC) and release of hemoglobin into the surrounding blood plasma. The body's ability to produce new RBCs to compensate for this excessive loss is stunted by the simultaneous bone-marrow suppressing effect of peg-interferon. This motivates us to formulate a suitable strategy to optimize the benefits of treatment and minimize the loss of red blood cell (RBC). According to Sulkowski [26], successful combination therapy with peg-interferon and ribavirin is contingent on maintaining adequate doses of both drugs throughout the treatment period . With this is mind, it is necessary to balance the side effect of hemolytic anemia while achieving SVR.

Earlier models of hepatitis C infection considered infected hepatocytes, target uninfected hepatocytes and free virus using ordinary differential equations. These models use parameters for the constant death and production of hepatocytes, loss of infected hepatocytes at a constant rate and target uninfected hepatocytes are infected at constant rate. In addition, the efficacy of treatment in blocking virion production and reducing new infectives are used as parameters without regard to drug dosage explicitly [9, 3]. The goal of this work was to model the different clinical outcomes of hepatitis C infection. The result was to understand the effect that drug efficacy on the dynamics of HCV. In addition, previous models have investigated other critical parameter values. Work has been done by Neumann et al. [4] modeling HCV dynamics and the effect interferon has on viral load. Using a system of three differential equations for target hepatocytes, infected hepatocytes and free virion, Neumann et al. showed that Hepatitis C viral infection is very dynamic and suggest that early monitoring of viral load can help achieve SVR. Moreover they showed that an early virological response (EVR) is important in achieving SVR. In addition, Dahari et al. [9] rigorously dealt with interferon and ribavirin combination treatment by comparing models and determining which one models the behavior observed in the clinic more accurately.

As an extension of Dahari's model, our model of hepatitis C infection incorporates the side effect of anemia. We use ordinary differential equations for target and infected hepatocytes, viral load, production and death of hepatocytes. We incorporate the side-effect of hemolytic anemia into this system of equations by considering the dynamics of the RBC population as a separate state variable. Also, we consider the amount of drug as a dynamic system rather than a constant parameter. We focus on the interaction of the red blood cell level with drug amount with the goal of finding an optimal drug treatment regimen to minimize the severity of anemia while still obtaining SVR. Analytic and numerical methods allow evaluation of several dosing regimens. In Section 27, we introduce our model and its parameters, in Section we analyze the model and calculate different reproductive numbers for HCV with treatment and without treatment under varying assumptions. In Section D we briefly discuss the results of our analytic work and how they motivate our numerical work. In addition, critical drug amount values are numerically calculated for the system and the results of simulations are shown. furthermore, we and understand the conditions under which the values C_{SVR} and C^* are the same or closer. In section E we discuss the analytic work in regards to the numerical calculations, numerical simulations and their biological meanings. In section F, we discuss future work to be done motivated from our work.

B Model

The state variables of the model include T, target healthy hepatocytes; I, infected hepatocytes; V, the viral load of free HCV; R, the red blood cell concentration in the body and C being the amount of drug (peg-interferon and ribavirin) in the body.

The model equations are:

$$\frac{dT}{dt} = s_T - d_T T - \frac{\alpha}{\alpha + C}\beta TV \tag{30}$$

$$\frac{dI}{dt} = \frac{\alpha}{\alpha + C}\beta TV - d_I I \tag{31}$$

$$\frac{dV}{dt} = p\frac{k}{k + C}I - \theta CV - d_V V \tag{32}$$

$$\frac{dR}{dt} = s_R - d_R R - \tau CR \tag{33}$$

$$\frac{dC}{dt} = \Lambda\frac{R^2}{A^2 + R^2} - hC \tag{34}$$

s_T is the rate of production of healthy hepatocytes from the bone marrow. d_T is the natural death rate of healthy hepatocytes. α is related to the efficacy of the ribavirin. It is essentially the amount of the drug at

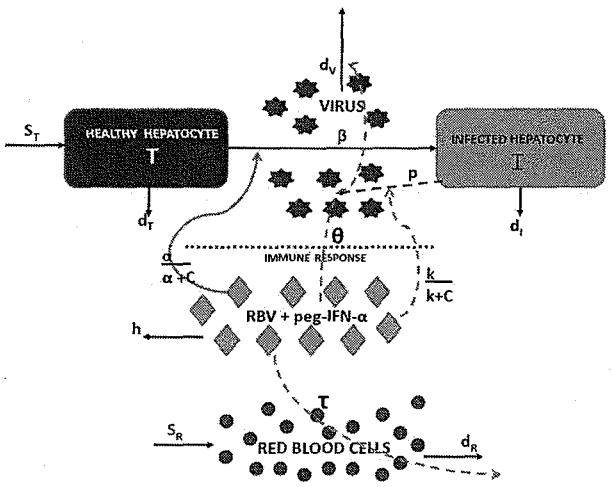

Figure 20: Figure 1: Compartmental Model

Parameter	Interpretation
s_T	natural production rate of hepatocytes
s_R	natural production rate of RBC
d_V	natural death rate of virus
d_T	natural death rate of hepatocytes
d_I	natural clearance rate of infected hepatocytes
d_R	natural death rate of RBC
α	specific drug amount
p	proliferation rate of virus
β	rate of new infections per virion
k	specific drug amount
θ	rate of cell death per unit drug
τ	sensitivity of RBC death to ribavirin
Λ	maximum dosage allowed per unit time
A	specific amount of RBC
h	rate of drug clearance

Table 12: Parameter Interpretation Table

which production of new infected cells is reduced by half, that is, when $C{=}\alpha$, we get $\frac{\beta}{2}$. If there is no drug in the body $\frac{\alpha}{\alpha+C}$, is equal to 1, the virus infects the healthy hepatocytes at a constant rate β. Here, β is the number of infections caused by one infected cell per unit time. As the amount of drug increases the production of infected cells decreases. βT is the number of healthy hepatocytes infected by one virion per unit of time. βTV is the total number of healthy hepatocytes infected by the amount of virus, V, per unit of time. The total number of infected hepatocytes, i.e. βTV, goes into the second class of hepatocytes which is the infected hepatocytes, $\frac{dI}{dt}$. d_I is the per-capita rate of clearance of infected hepatocytes by 'natural' death including the effect of immune response, per unit time. $d_I I$ is the total number of infected hepatocytes cleared per unit of time.

p is the per capita proliferation rate of virus, i.e. is the number of virions produced from bursting of one infected hepatocyte, per unit time. pI is the number of virions produced by the total population of infected hepatocytes per unit time. k is related to the efficacy of the interferon. It is essentially the amount of the drug in the body which reduces the production of new virions to half of the amount produced in absence of treatment. θ is a rate with unit $amount^{-1}time^{-1}$. "Ribavirin appears to exert an immunomodulatory rather than direct antiviral effect. The proposed mechanism of action is enhancement of HCV-specific T-cell immunity

by switching from a predominantly T-helper subtype 2 to T helper subtype 1 phenotype" [12]. θCV takes care of increased death of free virions depending on amount of ribavirin. d_V is the death rate of virion in absence of treatment. $d_V V$ is the total number of deaths of virions per unit time.

s_R is the rate of production of red blood cells in the bone marrow, which is estimated at 2 million per day in a healthy individual. d_R is the natural death rate of RBC. The rate of change in RBC in the body ($\frac{dR}{dt}$) depends on the amount of the drug C, present in the body. Again, how much drug is being administered to a patient depends on the concentration of RBC in the body. Hence, to take care of these factors we include the term, τRC where τ is the sensitivity of RBC death to drug.

Λ is the maximum level of drug in the body per unit time. $\frac{R^2}{A^2+R^2}$ is the function that incorporates the change of dosage of the drug depending on the RBC count at a certain time as a reduction factor. A is the RBC level at which medication is reduced to half. As the red blood cell count decreases the amount of drug administered has to be reduced also. The usefulness of $\frac{R^2}{A^2+R^2}$ is that the function has an inflection point, which accounts for the level of red blood cells at which the body becomes anemic. This function allows us to approximate a step function for the sudden change of dosage once the red blood cell level cross the anemic threshold. h is the rate at which the body clears out the drug, so hC is the clearance of drug depending on how much drug is present in the body at that moment. Model parameters are summarized in Table 12.

The model is essentially split into two separate systems because the red blood cell equation and drug amount equation is decoupled from the rest of the model. This allows us to analyze this model as two separate systems and as a cohesive system of equations. One of these subsystems is two dimensional and considers only the effect that drug amount and red blood cells count have on each other. The other is three dimensional and models the dynamics of target hepatocytes, infected hepatocytes, and free virion.

C Analytic Work

C.1 Analysis of (R, C) system

We determine the equilibrium of the decoupled $\frac{dR}{dt}$ and $\frac{dC}{dt}$ without regard to the complete system. Equating $\frac{dC}{dt} = 0$ we identify $C^* = \frac{\Lambda}{h}\frac{R^2}{A^2+R^2}$. Then we use C^* to find the abscissa of the equilibrium point, by equating $\frac{dR}{dt}$ to zero, resulting in the cubic equation

$$F(R) = (d_R + \frac{\tau\Lambda}{h})R^3 - S_R R^2 + d_R A^2 R - S_R A^2 = 0\,. \tag{35}$$

In light of this, we rescale the equation using $r = \frac{R}{A}$, $d = \frac{d_r}{\frac{S_R}{A}}$, and $a = \frac{\tau\frac{\Lambda}{h}}{\frac{S_R}{A}}$ to make our cubic equation depending on two parameters a and d which simplifies to

$$f(r) = (a+d)r^3 - r^2 + dr - 1 = 0 \tag{36}$$

Further details on these calculations can be found in the Appendix, section H.1. The equation (36) has exactly one positive real root.

Proof. If $r = 0$, $f(r) < 0$, and if r is large, $f(r) > 0$, so there is one or three real positive root. Using the rescaled equation, we analyze the possible roots in the parameter space. We consider $f(r)$ as a function of a and d, that is, $r^3 a + (r^3 + r)d = r^2 + 1$. Then, we use the original equation and the first derivative of this equation $3r^2 a + (3r^2 + 1)d = 2r$. Since if $f(r)$ has two roots, $f(r) = 0$ and at that point $f(r)$ should be tangent to the $x - axis$, thus $f'(r) = 0$. We apply linear algebra to this system of equations. If possible, let there exist two real solutions to $f(r) = 0$ and $f'(r) = 0$. Solving the system using the Gauss method, we find $a = -\frac{(r^2+1)^2}{2r^3} < 0$ and $d = \frac{r^2+3}{2r} > 0$. These solutions are biologically extraneous since all parameter values are positive and the bifurcation lies only in the second quadrant as shown in Figure C.1. We have determined that the system does not have a biologically relevant bifurcation point.

Hence, we conclude that the system cannot have two real roots thus there must exist either one or three positive real roots. We determine that there is only one positive real root by analyzing the bifurcation diagram in the first quadrant. Using this value and our parameter values, we find the exact value of C^*. For further details on exact method, refer to the Appendix section H.2. □

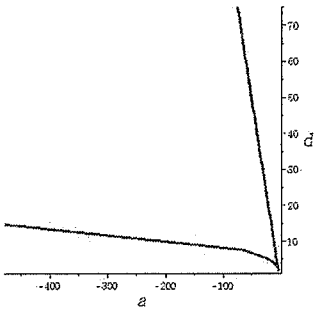

Figure 21: Parametric Plot of Bifurcation Curve, F(r)

Next, we analyze the local stability of this nontrivial equilibrium point. Using a Jacobian matrix,

$$J_{(R^*,C^*)} = \begin{bmatrix} -d_R - \tau C^* & -\tau R \\ 2\frac{A^2 \Lambda R}{\left(A^2+R^{*2}\right)^2} & -h \end{bmatrix}$$

Since $d_R > 0$, $C^* > 0$, $h > 0$, $\tau > 0$, we know $Tr(J) = -(d_R + \tau C^* + h) < 0$, and $det(J) = h(d_R + \tau C^*) + \frac{2\tau R^{*2} A^2 \Lambda}{A^2+R^{*2}} > 0$, thus we show (R^*, C^*) is locally asymptotically stable. For further details refer to the Appendix section H.3.

We have proven local stability; now our interest is in the global stability of the (R,C) system. To this end, we apply the Poincaré-Bendixson Theorem. The Poincaré-Bendixson Theorem states that there are three possibilities for the asymptotic behavior of solutions to a two dimensional system: a limit cycle, an unbounded solution or a stable equilibrium point. Now, using Dulac's criterion with $g = \frac{1}{R}$, we prove that there is no limit cycle. We also prove that $\limsup_t C(t) < \frac{\Lambda}{h}$ and $\limsup_t R(t) < \frac{s_R}{d_R}$; hence the solution cannot be unbounded. Thus local stability is extended to global stability for the unique equilibrium point. Further technical details are relegated to the Appendix section H.4. Consequently by a theorem of Thieme [29] the behavior of the (30) – (34) system is asymptotic to the behavior of the three dimensional subsystem (30) – (32) with the equilibrium values C^* and R^* substituted for the state variables C and R. In the next subsection, we will use this reduced system to determine the necessary equilibrium drug amount to eliminate HCV from the (T, I, V) system.

C.2 Analysis of (T, I, V)

We analyze the (T, I, V) subsystem and determine the desired critical drug amount, C_{SVR}, for which the disease free equilibrium of (T, I, V) is stable; we know it is different from the previously calculated C^* value. In terms of the biology of Hepatitis C treatment and the side effect of anemia, the analyses of (R,C) and (T,I,V) subsystems and their resulting critical drug amount values allows us to compare the drug amount that the body can handle in light of anemia, C^*, and the desired amount of drug in the body so that the disease becomes extinct in the body, C_{SVR}. With this goal in mind, we calculate the HCV reproductive number in the (T,I,V) subsystem under several assumptions and conditions.

In absence of Hepatitis C treatment, Callaway and Perelson [8] calculate the *Basic Reproduction Number* (BRN)

$$\mathfrak{R}_o = \frac{p\beta s_T}{d_V d_I d_T} . \tag{37}$$

We first analyze equations (30-32), taking C as asymptotic constant; in a sense we take it to be a parameter. Then the disease free equilibrium (DFE) is

$$(T_0, I_0, V_0) = (\frac{s_T}{d_T}, 0, 0)$$

Linearizing the system about the DFE, and imposing conditions for stability we calculate $\widehat{\Re}$ which we call the *Controlled Reproduction Number* (CRN).

$$\widehat{\Re} = \frac{\alpha}{\alpha+C}\frac{k}{k+C}\frac{d_V}{\theta C+d_V}\frac{s_T p\beta}{d_I d_T d_V} \tag{38}$$

$$\widehat{\Re} = \frac{\alpha}{\alpha+C}\frac{k}{k+C}\frac{d_V}{\theta C+d_V}\Re_o \tag{39}$$

Let,

$$q = d_V + \theta C \tag{40}$$

$$D = \frac{\alpha}{\alpha+C}\frac{k}{k+C}p\beta s_T \tag{41}$$

$$\rightarrow \widehat{\Re} = \frac{D}{d_I d_T q} \tag{42}$$

When $\widehat{\Re} < 1$, the DFE is stable, thus the infection is eliminated from the hepatocyte population. Since the effect of peg-interferon on the viral load, k, is much greater in magnitude compared to the effect of RBV on viral load, θ, for simplification of analysis we can take $\theta \approx 0$. Then the modified CRN is

$$\widehat{\Re_1} = \Re_o \frac{\alpha}{\alpha+C}\frac{k}{k+C} \tag{43}$$

which can be biologically translated as the pre-treatment BRN with some control parameters based on treatment. This allows us to control the BRN of the infection depending on drug-amount.

We have analyzed the disease free equilibrium to understand under which conditions the DFE is stable. Now our attention shifts to the endemic equilibrium since our biological interest is treatment of chronically HCV infected individuals. Turning to the endemic equilibrium, we solve to get:

$$T^* = \frac{s_T}{d_T\widehat{\Re}} \tag{44}$$

$$I^* = \frac{s_T}{d_I}(1-\frac{1}{\widehat{\Re}}) \tag{45}$$

$$V^* = d_T\frac{(\alpha+C)}{\alpha\beta}(\widehat{\Re}-1 \tag{46}$$

(see Appendix section H.5 for further details on equilibrium point of (T,I,V) subsystem).

Now we linearize about the endemic equilibrium and calculate eigenvalues which results in a cubic equations.
$P(\lambda) = -(d_T\widehat{\Re}+\lambda)(d_I+\lambda)(\theta C^* d_V+\lambda) + (d_T\widehat{\Re}+\lambda)\frac{\alpha\beta s_T p k}{d_T(\alpha+C^*)(k+C^*)\widehat{\Re}} - d_I d_T(\theta C^*+d_V)(\widehat{\Re}-1) = 0.$

Let, $q = (\theta C^* d_V)$, then

$P(\lambda) = -\lambda^3 + \lambda^2(d_I + d_T\widehat{\Re}+q) + \lambda d_T\widehat{\Re}(q+d_I) - d_I d_T q(1-\widehat{\Re}) = 0.$

We now use the Routh Hurwitz Criteriion to determine the stability. We get the following conditions:

i) $(d_I + d_T\widehat{\Re}+q) > 0$

ii) $-d_I d_T q(1-\widehat{\Re}) > 0$

iii) $(d_I + d_T\widehat{\Re}+q)d_T\widehat{\Re}(q+d_I) > -d_I d_T q(1-\widehat{\Re})$

However, the third condition implies the first one, and the second condition is true if and only if $\widehat{\Re} > 1$. If $\widehat{\Re} > 1$ is true and condition 3 is satisfied then the endemic equilibrium is stable. From condition 3, we have

$$\frac{D^2(q+d_I)}{{d_I}^2 d_T q^2} + D\left(\frac{(q+d_I)^2}{d_I d_T q} - \frac{1}{d_T}\right) + d_I q > 0 \tag{47}$$

Equating the left hand side of equation (80) to zero, we obtain two roots, $D_{1,2}$ as

$$D_{1,2} = \frac{-\left(\frac{(q+d_I)^2}{d_I d_T q} - \frac{1}{d_T}\right) \pm \sqrt{(\frac{(q+d_I)^2}{d_I d_T q} - \frac{1}{d_T})^2 - 4\frac{(q+d_I)}{d_I d_T q}}}{2\left(\frac{q+d_I}{d_I^2 d_T q^2}\right)}. \tag{48}$$

Let

$$Q = \left(\frac{(q+d_I)^2}{d_I d_T q} - \frac{1}{d_T}\right)^2 - 4\frac{(q+d_I)}{d_I d_T q}. \tag{49}$$

If Q is negative, then inequality (80) is always satisfied. Furthermore, since $4\frac{(q+d_I)}{d_I d_T q}$ is positive, if Q is positive, then $-\left(\frac{(q+d_I)^2}{d_I d_T q} - \frac{1}{d_T}\right)$ dominates the sign of the roots. Now,

$$\left(\frac{(q+d_I)^2}{d_I d_T q} - \frac{1}{d_T}\right) = \frac{1}{d_T}\left(\frac{(q+d_I)^2}{d_I q} - 1\right) \tag{50}$$

$$\rightarrow \frac{1}{d_I d_T q}\left((q-d_I)^2 + d_I q\right) > 0. \tag{51}$$

Thus, both roots are always negative. But D is a product of positive parameters, hence D is always positive. Therefore, the Routh-Hurwitz criterion always holds true and guarantees local stability of the endemic equilibrium. For further detail see Appendix section 8.5.4.

Now that we have found the conditions for which $\widehat{\Re}$ provides a stable equilibrium, we solve for the C value for which $\widehat{\Re} = 1$ using equation (38); this provides the critical drug amount, C_{SVR}. When we do this, the following cubic equation of C results: $G(C) = 0$, where

$$G(C) = \theta C^3 + (d_V + \theta(k+\alpha))C^2 + (\alpha k\theta + (k+\alpha)d_V)C + \alpha k d_V(1-\Re_o) \tag{52}$$

We observe that $G(0) < 0$ if and only if $\Re_o > 1$, in addition $G'(C) > 0$ for each C and $\lim_{C\to\infty} G(C) = +\infty$, therefore there exists one unique root, C_0. However, this calculation is very difficult, so in order to get a simpler approximation for C_{SVR} we take $\theta \approx 0$. We do this since we know that interferon treatment can be administered without ribavirin, represented by θ, since the virus can be cleared from the body with only peg-interferon. Also, in the model we assume ribavirin has a greater effect on red blood cell concentration than on viral load. The following results

$$0 = C^2 + (k+\alpha)C + \alpha k(1-\widehat{\Re}) \tag{53}$$

Solving the quadratic equation in C, we get only one biologically feasible dosage value,

$$C_0 = \frac{-(\alpha+k) + \sqrt{(\alpha+k)^2 - 4\alpha k(1-\Re_o)}}{2} \tag{54}$$

We note here that we introduce treatment into a infected population only if $\Re_o > 1$, implying that the body is incapable of clearing out the virus by itself. Therefore, $(1-\Re_o) < 0$ making $C_0 > 0$ always. We estimate possible values of this C_{SVR} by solving $G(C) = 0$ with numerical coefficients later in the paper.

D Numerical Simulation

D.1 Parameter Estimation

$\frac{\alpha}{\alpha+C}$ is the efficacy of the combination therapy. From Hermann et al. [14] we have that the efficacy of the therapy with peg-interferon and ribavirin is on an average 70 percent. Also the average amount of drug administered is chosen to be 1000 mg per day. Thus calculating α from these values we get 112.35 day^{-1}. Take $k = \alpha$, since the efficacy of the combination therapy cannot be considered as a sum of the efficacies of peg-interferon and ribavirin administered separately. This is because the combination therapy works at least three times more efficiently than if used individually, thus the values are the same. The purpose of having different names is to emphasize the separate modes of action of peg-interferon and ribavirin.

From Dahari et al. [9] the calculated total clearance rate of virus during combination therapy is 6.0 day^{-1}, and from [15] we get the clearance of virus in absence of treatment is 2.0 day^{-1}. Using these values and an equilibrium dosing of the drug we estimate θ. Plugging in the values of the parameters other than τ, and variables at equilibrium into the $\frac{dV}{dt}$ equation we solved out for it.

The dosage of RBV is reduced to half when the hemoglobin level falls below 10g/dl. From Mackey [20] we get the equilibrium concentration of RBC and from Hillman [16] we know that the average amount of hemoglobin

Table 13: Parameter Value Table

Parameter	Value	Reference
s_T	26000 cells mL^{-1} day^{-1}	[9]
s_R	8.382×10^8 cells mL^{-1} day^{-1}	[20]
d_V	2.0 day^{-1}	[15]
d_T	.01 day^{-1}	[9]
d_I	1.0 day^{-1}	[9]
d_R	.0231 day^{-1}	[20]
α	112.35 mg day^{-1}	estimated *
p	2.9 virions $cells^{-1}$ day^{-1}	[9]
β	2.25×10^{-5} mL $virion^{-1}$ day^{-1}	estimated from [9]
k	112.35 mg day^{-1}	estimated *
θ	.0034 mg^{-1} day^{-1}	estimated *
τ	1.80×10^{-4} mg^{-1} day^{-1}	estimated *
Λ	1000 mg day^{-1}	[7]
A	2.35714×10^5 cells mg^{-1}	estimated *
h	1.9 day^{-1}	[6]

* For further explanation of method refer below

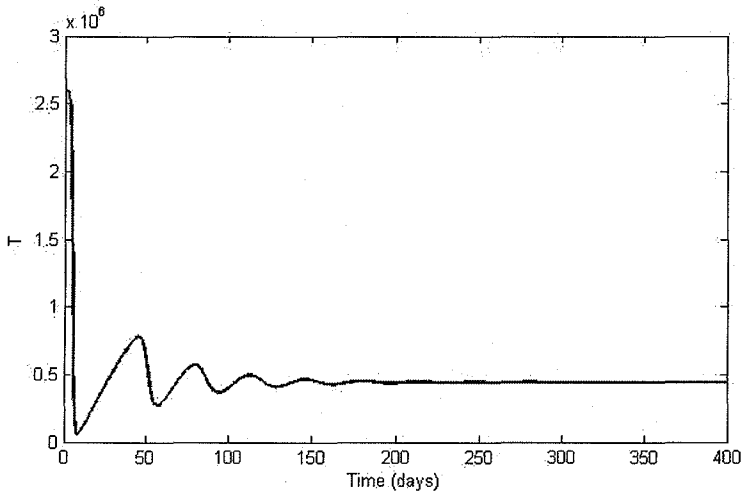

Figure 22: Target Cell Population over Days

in a person is 14g/dl. Thus calculating using these values we estimated A. Using these values, we simulate the complete system below.

From figure 22 we see that hepatocytes are dynamic from the beginning of treatment. The target cell population decreases around the first week, oscillates in quantity and reaches its steady state after approximately 150 days of drug administration. The values used for this simulation and the following ones have been mentioned above in table 13.

In figure D.1 we see the infected hepatocytes' behavior as a result of peg-interferon. Moreover, cell production decreases after approximately two weeks and starts stabilizing around the 50th day.

In figure 24 we see viral load also reaches equilibrium.

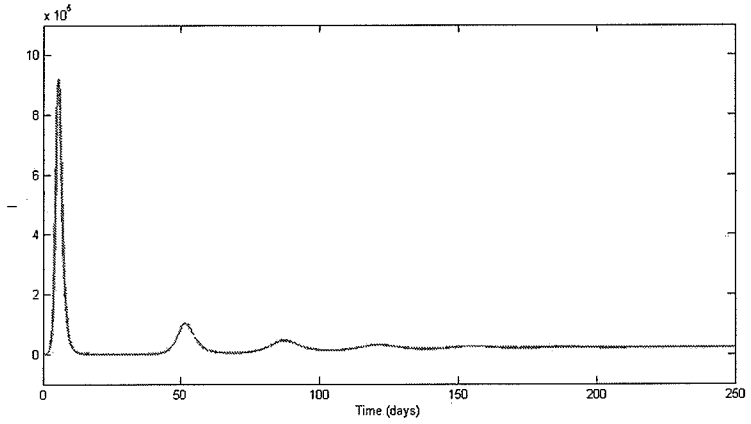

Figure 23: Infected Cells over Days

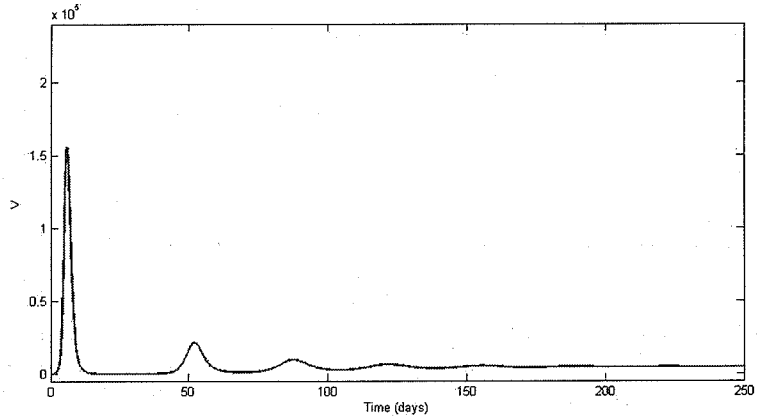

Figure 24: Viral Load over Days

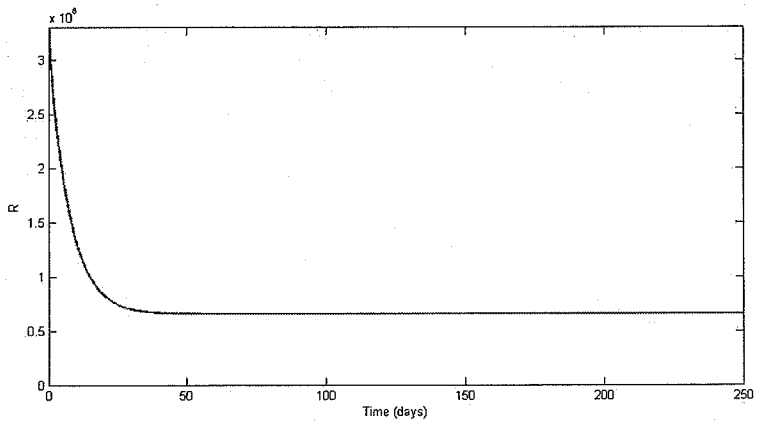

Figure 25: Red Blood Cell Concentration

In figure 25, red blood cell concentration decreases and reaches its equilibrium quantity without oscillations like the target cells dynamics.

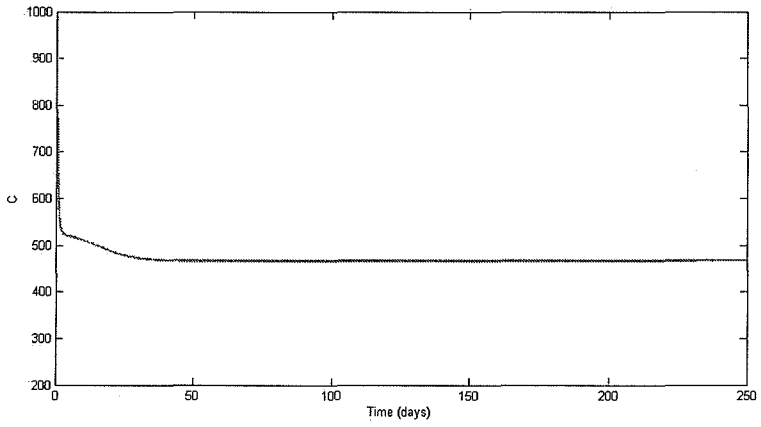

Figure 26: Drug Amount

In table 26, the amount of drug decreases to around 300 mg before reaching equilibrium at 475 mg.

E Discussion

Mathematical models have been used in the past to understand details of the mechanism of infection of HCV [4] with or without treatment. Models by Dahari et al. [9, 4] have estimated efficacies of antiviral peg-interferon by itself and in combination with ribavirin. Although this combination therapy is highly successful it has been associated with many adverse treatment related side-effects. The most common being hemolytic anemia. To combat hemolytic anemia, many patients are prescribed the drug epoetin. Epoetin contains a hormones that stimulates red blood cell production in bone marrow. In clinical trials an increase of two or threefold normal red blood cell production has been observed. Moreover, the increase in red blood cell production due to epoetin varies according to individual patients' immune response.

Our model is an extension of the basic model in Dahari et al. [9] which incorporates the RBC concentration and drug amount in the body as dynamical systems represented by two differential equations.Analysis of the decoupled equations separately gives two critical values of drug amount: C^* and C_{SVR}. These are the desired amount of drug amount to keep the RBC concentration at an equilibrium, and the necessary drug amount in the body to keep the virus in check, respectively. It is known from medical observation that there is a considerable amount of difference between C^* and C_{SVR} for most HCV patients. This is also reflected in our model for the set of estimated parameters stated previously. In this paper, we have explored numerically the effect epoetin has on increasing the C* value. That is, the increase in the red blood cell production that allows the body to handle a larger quantity of drug without causing anemia. Recall that $C^* = \frac{\Lambda}{h}\frac{R^{*2}}{A^2+R^{*2}}$ and R^* is the real root of 35. Now we substitute s_R by bs_R in $F(R)$ where b is the increment of red blood cells production due to epoetin. Note, that C^* is bounded by $\frac{\Lambda}{h}$ and thus to make C^* approach C_{SVR}, thus not only does b have to be increased but also the daily dosage, Λ, must be increased.

We find numerically that if dosage is increased to 1624 mg from 800-1200 mg daily and combined with enough epoetin to increase red blood cell production by 10 times, patients have received the "perfect" dosage of drugs that will eliminate the virus and also keep the red blood cell production at a healthy equilibrium. However, it is noted that these values are specific to the set of parameters values used in this paper, and need to be calculated for each individual patient. Knowledge of these values and their subsequent use will help physicians to calculate the amount of epoetin that needs to be administered to a patient to increase the RBC production sufficiently to avoid hemolytic anemia and the amount of combination drug therapy necessary to clear the virus from the body.

F Future Work

In this paper, the critical drug amount have been calculated along with their effect on the stability of the system. As an extension of this model, future work could be done in several directions. In terms of model construction, our model considers the amount of drug to be the dual amount of ribavirin and peg-interferon, however future work could be done where the drug amount of ribavirin and peg-interferon are separate state variables with different clearance rates h_1 and h_2. In addition our model did not consider the effect that peg-interferon has on bone marrow production of red bloods cells and instead considered s_R to be a constant when in fact it is a function of the amount of peg-interferon in the body. This type of model would also have to consider the synergistic effect that ribavirin and peg-interferon have being that ribavirin in not prescribed without interferon, and that the "whole is greater than the sum of its parts" in terms of HCV treatment.

Our model considers the drug amount separate from the drug efficacy of the drug which Dahari et al. [9] considers. It would be interesting to model HCV infection and the side effect hemolytic anemia with the efficacy of drug therapy and drug amount simultaneously being that efficacy is a function of drug amount. It is also possible to extend our model to be more complete by including a state variable for the immune system response considering that hepatitis C inhibits immune system response against it through different mechanisms [13]. In addition, since HCV has the ability to suppress immune response against it, it would be helpful to drug development to mathematically model the effect a new type of drug could have on this specific mechanism of the virus and how this could affect SVR rates and disease progression rates. Further, the motivating research question of this paper could be more deeply analyzed using optimal control theory because at each time step the viral load is changing along with the production rate of red blood cells. Further sensitivity analysis of parameters of our model would be useful to understand why some patients can spontaneously clear hepatitis C infection while others progress to chronic hepatitis C infection. Also, not all patients experience hemolytic anemia which suggests that their specific sub-genotype of hepatitis C virus has different values for d_I and d_V or that the system is highly sensitive to individuals' parameter values.

For future pharmaceutical development, it would be useful to develop medication that would affect the value of p. Before this could be done, it is necessary to calculate the critical value of p based on forcing the controlled reproduction number of the system to be less than 1. Thus, this would provide an unstable endemic equilibrium that would result in decreased viral load that eventually approached zero and would provide SVR. This result would be useful to clinicians and pharmaceutical companies in developing new hepatitis C therapies because it would provide the necessary level of drug effect in order to be a better therapy than the ones currently available. Moreover, research into the critical rate h of drug amount clearance should by studied to understand the affect that different dosing schedules would have on the dynamics and success rates of HCV therapy. Currently clinicians use hormonal stimulation of bone marrow production of red blood cells to counteract the effect of ribavirin-induced hemolytic anemia, and thus force C^* to be closer to C_{SVR}; however mathematical models could be used to understand the drug dynamics that would be needed to force C_{SVR} to be closer to C^* which could help motivate new drug therapies research.

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H Appendix

H.1 Finding Equilibrium Points for (R, C) system

Equating $\frac{dC}{dt} = 0$, we get $C^* = \frac{\Lambda R^2}{h(A^2+R^2)}$.

Plugging in C^* into $\frac{dR}{dt} = 0$, we get

$$s_R - d_R R - \tau C^* R = s_R - d_R R - \frac{\tau \Lambda R^3}{h(A^2+R^2)}.$$

Multiplying by $h(A^2+R^2)$ and then dividing by h results in

$$F(R) = \left(d_r + \frac{\tau}{h}\Lambda\right) R^3 - s_R R^2 + d_R A^2 R - S_R A^2.$$

Next, dividing by A^3 throughout, we get

$$\left(d_R + \tau\frac{\Lambda}{h}\right)\left(\frac{R}{A}\right)^3 - \frac{s_R}{A}\left(\frac{R}{A}\right)^2 + d_R\left(\frac{R}{A}\right) - \frac{s_R}{A} = 0$$

Again dividing throughout by $\frac{s_R}{A}$, we get

$$\frac{\left(d_R + \tau\frac{\Lambda}{h}\right)\left(\frac{R}{A}\right)^3}{\left(\frac{s_R}{A}\right)} - \left(\frac{R}{A}\right)^2 + \frac{d_R}{\left(\frac{s_R}{A}\right)}\left(\frac{R}{A}\right) - 1 = 0.$$

Now we rescale using $r = \frac{R}{A}$, $d = \frac{d_R}{(\frac{s_R}{A})}$,$a = \frac{\tau\frac{\Lambda}{h}}{(\frac{s_R}{h})}$. Then we get

$$f(r) = (a+d)r^3 - r^2 + dr - 1 = 0.$$

Here note $f(0) = -1$, $\lim_{r\to\infty} f(r) = +\infty > 0$, and most importantly $f(r) < 0$ if $r \leq 0$.

H.2 Bifurcation Analysis for $(\mathbf{R}, \mathbf{C})$ system

From the previous section we have,

$$f(r) = (a+d)r^3 - r^2 + dr - 1 = 0.$$

Now the possible cases are as follows: We know that at a bifurcation point,$f(r) = 0$ and $f'(r) = 0$, since at this point the sub-system will have two positive real root, thus for our system

$$f(r) = 0 \Rightarrow ar^3 + d(R^2+R)d = r^2 + 1, \tag{55}$$
$$f'(r) = 0 \Rightarrow 3ar^2 + d(3r^2+1) = 2r. \tag{56}$$

This is a linear system with respect to a and d. Hence we can write:

$$\begin{bmatrix} r^3 & r^3+r \\ 3r^2 & 3r^2+1 \end{bmatrix}\begin{bmatrix} a \\ d \end{bmatrix} = \begin{bmatrix} r^2+1 \\ 2r \end{bmatrix}.$$

Now, since the determinant of the coefficient matrix is non-zero, $(r \neq 0)$ we can calculate its inverse to get

$$\begin{bmatrix} a \\ d \end{bmatrix} = \left(\frac{-1}{2r^3}\right)\begin{bmatrix} 3r^2+1 & -r^3-r \\ -3r^2 & r^3 \end{bmatrix}\begin{bmatrix} r^2+1 \\ 2r \end{bmatrix}.$$

Therefore,

$$a = -\frac{(r^2+1)^2}{2r^3} < 0. \tag{57}$$
$$d = \frac{r^4+3r^2}{2r^3} = \frac{r^2+3}{2r} > 0. \tag{58}$$

Thus our bifurcation line lies in the second quadrant of the parameter plane. However, motivated by biological reasons, our region of interest lies in the first quadrant where $a > 0$ and $d > 0$. Plugging in $(a, d) = (1, 1)$ in $f(r)$ we get $f(r) = 2r^3 - r^2 + r - 1$. In this case, $f(r) = 0$ has only one positive real root. Hence, we conclude that we have only one equilibrium point throughout the first quadrant.

H.3 Local Stability of (R, C) system

$$\frac{dR}{dt} = s_R - d_R R - \tau C R$$
$$\frac{dC}{dt} = \Lambda \frac{R^2}{(A^2 + R^2)} - hC$$

From the previous results, we derive that

$$J_{(R^*, C^*)} = \begin{bmatrix} -d_R - \tau C^* & -\tau R \\ 2\frac{A^2 \Lambda R}{(A^2 + R^{*2})^2} & -h \end{bmatrix}$$

Since $d_R > 0$, $C^* > 0$, $h > 0$, $\tau > 0$.
$\Rightarrow Tr(J) = -(d_R + \tau C^* + h) < 0$, and $det(J) = h(d_R + \tau C^*) + \tau R^* \frac{\tau A^2 R^* \Lambda}{A^2 + R^{*2}} > 0 \therefore (R^*, C^*)$ is locally asymptotically stable.

H.4 Global Stability of (R, C)

We then use a result by H. Thieme [29] so that we can find the equilibrium points of the (T,I,V) system. According to the Poincaré-Bendixson Theorem there are three possibilities for end behavior solutions to our system: a limit cycle, an unbounded solution or a globally stable equilibrium point. We use Dulac's criterion and Poincaré - Bendixson Theorem to prove global stability which allows us to say that $C(t)$ is asymptotically constant.

Dulac's Criterion:

Let $g = \frac{1}{R} \rightarrow \nabla(g\dot{x}) = \frac{\partial}{\partial R}(\frac{s_R}{R} - d_R \tau C) + \frac{\partial}{\partial C}(\Lambda \frac{R}{A^2 + R^2} - \frac{hC}{R}) \Rightarrow = -\frac{s_R}{R^2} = \frac{h}{R} < 0 \therefore$ there is no limit cycle.

We prove that $C < \frac{\lambda}{h}$ and $R < \frac{s_R}{d_R}$, hence the solution cannot be unbounded thus we have a globally stable equilibrium. We see that local stability has extended to global stability.

H.5 Analysis of (T, I, V) System

H.5.1 Disease Free Equilibrium

In this section we use our results from section C.2, and use a result from H. Thieme [29] to analyze the three dimensional system .

$$\frac{dT}{dt} = s_T - d_T T - \frac{\alpha}{\alpha + C} \beta T V = 0 \tag{59}$$
$$\frac{dI}{dt} = \frac{\alpha}{\alpha + C} \beta T V - d_I I = 0 \tag{60}$$
$$\frac{dV}{dt} = p \frac{k}{k + C^*} I - \theta C V - d_V V = 0 \tag{61}$$

To determine the DFE, we let $I = 0$ and $V = 0$ and solve for $\frac{dV}{dt} = 0$, $\frac{dI}{dt} = 0$, $\frac{dT}{dt} = 0$. It is clear by inspection that the disease free equilibrium (DFE) is $(\frac{S_T}{d_T}, 0, 0)$.

H.5.2 Stability Analysis of DFE

From the previous section we know that the DFE is $(\frac{S_T}{d_T}, 0, 0)$; now we will analyze the stability of the DFE. Using the Jacobian matrix,

$$J_{(T^*, I^*, V^*)} = \begin{bmatrix} -d_T - \frac{\alpha\beta V}{\alpha+C} & 0 & -\frac{\alpha\beta T}{\alpha+C} \\ \frac{\alpha\beta V}{\alpha+C} & -d_I & \frac{\alpha\beta T}{\alpha+C} \\ 0 & \frac{pk}{k+C} & -\theta C - d_V \end{bmatrix}.$$

Substituting $(T^*, 0, 0)$ for $(\frac{s_T}{d_T}, 0, 0)$ yields

$$J_{(\frac{s_T}{d_T}, 0, 0)} = \begin{bmatrix} -d_T & 0 & -\frac{\alpha\beta \frac{s_T}{d_T}}{(\alpha+C)} \\ 0 & -d_I & \frac{\alpha\beta \frac{s_t}{d_T}}{(\alpha+C)} \\ 0 & \frac{pk}{k+C} & -\theta C - d_V \end{bmatrix}.$$

Characteristic Equation is:

$$(-d_T - \lambda) \begin{vmatrix} -d_I - \lambda & \frac{\alpha\beta \frac{s_T}{d_T}}{\alpha+C} \\ \frac{pk}{k+C} & -\theta C - d_V - \lambda \end{vmatrix} = 0$$

We have that $\lambda_1 = -d_T$, now we consider the other sub-matrix and find its eigenvalues.

$(d_I + \lambda)(\theta C + d_V + \lambda) - (\frac{\alpha\beta \frac{s_T}{d_T}}{\alpha+C})(\frac{pk}{k+C}) = 0$

$\lambda^2 + (d_I + \theta C + d_V)\lambda + d_I(\theta C + d_V) - (\frac{\alpha\beta \frac{s_T}{d_T}}{\alpha+C})(\frac{pk}{k+C})$

Let $\delta = d_I(\theta C + d_V) - (\frac{\alpha\beta \frac{s_T}{d_T}}{\alpha+C})(\frac{pk}{k+C})$. Now we apply the quadratic formula,

$$\lambda_{2,3} = \frac{-(d_I+\theta C+d_V) \pm \sqrt{(d_I+\theta C+d_V)^2 - 4\delta}}{2} < 0$$

For stability of the DFE, we want all the eigenvalues to have negative real parts. If $\sqrt{(d_I + \theta C + d_V)^2 - 4\delta}$ is imaginary, then we are done as $-(d_I+\theta C+d_V)$ is always negative. If $-(d_I+\theta C+d_V) - \sqrt{(d_I + \theta C + d_V)^2 - 4\delta}$ is real then it is negative. Thus the only case that needs to be considered is $-(d_I+\theta C+d_V) + \sqrt{(d_I + \theta C + d_V)^2 - 4\delta}$. Thus

$-(d_I + \theta C + d_V) + \sqrt{(d_I + \theta C + d_V)^2 - 4\delta} < 0$

$\Leftrightarrow -(d_I + \theta C + d_V) < -\sqrt{(d_I + \theta C + d_V)^2 - 4\delta}$

$\Leftrightarrow (d_I + \theta C + d_V) > \sqrt{(d_I + \theta C + d_V)^2 - 4\delta}$

$\Leftrightarrow (d_I + \theta C + d_V)^2 > (d_I + \theta C + d_V)^2 - 4\delta.$

We can cancel out like terms from both sides, since they are both positive.

$\Leftrightarrow -4\delta < 0$

$\Leftrightarrow \delta > 0$

$\Leftrightarrow d_I(\theta C + d_V) - \frac{\alpha\beta s_T}{d_T(\alpha+C)} \frac{pk}{k+C} > 0$

$\Leftrightarrow \widehat{\Re} = \frac{\alpha\beta s_T pk}{d_I d_T(\alpha+C)(k+C)(\theta C+d_V)} < 1.$

Thus the DFE is locally asymptotically stable if and only if $\widehat{\Re} < 1$.

H.5.3 Endemic Equilibrium

Now, we solve for the endemic equilibrium points, using $V^* \neq 0$ and $I^* \neq 0$.

$$\begin{aligned}
\frac{dI}{dt} &= \frac{\alpha}{\alpha+C}\beta TV - d_I I = 0 && (62)\\
\Rightarrow I^* &= \frac{\alpha}{\alpha+C}\frac{\beta T^* V^*}{d_I} && (63)\\
\frac{dV}{dt} &= p\frac{k}{k+C}I - \theta CV - d_V V = 0 && (64)\\
0 &= \frac{p\alpha k\beta}{(k+C)(\alpha+C)}T^*V^* - \theta CV^* - d_V V^* && (65)\\
V^* \neq 0 \Rightarrow T^* &= \frac{d_I(d_V+\theta C)(k+C)(\alpha+C)}{k\alpha p\beta} && (66)
\end{aligned}$$

From, $\frac{dT}{dt} = 0$, we see that

$$\begin{aligned}
s_T - d_T T &= \frac{\alpha}{\alpha+C}\beta TV, && (67)\\
\Rightarrow V^* &= \frac{s_T}{T^*\beta\frac{\alpha}{\alpha+C}} - \frac{d_T(\alpha+C)}{\alpha\beta}, && (68)\\
\Rightarrow V^* &= \frac{s_T kp}{d_I(d_V+\theta C)(k+C)} - \frac{d_T(\alpha+C)}{\beta\alpha}. && (69)
\end{aligned}$$

Thus, the endemic equilibrium is

$$(T^*, I^*, V^*) = \left(\frac{d_I(d_V+\theta C)(k+C)(\alpha+C)}{k\alpha p\beta}, \frac{\alpha}{\alpha+C}\frac{\beta T^* V^*}{d_I}, \frac{s_T kp}{d_I(d_V+\theta C)(k+C)} - \frac{d_T(\alpha+C)}{\beta\alpha}\right)$$

Then simplifying using $\widehat{\Re}$, the endemic equilibrium is

$$\begin{aligned}
T^* &= \frac{s_T}{d_T\widehat{\Re}} && (70)\\
I^* &= \frac{s_T}{d_I}\left(1 - \frac{1}{\widehat{\Re}}\right) && (71)\\
V^* &= \frac{(\alpha+C)d_T}{\alpha\beta}\left(\widehat{\Re}\right) && (72)
\end{aligned}$$

H.5.4 Stability Analysis of Endemic Equilibrium

$$J_{(T^*,I^*,V^*)} = \begin{bmatrix} -d_T - \frac{\alpha\beta V}{\alpha+C^*} & 0 & -\frac{\beta\alpha T^*}{\alpha+C^*} \\ \frac{\alpha\beta V^*}{\alpha+C^*} & -d_I & \frac{\alpha\beta T^*}{\alpha+C^*} \\ 0 & \frac{pk}{k+C^*} & -\theta C^* - d_V \end{bmatrix}$$

Substituting (T^*, I^*, V^*) for definitions (70)–(72), the following Jacobian matrix results

$$\widehat{\mathfrak{J}} = \begin{bmatrix} -d_T - d_T\left(\widehat{\Re} - 1\right) - \lambda & 0 & -\frac{\alpha\beta\, s_T}{d_T\widehat{\Re}(\alpha+C)} \\ d_T\left(\widehat{\Re} - 1\right) & -d_I - \lambda & \frac{\alpha\beta\, s_T}{d_T R(\alpha+C)} \\ 0 & \frac{pk}{k+C} & -\theta C - d_V - \lambda \end{bmatrix}$$

From this matrix, we determine the characteristic equation:

$P(\lambda) = -(d_T\widehat{\Re} + \lambda)(d_I + \lambda)(\theta C^* d_V + \lambda) + (d_T\widehat{\Re} + \lambda)\frac{\alpha\beta s_T pk}{d_T(\alpha+C^*)(k+C^*)\widehat{\Re}} - d_I d_T(\theta C^* + d_V)(\widehat{\Re} - 1).$

Let, $q = (\theta C^* d_V)$, then

$P(\lambda) = -\lambda^3 + \lambda^2(d_I + d_T\widehat{\Re} + q) + \lambda d_T\widehat{\Re}(q + d_I) - d_I d_T q(1 - \widehat{\Re}) = 0.$

Now we apply the Routh Hurwitz Criterion to determine stability.

Let,

$$q = d_V + \theta C \tag{73}$$

$$D = \frac{\alpha}{\alpha + C}\frac{k}{k + C}p\beta s_T \tag{74}$$

$$\rightarrow \widehat{\Re} = \frac{D}{d_I d_T q} \tag{75}$$

When $\widehat{\Re} < 1$, the DFE is stable, thus the infection is eliminated from the hepatocyte population. Since the effect of peg-interferon on the viral load, k, is much greater in magnitude compared to the effect of RBV on viral load, θ, for simplification of analysis we can take $\theta \approx 0$. Then the modified CRN is

$$\widehat{\Re_1} = \Re_0 \frac{\alpha}{\alpha + C}\frac{k}{k + C} \tag{76}$$

which can be biologically translated as the pre-treatment BRN with some control parameters based on treatment. This allows us to control the BRN of the infection depending on drug-amount.

We have analyzed the disease free equilibrium to understand under which conditions the DFE is stable. Now our attention shifts to the endemic equilibrium since our biological interest is treatment of chronically HCV infected individuals. Turning to the endemic equilibrium, we solve to get:

$$T^* = \frac{s_T}{d_T\widehat{\Re}} \tag{77}$$

$$I^* = \frac{s_T}{d_I}(1 - \frac{1}{\widehat{\Re}}) \tag{78}$$

$$V^* = d_T\frac{(\alpha + C)}{\alpha\beta}(\widehat{\Re} - 1 \tag{79}$$

(see Appendix section H.5 for further details on equilibrium point of (T,I,V) subsystem).

Now linearizing about the endemic equilibrium and applying the Routh-Hurwitz criterion to the cubic eigenvalue equations, we get the following conditions:

i) $(d_I + d_T\widehat{\Re} + q) > 0$

ii) $-d_I d_T q(1 - \widehat{\Re}) > 0$

iii) $(d_I + d_T\widehat{\Re} + q)d_T\widehat{\Re}(q + d_I) > -d_I d_T q(1 - \widehat{\Re})$

However, the third condition implies the first one, and the second condition is true if and only if $\widehat{\Re} > 1$. If $\widehat{\Re} > 1$ is true and condition 3 is satisfied then the endemic equilibrium is stable. From condition 3, we have

$$\frac{D^2(q + d_I)}{{d_I}^2 d_T q^2} + D\left(\frac{(q + d_I)^2}{d_I d_T q} - \frac{1}{d_T}\right) + d_I q > 0 \tag{80}$$

Equating the left hand side of equation (80) to zero, we obtain two roots, $D_{1,2}$ as

$$D_{1,2} = \frac{-\left(\frac{(q+d_I)^2}{d_I d_T q} - \frac{1}{d_T}\right) \pm \sqrt{(\frac{(q+d_I)^2}{d_I d_T q} - \frac{1}{d_T})^2 - 4\frac{(q+d_I)}{d_I d_T q}}}{2\left(\frac{q+d_I}{d_I^2 d_T q^2}\right)}. \tag{81}$$

Let,

$$Q = \left(\frac{(q + d_I)^2}{d_I d_T q} - \frac{1}{d_T}\right)^2 - 4\frac{(q + d_I)}{d_I d_T q}. \tag{82}$$

If Q is negative, then inequality (80) is always satisfied. Furthermore, since $4\frac{(q+d_I)}{d_I d_T q}$ is positive, if Q is positive, then $-\left(\frac{(q+d_I)^2}{d_I d_T q} - \frac{1}{d_T}\right)$ dominates the sign of the roots. Now,

$$\left(\frac{(q + d_I)^2}{d_I d_T q} - \frac{1}{d_T}\right) = \frac{1}{d_T}\left(\frac{(q + d_I)^2}{d_I q} - 1\right) \tag{83}$$

$$\Leftrightarrow \frac{1}{d_I d_T q}\left((q - d_I)^2 + d_I q\right) > 0. \tag{84}$$

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