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Platelet-driven routes to chaos in a model of hepatitis

Martin R. Nelson [a,*], Jonathan M. Gibbins [b], Joanne L. Dunster [b]

[a] School of Science and Technology, Department of Physics and Mathematics, Nottingham Trent University, Nottingham, NG11 8NS, UK
[b] Institute for Cardiovascular and Metabolic Research, University of Reading, Reading, RG6 6AS, UK

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ABSTRACT

Hepatitis is a general term used to describe inflammation in the liver, the regulation of which involves a complex array of interactions between liver cells, inflammatory mediators and cells of the immune system (macrophages and neutrophils, in particular). There is increasing evidence that platelets (small blood cells that are primarily responsible for clotting) affect numerous mechanisms that underlie hepatitis, including the rates of cell migration, liver damage and mediator production, giving rise to a complex and diverse range of inflammatory outcomes. In particular, platelet activation has the scope to promote both healthy and chronic outcomes in a highly unpredictable and potentially state-dependent manner that is not currently well-understood. In this paper, we take an existing model of the inflammatory dynamics associated with hepatitis, and introduce platelets and their effects. We interrogate this new model via numerical simulations in Matlab and bifurcation analysis in XPPAUT, and find that amplification of certain key interactions by platelets can stimulate chaotic dynamics that correspond to complex chronic outcomes. We initially introduce a single parameter to represent the scale of the platelet stimulus and, through bifurcation analysis, illustrate that as this parameter is gradually increased we see the emergence of a period-doubling cascade that results in chaotic dynamics. We then explore individual mechanisms in more detail and illustrate that, while neutrophils can promote chaos in some settings, routes to chaos are primarily driven by macrophage-related platelet stimuli. Finally, we briefly comment upon the implications of our observations in terms of the ongoing hunt for new therapeutic interventions in hepatitis, as well as other inflammation-related medical conditions.

1. Introduction

Platelets are a key component of the blood clots that stop bleeding and, when produced inappropriately, can contribute to heart attacks and strokes. But, beyond this role in hemostasis and cardiovascular disease, there is a growing body of evidence that platelets influence a variety of inflammatory diseases, including those that occur in the liver that are termed hepatitis [1,2]. Given the liver's role in the synthesis of many hemostatic proteins, anti-platelet drugs (the cornerstone in the treatment of thrombotic disorders) are normally ceased under liver disease. However, there has been a recent reanalysis of symptoms associated with hepatitis and several studies now demonstrate that liver disease is associated with thrombosis, promoting the hypothesis that platelets may represent a promising therapeutic approach for inflammation of the liver [2,3]. However, clinical and experimental studies are contradictory, with platelets being shown to exert both pro-inflammatory and anti-inflammatory roles in the liver, pointing to gaps in our understanding of the mechanisms involved in platelet-mediated modulation of liver diseases [2,4].

In a previous study [5], we developed a mathematical model of hepatitis, accounting for cellular populations resident in the liver (hepatocytes and stellate cells) and their interactions with immune cells (neutrophils and macrophages) and inflammatory mediators (cytokines) as occurs under inflammatory conditions. The model is bistable with a trivial solution representing a healthy response (in which inflammation subsides) and a positive steady state representing chronic liver disease that has positive levels of pro-inflammatory components and liver cells being replaced by extracellular matrix. We used bifurcation analysis to investigate the manner in which variation of key model parameters drives switching between these outcomes. In particular, our analysis revealed the rates of apoptosis of neutrophils and hepatoctyes, phagocytosis of apoptotic cells by macrophages, and production of both pro- and anti-inflammatory mediators being the key parameters affecting the switch between bistability and guaranteed restoration of health, and also illustrated that variations in the rate of hepatocyte apoptosis can induce oscillatory dynamics.

In this article, we explore the diverse range of platelet effects in hepatitis. Platelets normally exist in an inert state but quickly respond

* Corresponding author.
E-mail addresses: martin.nelson@ntu.ac.uk (M.R. Nelson), j.m.gibbins@reading.ac.uk (J.M. Gibbins), j.l.dunster@reading.ac.uk (J.L. Dunster).

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to extracellular signals to activate and influence their environment, which is equivalent to a wide-spread upscaling of many of the feedbacks incorporated in our original model. Here we investigate how increasing levels of platelet activation not only affect the switch between resolved (healthy) and chronic inflammatory outcomes, but can also result in chaotic dynamics. In Section 2 below, we describe how the model of [5] can be extended to incorporate these platelet effects. We begin with a simple approach in which we assume that all platelet-affected feedbacks are amplified to the same degree, parameterised by scaling parameter ρ. As such, a single additional parameter is introduced into the model, but we show that this can have large-scale affects as it amplifies several of the model's interactions simultaneously. Since the model then encompasses eleven nonlinear ODEs and twenty-four parameters, the scope for analytical progress is limited (beyond calculation of some simple steady states). We therefore focus our analysis upon numerical simulation of the model in Matlab (using standard ODE solvers such as ode45) and bifurcation analysis conducted in XPPAUT. The latter of these uses continuation methods to track the model's steady state and periodic solutions as model parameters are varied, as described in [6]. We highlight that all of the Matlab and XPPAUT codes used in this article have been made available online at https://github.com/martinrnelson/PlateletChaosHepatitis. In Section 3, we summarise the key behaviours of the original model of [5], before (in Section 4) comparing these to the much more diverse dynamics attained through the incorporation of platelets into the model. In Section 5, we then adopt a more detailed implementation of the platelet-induced scaling, in which we separately stimulate the model's key interactions to ascertain which facets of the model are primarily responsible for inducing chaotic dynamics. We close with a brief discussion of our model's key findings, including in the context of the ongoing scope for identification of new therapeutic interventions.

2. The model

We revisit an existing model of hepatitis [5], which describes interactions of active and apoptotic neutrophils (n, a), inflammation-promoting and inflammation-resolving macrophages (m_i, m_r) and pro- and anti-inflammatory mediators (c, g) with the liver tissue itself, as illustrated in Fig. 1. Liver tissue is primarily composed (approximately 80%, by weight) of hepatocytes, which are responsible for performing the majority of the liver's functions and can exist in two forms: either active (in their healthy state, performing key functions as normal) or damaged (in which they can stimulate inflammatory damage). In this model, we denote these two cell populations by h (active) and h_a (apoptotic/damaged) respectively; loss of damaged hepatocytes leads to production of either new hepatocytes or extra-cellular matrix (ECM, e), and (after suitable nondimensionalisation) the model assumes that the total size of the liver is conserved such that $h + h_a + e = 1$. The production of ECM is stimulated by hepatic stellate cells, which exist in the liver in a quiescent (dormant) state (s), but under the influence of pro-inflammatory mediators can switch to a myofibroblast-like phenotype (s_a) via a (reversible) process termed activation. These active cells are well known as the major source of the fibrillar collagens that comprise ECM, and are associated with liver fibrosis.

This existing model excludes the roles of platelets; however, platelets are now known to interact both with the liver's resident cellular population and with cells of the immune system to exert regulatory functions and influence the cytokine milieu [1,4]. Platelets have been shown to increase neutrophil and macrophage recruitment by paving the way through the vascular endothelium into the liver [1,7,8], promote an anti-inflammatory macrophage phenotype [9], drive the activation of hepatic stellate cells [10], and stimulate the production of pro-inflammatory mediators from neutrophils and anti-inflammatory mediators from macrophages [9,11,12]. We initially take a very simple approach to understanding the manner in which platelets upscale key inflammatory mechanisms, by introducing a single scaling parameter ρ, which multiplies the relevant terms in the ODE model. We therefore make the general assumption that all platelet-affected mechanisms are upscaled equally as platelet effects take hold; while this is not necessarily realistic, it is a useful starting point for our analysis, and we further explore the implications of this later in this manuscript. With the parameter ρ included, our model's governing equations are as follows:

$$\frac{dn}{dt} = \rho\,\frac{c}{1+g} - \nu\frac{1+\frac{g}{\beta_g}}{1+\frac{c}{\beta_c}}n, \tag{1a}$$

$$\frac{da}{dt} = \nu\frac{1+\frac{g}{\beta_g}}{1+\frac{c}{\beta_c}}n - \rho\,\gamma_a a - \phi\,a\,(m_i + \phi_2\,m_r), \tag{1b}$$

$$\frac{dm_i}{dt} = \rho\,c - \rho\,k_{m1}\,\phi\,a\,m_i + k_{m2}\,m_r - \gamma_m\,m_i\left(1+\gamma_{m2}\,m_r\right), \tag{1c}$$

$$\frac{dm_r}{dt} = \rho\,k_{m1}\,\phi\,a\,m_i - k_{m2}\,m_r - \gamma_m\,m_r, \tag{1d}$$

$$\frac{dc}{dt} = \rho\,\gamma_a\frac{a^2}{\beta_a^2+a^2} + \rho\,k_n\frac{n^2}{\beta_n^2+n^2} + h_a + k_m\,m_i - c, \tag{1e}$$

$$\frac{dg}{dt} = \rho\,k_g\,m_r + k_h\,h - \gamma_g\,g, \tag{1f}$$

$$\frac{dh}{dt} = \chi_h\phi\,s\,h_a(m_i + \phi_2\,m_r) + \gamma_e\,e - \nu_2\,h\,c + \gamma_h\,h_a\,s, \tag{1g}$$

$$\frac{dh_a}{dt} = \nu_2\,h\,c - \chi_h\phi\,h_a(m_i + \phi_2 m_r) - \gamma_h\,h_a, \tag{1h}$$

$$\frac{de}{dt} = \chi_h\phi\,s_a\,h_a(m_i + \phi_2 m_r) - \gamma_e\,e + \gamma_h\,h_a\,s_a, \tag{1i}$$

$$\frac{ds}{dt} = r_2\,s_a(1+g) - \rho\,r_1\,sc, \tag{1j}$$

$$\frac{ds_a}{dt} = \rho\,r_1\,s\,c - r_2\,s_a(1+g). \tag{1k}$$

This system is solved subject to the initial conditions $n(0) = a(0) = m_i(0) = m_r(0) = g(0) = h_a(0) = s_a(0) = e(0) = 0$, $h(0) = s(0) = 1$ and $c(0) = c_0$, for parameter c_0, which represents an initial stimulus of inflammatory damage. That is, we initialise the model at a healthy state in which all inflammatory components other than c are zero, all hepatocytes are active and all stellate cells are quiescent.

3. Dynamics in the absence of platelet stimuli ($\rho = 1$)

The model of (1) has the potential to generate various outcomes, depending on model parameters. In particular, (1) exhibits a trivial steady state with $h = s = 1$, $g = k_h/\gamma_g$ and all other variables equal to zero. Since this steady state corresponds to all pro-inflammatory components having been eliminated, it corresponds to a healthy outcome (*i.e* guaranteed resolution of inflammation). For some model parameters, the model can also yield chronic outcomes in which pro-inflammatory components take positive values; these include both steady state solutions and periodic orbits. (We will show below that stimulation of platelet effects can also supplement this range of outcomes with chaotic behaviours.)

It is relatively straightforward to show [5] that the healthy steady state is stable provided that

$$\rho k_m < \gamma_m\left(1 - \frac{\nu_2}{\gamma_h}\right), \tag{2}$$

with the possibility of attaining a healthy (resolved) outcome being determined by a balance between the inflammation-promoting influence of macrophages (k_m), the strength of damage to hepatocytes (ν_2), and the rates of removal of these cells (γ_m, γ_h). Since one of the roles of platelets is to upscale macrophage recruitment, the former of these mechanisms additionally scales with ρ. The stability of the chronic steady states, however, is more complex. Prior works [5,13,14] have identified the rates of neutrophil apoptosis (ν) and removal (phagocytosis) of apoptotic neutrophils by macrophages (ϕ) as the two key parameters in determining the stability of chronic steady states. Fig. 2 illustrates bifurcation diagrams (produced via XPPAUT; code

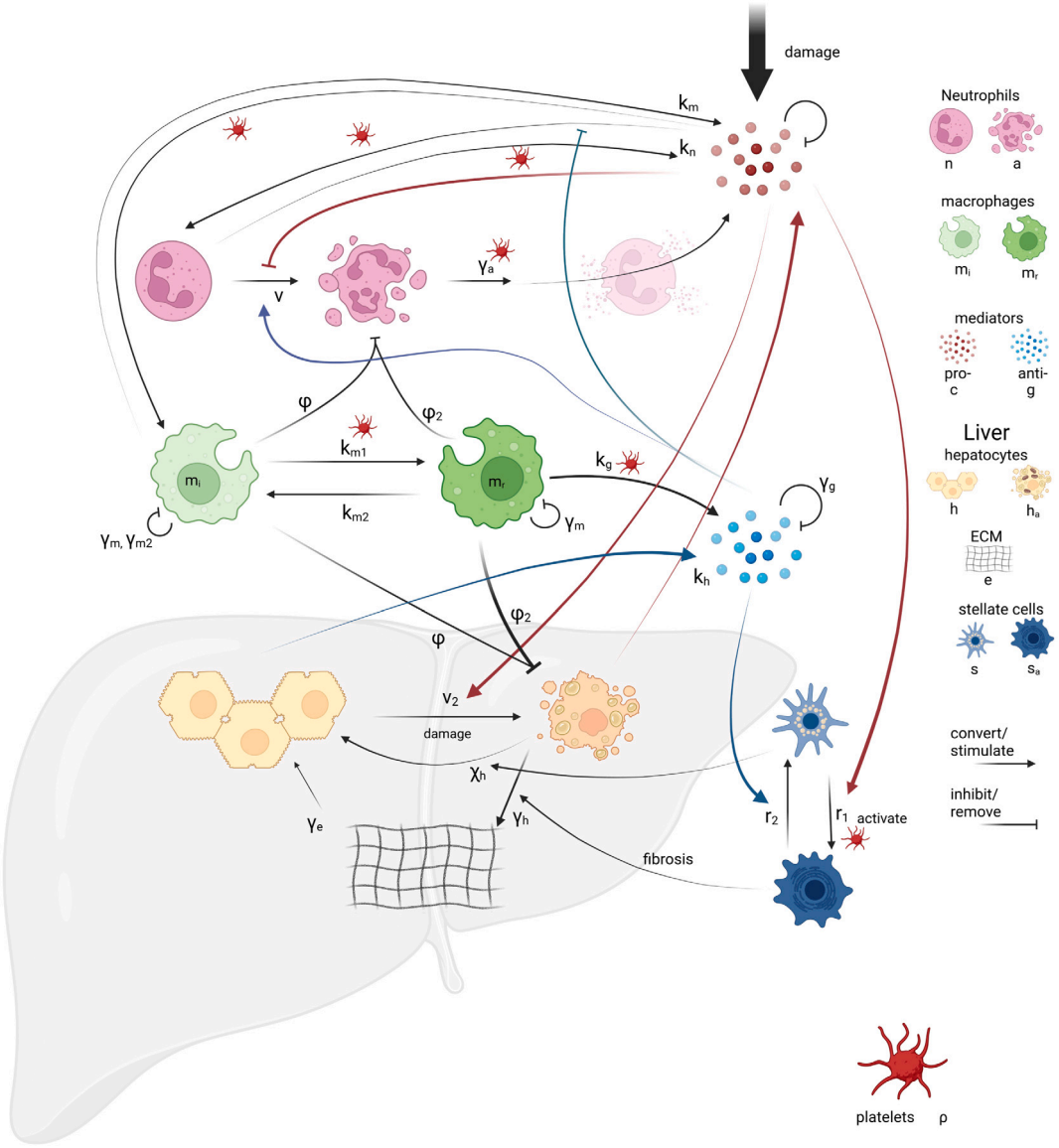

Fig. 1. Interactions between the cells and mediators captured in our model of hepatitis, with those that are stimulated by platelets highlighted. Damage causes a rise in pro-inflammatory mediators, increasing influx of neutrophils and macrophages. Neutrophils (in active or apoptotic form) cause a rise in pro-inflammatory mediators through the release of their toxic content. Macrophages exist in pro- or anti-inflammatory phenotypes which release the associated mediators; both phenotypes remove damaged or dead cells. Pro-inflammatory stimuli damage hepatocytes and activate stellate cells, the latter stimulating the production of extracellular matrix (ECM). Dead hepatoctytes are removed by macrophages, being replaced by healthy hepatocytes or ECM, depending on the activation state of stellate cells. Dimensionless parameters associated with particular processes are placed next to the relevant arrows and summarised in Table 1. Platelets increase rates of neutrophil and macrophage influx, the activation of stellate cells, a switch in macrophage phenotype, an increase in anti-inflammatory mediators from macrophages and pro-inflammatory mediators from neutrophils.
Source: Figure adapted from [5] with permission.

online) that summarise the model's outcomes for $\rho = 1$. This Figure summarises the key results of [5]. Typically, for realistic values of the model's other parameters (Table 1), if ϕ is small the chronic steady state is stable, with macrophages unable to combat the pro-inflammatory feedback of neutrophils. As ϕ is increased, the chronic steady state is generally destabilised via a Hopf bifurcation, representing a shift from a region of bistability (B) to a region of monostability with resolution of damage guaranteed (M:Res), as shown in Fig. 2(a). (We note that for the parameter values of Table 1, the healthy steady state is stable.)

In Fig. 2(b), we track the position of the Hopf bifurcation in (ϕ, ν)-space, dividing parameter space into regions of guaranteed resolution and bistability (with chronic outcomes permissible). As above, we see that the model is always bistable for small ϕ. Similarly, if ν is small, the pro-inflammatory feedback from apoptotic neutrophils is sufficiently weak that macrophage recruitment is insufficient to resolve inflammatory damage and chronic outcomes result, with inflammatory damage primarily being sustained by feedbacks from the active neutrophil population. Manipulating the strengths of other key feedbacks in the model has the effect of shifting this boundary between bistability and monostability; in general, we are particularly interested in manipulations which lead to the size of the region of monostability (guaranteed resolution) being enlarged, which are akin to the intended affects of therapeutic interventions. We illustrate this for decreasing k_n (pro-inflammatory feedback from active neutrophils) and increasing k_h (anti-inflammatory feedback from hepatocytes) in Figs. 2(c,d) respectively.

Fig. 2(e) shows that increasing the parameter k_m, which represents pro-inflammatory mediator production by inflammatory macrophages, not only affects the stability of the healthy state via (2), but also shifts the Hopf bifurcation in Fig. 2(a) to the right, until it collides

Table 1
List of nondimensional model parameters and default values. We take $\phi = 0.01$ by default throughout, in order to ensure that both chronic and healthy steady states are stable; all other parameters are taken directly from [5].

Parameter	Description	Values
ν	Rate of neutrophil apoptosis	0.1
ν_2	Rate of hepatocyte apoptosis	0.01
ϕ	Rate of phagocytosis by inflammatory macrophages (m_i)	0.01
ϕ_2	Increased rate of phagocytosis due to m_r macrophages	10
χ_h	Proliferation of hepatocytes	1
ρ	Scaling parameter representing platelet effects	1
Decay rates		
γ_a	Apoptotic neutrophils (necrosis)	1
γ_m	Macrophages (inflammatory; m_i)	0.01
γ_{m2}	Macrophages (resolving; m_r)	0.01
γ_g	Anti-inflammatory mediators	1
γ_h	Hepatocytes (necrosis)	0.1
γ_e	ECM	0.1
Rate parameters		
k_n	Pro-inflammatory mediator production by active neutrophils	0.01
k_g	Anti-inflammatory mediator production by m_r macrophages	0.1
k_h	Anti-inflammatory mediator production by hepatocytes	0.1
k_m	Pro-inflammatory mediator production by m_i macrophages	0.0001
k_{m1}	Macrophage phenotype switching (m_i to m_r)	30
k_{m2}	Macrophage phenotype switching (m_r to m_i)	0.3
r_1	Stellate cell activation	1
r_2	Active stellate cells reverting to quiescence	1
Miscellaneous		
β_a	Saturation constant	0.1
β_n	Saturation constant	0.1
β_c	Saturation constant	0.12
β_g	Saturation constant	0.01

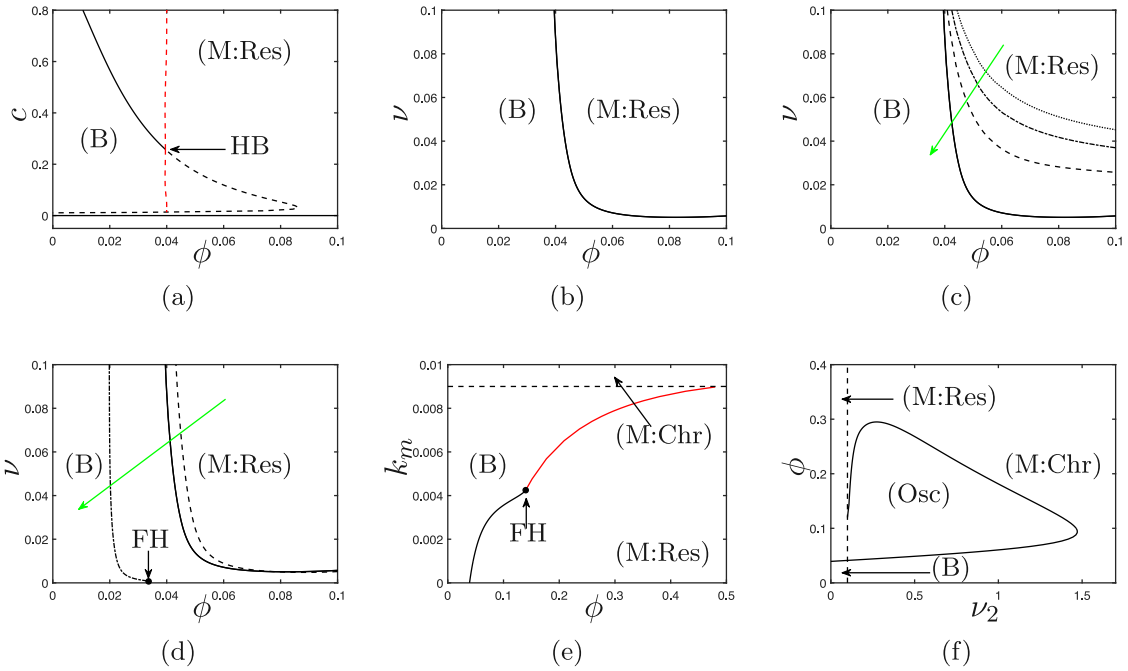

Fig. 2. Summary of the model's behaviours for $\rho = 1$; (a) Bifurcation diagram showing c as a function of ϕ, with the chronic steady state being destabilised via a Hopf bifurcation (HB) as ϕ increased. Tracking HB in (ϕ, ν)-space allows us to demark regions of bistability (B) and monostability with guaranteed resolution of damage (M:Res), as shown in (b). Manipulating the strength of inflammatory feedbacks shifts this boundary in parameter space, as illustrated for (c) decreasing k_n and (d) increasing k_h (by traversing the plots in the direction of the green arrows). (In (c), $k_n = 0.1$ (dotted), $k_n = 0.07$ (dash-dotted), $k_n = 0.04$ (dashed) and $k_n = 0.01$ (solid). In (d), $k_h = 0.01$ (dashed), $k_h = 0.1$ (solid) and $k_h = 1$ (dash-dotted).) (e) Increasing k_m results in the Hopf bifurcation (black) shifting right in (a) until it collides with the nearby saddle–node (red) via a fold-Hopf bifurcation (FH), and for larger increases can also destabilise the healthy steady state. (f) Manipulation of the rate of hepatocyte damage (ν_2) can also destabilise the healthy steady state, resulting in a monostable region with guaranteed chronicity (M:Chr), but can additionally give rise to branches of periodic chronic solutions (Osc). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Source: These figures reproduce the corresponding figures presented in [5] with permission.

with a neighbouring saddle node, and is then eliminated via a fold-Hopf bifurcation. (In Fig. 2(e), we show the Hopf position in black and the saddle–node position in red, plotting only the portion of the saddle–node branch that bounds the bistable region for clarity.)

Interestingly, the parameter ν_2, which represents the rate of hepatocyte damage, plays a significant role in determining resulting outcomes, as shown in Fig. 2(f). Not only can increased values of ν_2 destabilise the healthy steady state via (2) to result in guaranteed chronic damage; for some values of ϕ, increases in ν_2 can also result in oscillatory chronic solutions (labelled 'Osc' in Fig. 2(f)), reminiscent of inflammatory conditions with relapsing-remitting characteristics. Below, we will examine how ν_2 also contributes to the potential for chaotic solutions.

4. Platelet effects

In this section, we investigate the manner in which upscaling the interactions highlighted in Fig. 1, via the platelet parameter ρ, affects the outcomes of our model and the corresponding dynamics. Foremost, from (2), we know that variations in ρ have a direct effect on the stability of the healthy steady state; an increase in ρ (for all other parameters fixed) can result in a destabilising of the healthy steady state, thus guaranteeing chronic outcomes and supporting recent investigations into the use of anti-platelet treatments for hepatitis [2,3,15]. Below, we illustrate that, in addition to this, variation of ρ can also result in significant changes to the nature and severity of chronic outcomes.

Fig. 3 shows simulations that represent typical chronic results as ρ is increased, obtained for the parameter values of Table 1. While resolving outcomes are possible for these parameter choices, in Fig. 3 we focus upon understanding platelet-driven changes to chronic outcomes. Comparing panels (a) and (b) of Fig. 3, we see that increasing ρ from $\rho = 1$ to $\rho = 2$ results in a long-term outcome that comprises similar levels of neutrophil recruitment and pro-inflammatory mediators, with little change to the activity of liver cells (hepatocytes, stellate cells); however, we see much stronger levels of macrophage recruitment (particularly pro-inflammatory macrophages), and a corresponding increase in the production of anti-inflammatory mediators in order to mitigate against the resultant potential damage. For more significant increases in ρ (Fig. 3(c,d)), we see greater activation of stellate cells in particular, resulting in more hepatocytic activity and oscillatory inflammation dynamics in general. The period of these oscillatory solutions can be seen to have a strong dependence upon ρ.

In Fig. 4, we present a bifurcation analysis that further elucidates the role ρ plays in facilitating the oscillatory dynamics of Fig. 3. Fig. 4(a) shows that for $\rho \lesssim 2.45$, the model is bistable with both healthy and chronic steady-state outcomes permissible. At $\rho \simeq 2.45$, the chronic steady state undergoes a supercritical Hopf bifurcation (HB), giving rise to stable periodic solutions such as that shown for $\rho = 3$ in Fig. 5(a). As ρ is increased, this branch of chronic oscillatory solutions undergoes a period doubling bifurcation (PD1) at $\rho \simeq 5.3$, at which point the original orbit becomes unstable, and a new stable orbit with twice the period is created (as illustrated for $\rho = 5.5$ in Fig. 5(b)). The point PD1 is the first in a sequence of period doubling bifurcations, the first four of which are shown in Fig. 4(a). As ρ increases through these points, periodic solutions obtain longer periods and more complex profiles, which are conveniently visualised by plotting trajectories in any two-dimensional slice of phase-space, as illustrated in (c, g)-space in the right-hand panels of Fig. 5; each period doubling bifurcation results in at least one additional loop in these plots.

This cascade of period doubling bifurcations generates a number of branches of periodic solutions, which all ultimately become unstable for some value of ρ, giving rise to chaotic solutions in those areas of parameter space for which all chronic branches are unstable. This period doubling route to chaos is illustrated in Fig. 4(c), and a typical chaotic solution is illustrated in Fig. 6. In Fig. 6(b), we colour the chaotic attractor according to the proportion of the liver that is comprised of active hepatocytes (h), noting that small h corresponds to high levels of liver damage. A typical trajectory corresponds to orbiting the attractor in an anti-clockwise direction, and we generally observe that peaks in pro-inflammatory mediators (c) are quickly followed by peaks in anti-inflammatory mediators (g) as the immune system attempts to mitigate against further damage. Starting, for example, from a configuration in which the extent of liver damage is low (*i.e.* h is large, shown in yellow in Fig. 6(b)), we have high rates of production of anti-inflammatory mediators via the k_h term in (1f), but also high levels of hepatocyte apoptosis via the ν_2 term in (1g), which in turn increases pro-inflammatory mediator concentrations via (1e). However, as anti-inflammatory mediators accumulate, these suppress the pro-inflammatory contributions of neutrophils, and also reduce stellate cell activation, which in turn stimulates recruitment of new active hepatocytes to allow the liver to recover. We therefore obtain complex switching behaviour that shifts the system between high and low levels of inflammation. We note, in particular, that the chaotic solution of Fig. 6 includes periods in which up to 70% of the liver is comprised of apoptotic hepatocytes or ECM, while the periodic solutions of Fig. 5 involve much lower levels of liver damage (with less than 10% of the liver being comprised of apoptotic hepatocytes or ECM). The chaotic behaviour induced by platelets, here, therefore corresponds to a significant worsening of the inflammatory condition.

Examining the dynamics for larger values of ρ (as shown in Fig. 4(b)) reveals that the branches of unstable periodic orbits eventually coalesce via a sequence of period-halving and torus bifurcations, eventually yielding a single stable periodic orbit once again. (This occurs at approximately $\rho = 25$, for the parameters of Table 1.) These stable period orbits are then eliminated via a second supercritical Hopf bifurcation (for $\rho \simeq 35$ here), at which point the chronic steady state regains stability. For choices of ρ larger than this, chronic steady state outcomes are permissible, although it is notable that these are less severe in magnitude than those corresponding to our baseline simulations for $\rho = 1$; very strong platelet effects can therefore act to improve upon chronic outcomes, which may help to explain some of the contradictory evidence surrounding platelet effects in the liver — some deleterious and some reparative [2,4]. The two Hopf bifurcations shown in Fig. 4(b) bound a central region of complex oscillatory and chaotic behaviours (Fig. 4(d)).

For a more broad range of parameter choices, we can elucidate where in parameter space we expect to observe oscillations and chaos by tracking the coordinates of the two Hopf bifurcations in Fig. 4 as a function of other key model parameters. In Fig. 7(a–c) we illustrate the manner in which variations of the hepatocyte damage parameter, ν_2, the macrophage phagocytosis parameter, ϕ, and the rate of pro-inflammatory feedback from macrophages, k_m, affect the positions of these Hopf bifurcations, and hence the scale of our region of chaotic dynamics. (We focus upon these parameters in particular since they were identified to have particularly significant impact upon the dynamics in the original model of [5].) We also illustrate calculations of the largest Lyapunov exponent (Fig. 7(d–f)), with areas of parameter space in which the largest Lyapunov exponent is positive (indicated by bright colours in the figure) indicating chaotic dynamics. We observe that an increase in the rate of hepatocyte damage (ν_2) results in the two Hopf bifurcations moving closer to one another, narrowing the corresponding region of chaotic or oscillatory dynamics (Fig. 7(a,d)), while also having the potential to destabilise the healthy steady state according to (2). For very high values of ν_2, hepatocyte damage overwhelms any temporal dynamics, resulting in a chronic steady state being obtained with certainty. (We note that increasing ν_2 was also shown to eliminate oscillations in the original model in the absence of platelets [5], corresponding to traversing to the right in Fig. 2(f) above.)

The extent to which platelets can drive chronic and chaotic outcomes is perhaps most evident in Fig. 7(b,e), which explores the relationship between platelet effects (ρ) and the rate of macrophage phagocytosis (ϕ). In the original model of [5], and as shown in Fig. 2, for $\rho = 1$, the parameter ϕ plays a key role in switching between regions of bistability and guaranteed resolution of damage; that is, for ϕ large, the rate at which macrophages phagocytose apoptotic neutrophils and hepatocytes is sufficiently high that the pro-inflammatory

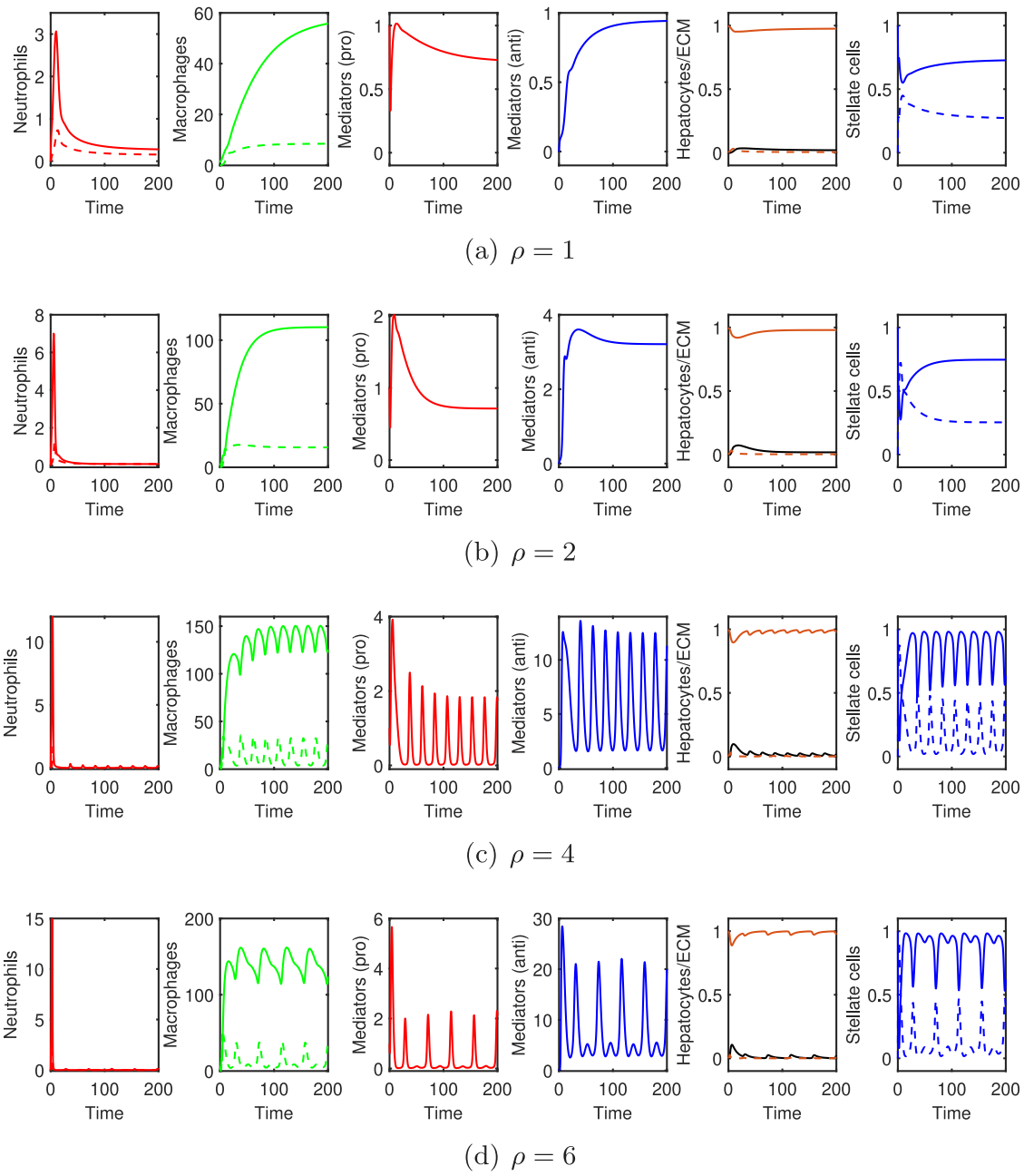

Fig. 3. Simulation showing the effects of platelets on hepatitis, for all unspecified parameters as in Table 1. Solid/dashed lines respectively illustrate active/apoptotic neutrophils, inflammatory/restorative macrophages, active/apoptotic hepatocytes and quiescent/activated stellate cells; ECM levels are shown in black.

stimulus is successfully mitigated against. However, Fig. 7(b,e) illustrates that increases in ρ can result in wide-spread oscillatory or chaotic dynamics representing chronic outcomes in a region of parameter space in which the original model predicts guaranteed restoration of health. This observation is due to the fact that increasing ρ affects a full suite of interactions in the model, many of which result in an upregulation of pro-inflammatory feedbacks; however, the phagocytic activity of macrophages (parameterised by ϕ) can only combat the pro-inflammatory mechanisms related to neutrophil/hepatocyte apoptosis, with the remaining additional stimuli being fundamentally unchecked by the macrophage response. While increasing ρ does have an indirect affect upon phagocytosis rates via increased switching of macrophages to the restorative phenotype, these appear to be outweighed by the upscaled pro-inflammatory feedbacks of inflammatory macrophages and active neutrophils, in particular, moving the model from a healthy configuration to one of chronic (oscillatory/chaotic) outcomes.

We have seen in (2) above that variations in ρ and k_m both have the ability to destabilise the healthy steady state and that, in the absence of platelets, varying k_m also affects the existence/stability of chronic steady-state outcomes (Fig. 2(e)). From Fig. 7(c,f), we see that increasing k_m also results in a narrowing of the region of oscillatory or chaotic solutions, until they are eliminated entirely and we cross in to a region in which the model is bistable (in the sense that both chronic and healthy steady state outcomes are permissible). For more substantial increases in k_m or ρ, the healthy steady state is destabilised via (2) (as we cross the red dashed line in Fig. 7(c)) resulting in a guaranteed chronic steady state outcome.

For the ranges of parameters investigated in Fig. 7, we observe the general trend that stimulating very strong platelet effects has the result of destabilising the healthy steady state via (2) and driving the model toward chronic steady-state outcomes; however, the severity of these chronic outcomes is generally much lower than those obtained in the

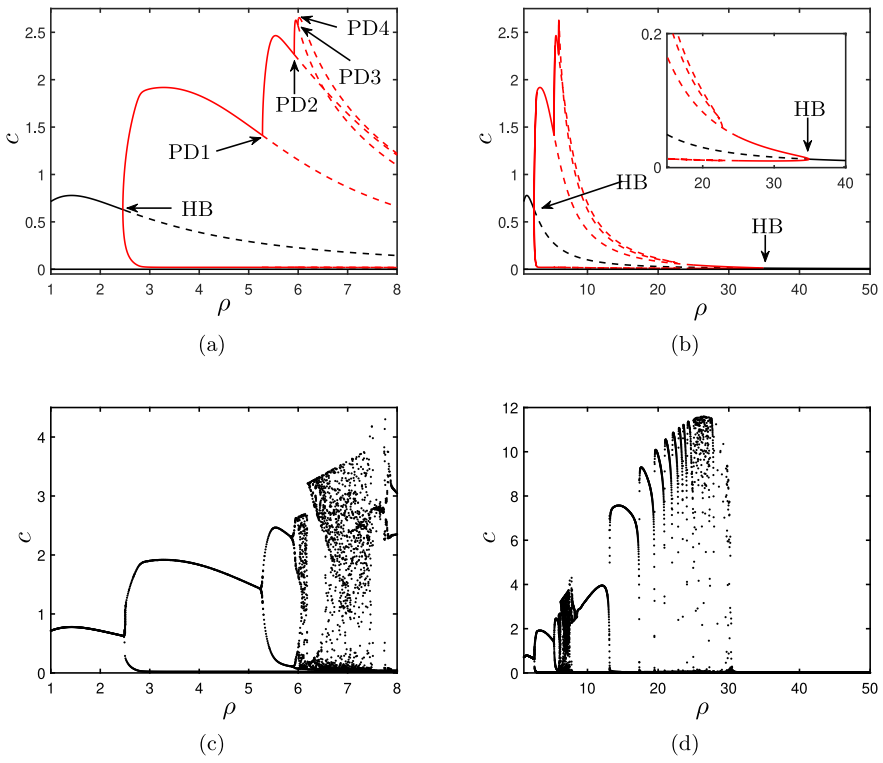

Fig. 4. Bifurcation diagrams showing the effect of the platelet parameter ρ, for the parameters of Table 1. In (a,b), the chronic steady state (black) becomes unstable via a Hopf bifurcation at HB, giving rise to periodic solutions (red) whose periods increase via a sequence of period doubling bifurcations, the first four of which are marked PD1–PD4 in (a). This period doubling cascade ultimately results in a window of chaotic behaviours for approximately $\rho \in [6.07, 25]$, with a cascade of period-halving and torus bifurcations eventually giving rise to periodic solutions once again (for approximately $\rho \in [25, 34.8]$). These periodic solutions are eliminated via a second Hopf bifurcation at $\rho \simeq 34.8$, giving rise to stable steady state solutions for larger ρ. (c) and (d) illustrate the corresponding maxima and minima of oscillations in c for varying ρ, showing a period-doubling route to chaos. (Panels (a,b) are produced via continuation in XPPAUT, while panels (c,d) arise from numerical simulations conducted in Matlab; codes online.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

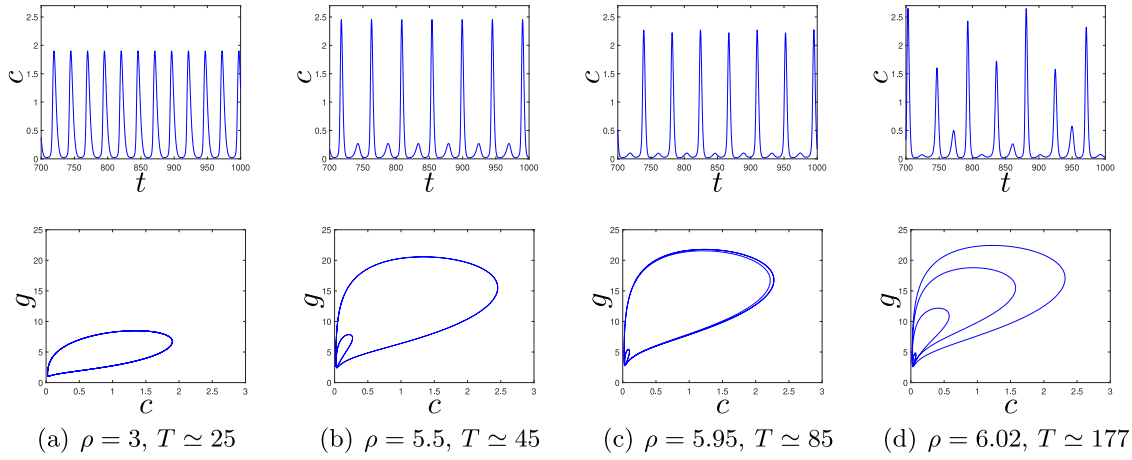

Fig. 5. Periodic solutions for various choices of ρ, illustrating period doubling for the parameters of Table 1. T denotes the period of these solutions.

original model in the absence of platelet effects. For intermediate levels of platelet stimulation, we see a vast array of oscillatory and chaotic dynamics which depend strongly on the values of the model's other parameters. In those regions of parameter space that exhibit chaotic solutions, we estimate the Kaplan–Yorke dimension of the corresponding chaotic attractor to lie approximately in the range $[2, 2.5]$. This diverse range of outcomes is consistent with platelets being reported to have a complex range of both restorative and inflammation-promoting effects on observed outcomes in previous literature [2,4].

5. Elucidating individual platelet mechanisms

We here briefly address the question of how the numerous mechanisms affected by platelet stimulation in the system (1) individually affect the scope for chaotic outcomes. To do so, we introduce individual parameters $\rho_1, \ldots, \rho_7$ to denote the platelet-driven scalings associated with neutrophil recruitment (ρ_1), neutrophil apoptosis (ρ_2), recruitment of inflammatory macrophages (ρ_3) and their transition to the restorative state (ρ_4), production of pro- and anti-inflammatory mediators by

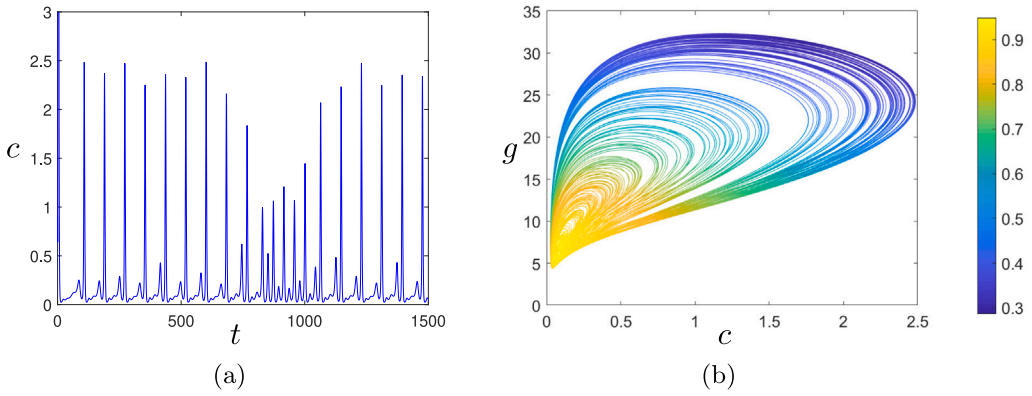

Fig. 6. A typical chaotic solution (a) and the corresponding chaotic attractor plotted in (c, g)-space (b), for $\rho = 8$, $\nu_2 = 0.5$ and all other parameters as in Table 1. In (b), the attractor is coloured according to the proportion of active hepatocytes in the liver (h); dark blue areas correspond to the greatest liver damage with approximately 70% of the liver comprised of apoptotic hepatocytes or ECM; bright yellow areas correspond to the least damage, approximately 95% of the liver being comprised of active hepatocytes. We note that chaotic solutions can give rise to much greater levels of liver damage than is given by the corresponding oscillatory solutions of Fig. 5, for which h lies in the range $[0.9, 1]$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

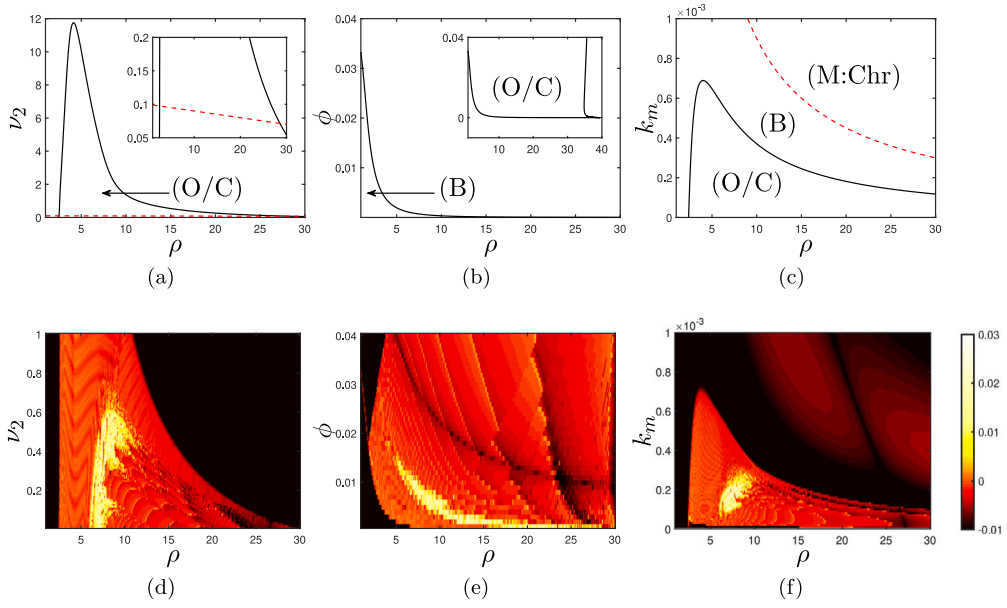

Fig. 7. Bifurcation diagrams showing the position of Hopf bifurcations (solid black curves), together with the stability condition for the healthy steady state (Eq. (2); red dashed curves) as functions of the parameters ν_2, ϕ and k_m (a–c), together with computations of corresponding largest Lyapunov exponents (d–f). All unspecified parameters are as in Table 1. In (a–c), (M:Chr) = monostable, chronic steady state; (B) = bistable (chronic and healthy steady states stable); (O/C) = oscillatory/chaotic solutions. In (d–f), bright regions indicate regions of chaotic behaviour, in which the largest Lyapunov exponent is positive. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

neutrophils and restorative macrophages respectively (ρ_5 and ρ_6) and activation of stellate cells (ρ_7). Thus, we examine the following system:

$$\frac{dn}{dt} = \rho_1 \frac{c}{1+g} - \nu \frac{1+\frac{g}{\beta_g}}{1+\frac{c}{\beta_c}} n, \tag{3a}$$

$$\frac{da}{dt} = \nu \frac{1+\frac{g}{\beta_g}}{1+\frac{c}{\beta_c}} n - \rho_2 \gamma_a a - \phi\, a\, (m_i + \phi_2\, m_r), \tag{3b}$$

$$\frac{dm_i}{dt} = \rho_3\, c - \rho_4\, k_{m1}\, \phi\, a\, m_i + k_{m2}\, m_r - \gamma_m\, m_i \left(1 + \gamma_{m2}\, m_r\right), \tag{3c}$$

$$\frac{dm_r}{dt} = \rho_4\, k_{m1}\, \phi\, a\, m_i - k_{m2}\, m_r - \gamma_m\, m_r, \tag{3d}$$

$$\frac{dc}{dt} = \rho_2\, \gamma_a \frac{a^2}{\beta_a^2 + a^2} + \rho_5\, k_n \frac{n^2}{\beta_n^2 + n^2} + h_a + k_m\, m_i - c, \tag{3e}$$

$$\frac{dg}{dt} = \rho_6\, k_g\, m_r + k_h\, h - \gamma_g\, g, \tag{3f}$$

$$\frac{ds}{dt} = r_2\, s_a (1+g) - \rho_7\, r_1\, sc, \tag{3g}$$

$$\frac{ds_a}{dt} = \rho_7\, r_1\, s\, c - r_2\, s_a (1+g), \tag{3h}$$

with h, h_a and e governed by ((1)g–i) as before.

Our analysis of the scope for chaotic outcomes in this model is now substantially more complicated since (assuming that the original model parameters of Table 1 remain fixed) we are now tasked with exploring seven-dimensional parameter space. To do so, we employ two approaches below. Firstly, we consider the case in which all but one of the ρ_i are held fixed at one and examine the effect of varying each of the ρ_i independently via bifurcation analysis. Secondly, in order to explore our seven-dimensional parameter space more fully, we employ a Metropolis–Hastings (MH) algorithm in which we seek the maximal value of the largest Lyapunov exponent (LLE) as a function of $\rho_1, \ldots, \rho_7$. Having identified this 'most chaotic' region of parameter space, we then examine the local dependence of each of our platelet parameters

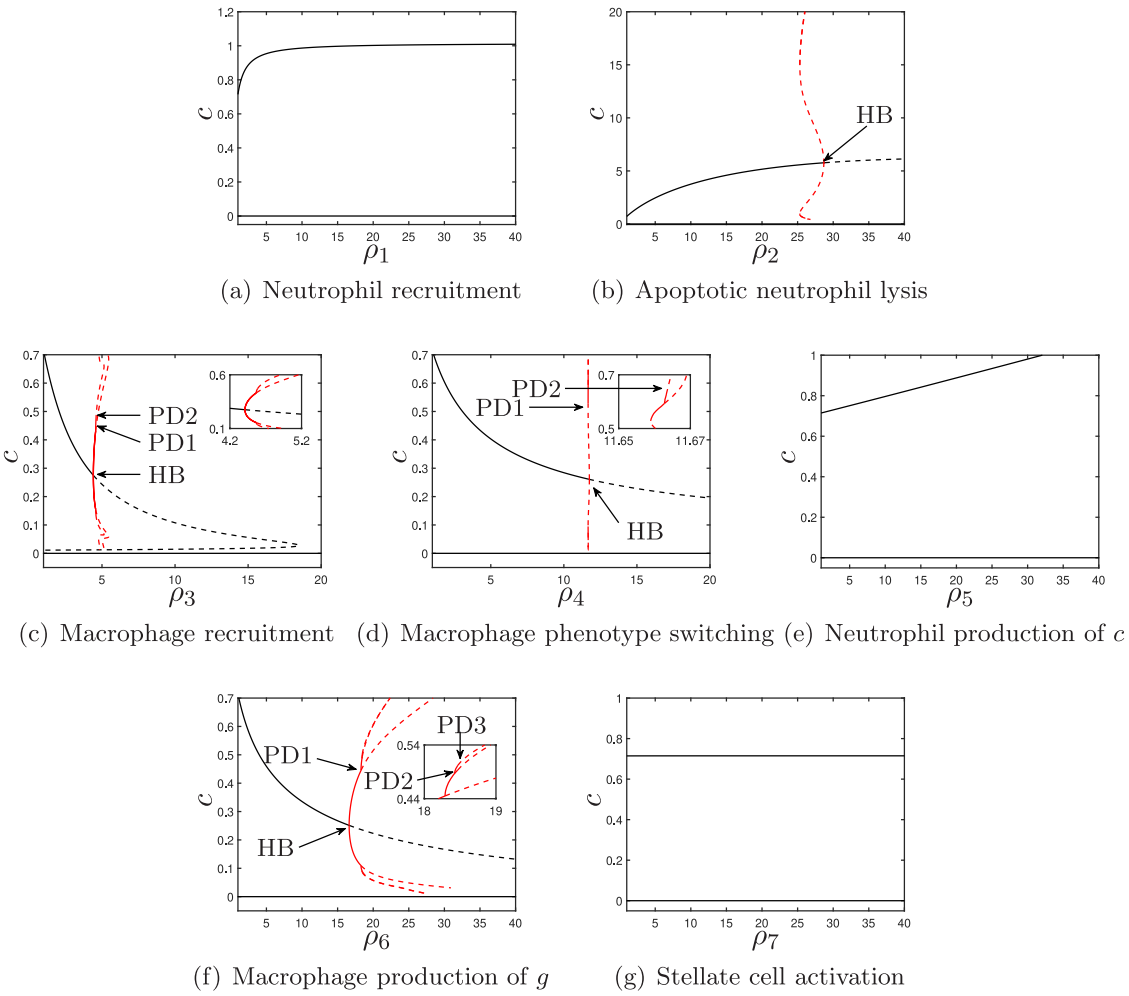

Fig. 8. Bifurcation diagrams illustrating the influence of platelet-driven stimulation of individual mechanisms in the model, via the parameters $\rho_1, \ldots, \rho_7$. We fix $\rho_i = 1$ (no platelet stimulation) for all i other than that on the horizontal axis in each panel. The period-doubling route to chaos is driven by macrophage-based mechanisms only (panels c, d and f), with neutrophil and stellate cell feedbacks having no role in the route to chaos, for these parameters.

$\rho_1, \ldots, \rho_7$ by examining variations around this new baseline set of parameters. Motivated by the ranges over which chaotic dynamics occur in Fig. 4, throughout both of these analyses, we restrict our investigation to the region $\rho_i \in [0, 40]$ for all $i \in [1, 7]$.

Fig. 8 illustrates bifurcation diagrams in which we vary each of $\rho_1, \ldots, \rho_7$ independently, holding the remaining ρ_i at one and all other parameters fixed at the values of Table 1. Our goal here is to understand which of the seven distinct platelet mechanisms above play a role in giving rise to the period doubling cascade of Fig. 4. Fig. 8 suggests that the platelet-driven routes to chaos observed above are driven entirely by macrophage effects, with independent stimulation of macrophage recruitment (ρ_3), macrophage phenotype switching from the inflammatory m_i phenotype to the restorative m_r phenotype (ρ_4), and macrophage production of anti-inflammatory mediators (ρ_6) all giving rise to a cascade of period doubling bifurcations. While stimulating the rate of lysis of apoptotic neutrophils (ρ_2) can provide a switch from bistability to guaranteed resolution of damage (via a subcritical Hopf bifurcation), as per the corresponding influence of γ_a itself in the original model, stimulation of neutrophil feedbacks or stellate cell activation have no independent impact upon the scope for chaotic outcomes.

Fig. 9 illustrates the results of a MH simulation with 10^6 iterations of sampling on the domain $\rho_i \in [0, 40]$ for all $i \in [1, 7]$. The scatter plots in the upper-right of Fig. 9 provide an indication (via 2D slices of 7D space) of the regions of parameter space in which we find chaotic outcomes. Grey points represent those whose largest Lyapunov exponents are positive (*i.e.* chaotic points), which comprise approximately 53% of those sampled. Red points demark those that lie in the top 5% of those points sampled when ordered by LLE, *i.e.* those with largest LLE, for which LLE $\gtrsim 0.009$. The single point sampled that has maximal LLE is shown by black squares, *i.e.* the point $\rho_1 = 30.95$, $\rho_2 = 21.94$, $\rho_3 = 14.25$, $\rho_4 = 6.50$, $\rho_5 = 24.41$, $\rho_6 = 8.29$ and $\rho_7 = 36.91$ (to two decimal places), with LLE $\simeq 0.036$. In the lower-left panels of Fig. 9, we fix all ρ-values at these values (demarked by the black squares) as a baseline, and then vary pairs of parameters (ρ_i, ρ_j) in order to understand the local sensitivity to each of these parameters. (Our aim here is to elucidate the extent to which fixing $\rho_i = 1 \forall i$ as a baseline in Fig. 8 impacts upon our overall conclusions.) Here, once again, we observe that the model appears to have very weak sensitivity to the parameters ρ_5, representing production of pro-inflammatory mediators by neutrophils, and ρ_7, representing stellate cell activation. While neutrophil feedbacks, in general, seem to play a less significant role in inducing chaotic dynamics than macrophage feedbacks do, there is some scope for neutrophil behaviours to exacerbate chaotic outcomes as they operate in tandem with macrophages. Intuitively, there is a natural relationship between the rates of neutrophil recruitment and lysis, as indicated by the plots of ρ_1 versus ρ_2. It appears that chaotic dynamics are most prevulent if platelets stimulate both of these behaviours at similar rates. Largely, the parameters related to platelet stimulation of macrophage effects (ρ_3, ρ_4, ρ_6) seem to underpin the greatest amount of structure in the LLE-landscape, suggesting that macrophages are the primary driver of the chaotic dynamics observed here.

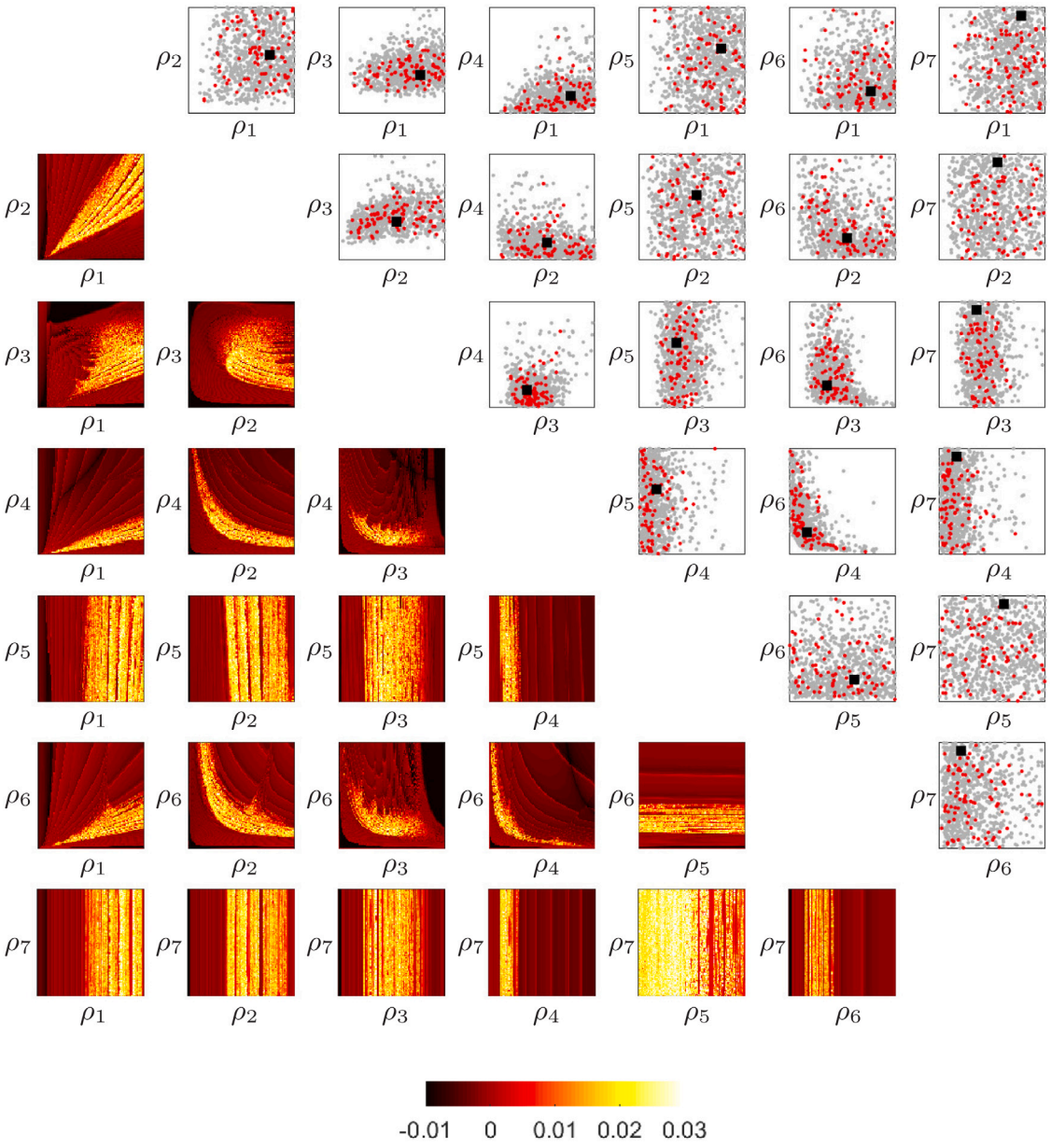

Fig. 9. Upper right panels: 2D slices of parameter space sampled by a Metropolis–Hastings algorithm seeking maximal values of the largest Lyapunov exponent (LLE) as we vary the parameters $\rho_1, \ldots, \rho_7$. We perform a simulation of 10^6 iterations. Grey points represent those points sampled that have positive LLE (approx 53% of those sampled). Red points are those in the top 5% when ordered by LLE (with LLE $\gtrsim 0.009$). Black squares indicate the single point of those sampled with the maximal LLE, namely: $\rho 1 = 30.95$, $\rho_2 = 21.94$, $\rho_3 = 14.25$, $\rho_4 = 6.50$, $\rho_5 = 24.41$, $\rho_6 = 8.29$ and $\rho_7 = 36.91$ (to two decimal places), with LLE = 0.036. Bottom left panels: the results of fixing baseline values of $\rho_1, \ldots, \rho_7$ at the aforementioned values, and then varying pairs of parameters ρ_i, ρ_j for $i, j \in [1, 7]$ with all other ρ-values fixed, with colours representing LLE. All plots are on the domain $[0, 40] \times [0, 40]$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

6. Discussion

Our study here has taken an existing model [5] of the inflammatory dynamics in hepatitis and examined how these dynamics are affected by the upscaling of key feedbacks due to platelet effects. Initially, we implemented a simplistic description of these platelet-driven effects by introducing a single scaling parameter, ρ, which uniformly magnifies the roles of neutrophil recruitment/apoptosis, macrophage recruitment and phenotype switching, inflammatory mediator production and activation of stellate cells in the presence of platelets. Our analysis revealed that increases in ρ can not only destabilise the steady state that corresponds to full resolution of inflammation (via (2)), but can also influence the nature of periodic solutions and give rise to chaotic outcomes via a period doubling cascade (Figs. 4–6). The existence of chaotic outcomes depends, intuitively, on a similar delicate balance of parameter values that controls the existence of periodic solutions in the original model (Fig. 7). In particular, the parameters ν_2 and k_m, which represent the rates of hepatocyte damage and pro-inflammatory mediator production by macrophages, play a strong role in bounding regions of chaos through controlling the locations of related Hopf bifurcations. For ν_2 or k_m sufficiently large, the magnitude of inflammatory damage is big enough that both fully-resolved (healthy) and chaotic solutions are eliminated, and the model is biased toward a chronic steady state. In addition, it is interesting to note that in previous models the parameter ϕ, which represents the rate of removal of apoptotic neutrophils by macrophages, has been implicated as one of the most dominant parameters in controlling the switch between bistability and guaranteed resolution of damage [5,13,14]. However, in the presence of platelets, this parameter has a much weaker role, with chaotic outcomes persisting for large ρ in areas of parameter space that would

have otherwise yielded healthy outcomes (see Figs. 2(b) and 7(b,e)). Given macrophages' key role in controlling inflammation, they are an attractive therapeutic target [16–18]; however, this weakening in their effect in the liver hints that a dual approach, alongside anti-platelet medication, may be useful in controlling hepatitis.

Having analysed the period-doubling route to chaos that platelet interactions provide in our simple model, we then modified our parameterisation of these platelet effects to allow relevant feedbacks to be stimulated on individual scales, in order to elucidate which of the various platelet-related mechanisms are responsible for facilitating chaotic dynamics. We hence introduced seven separate platelet scaling parameters $\rho_1, \ldots, \rho_7$ and analysed their individual roles. Through bifurcation analyses conducted for each individual ρ_i (Fig. 8) and a broader exploration of parameter space in which we use a Metropolis–Hastings algorithm to seek maximal Lyapunov exponents (Fig. 9), we concluded that the primary drivers of chaotic dynamics seem to relate to the actions of macrophages, with platelet-stimulated macrophage recruitment, phenotype switching and production of anti-inflammatory mediators resulting in Hopf and period-doubling bifurcations that trigger routes to chaos. The model is much less sensitive to stimulation of neutrophil-related interactions, although we have observed that increased rates of apoptotic neutrophil lysis, in particular, can exacerbate chaotic dynamics for some choices of macrophage-related parameters.

We note that the model offered here has a number of deficiencies. Platelets are known to have numerous effects on the cells involved in hepatitis [2]. While we have included those mechanisms that we think are most supported by literature, this is a fast evolving field and there are over one hundred different forms of hepatitis with evidence that platelet responses differ according to the type of damage and the form of hepatitis [2]. A model tailored to specific liver disease could help unravel some of these contradictions; however, we note that construction and validation of a sufficiently robust model requires a greater volume of experimental data than is currently available. The model presented here also omits any description of the dynamics of platelet production (thrombopoiesis) or clearance (a mechanism that is itself influenced by the liver [19]), instead focusing on a simple parameterisation of the downstream effects of platelets on inflammatory feedbacks. Platelets are produced by precursor cells called megakaryocytes that reside in the bone marrow, with the rate of platelet production being carefully controlled by relevant hormones, thrombopoietin in particular [20]. Reduced platelet counts (termed thrombocytopenia) are linked to liver disease [21] and dysregulation of thrombopoiesis has itself been previously linked with chaotic dynamics in platelet levels, not captured here [22–24]. Explicit coupling of thrombopoiesis dynamics to the hepatitic dynamics described here presents one possible target for future study. We note, in particular, that while our model assumes linear upscalings of these platelet-influenced feedbacks, a more detailed model of platelets themselves may reveal a more complex range of nonlinear responses than is described here. Furthermore, we note that the liver is highly structured with hepatocytes organised into lobules that are fed by a central unidirectional blood supply. It is conceivable, therefore, that spatial effects related to inflammation travelling between lobules could further complicate the dynamics observed here. Since our ODE model neglects such spatial effects, there is scope for future work to examine PDE or agent-based models that incorporate this spatial structure. We note that, in a more generic inflammatory context, the previous agent-based models of [25] have shown particular promise as regards careful calibration of relevant cell migration dynamics against experimental data; applying this approach to hepatitis remains an area of ongoing exploration.

While there remain many aspects of platelet dynamics that are not fully understood, both in isolation and in various disease scenarios, our model provides an illustration of just how complex platelet-driven dynamics can be. There is currently great interest in understanding not only platelet effects in the liver, but also how liver damage can influence platelet effects in a wider context, such as in thrombosis and wound healing. The hepatitis model presented here reveals the significant scope for modelling and dynamical systems analysis in unravelling these complex interactions, with the potential to elucidate some of the contradictory evidence in the current literature as is necessary in the ongoing hunt for new therapeutic interventions.

CRediT authorship contribution statement

Martin R. Nelson: Software, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Visualisation. **Jonathan M. Gibbins:** Conceptualization, Writing – original draft, Writing – review & editing. **Joanne L. Dunster:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Visualisation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: J.L. Dunster reports financial support was provided by British Heart Foundation. J.L. Dunster reports financial support was provided by Medical Research Council.

Data availability

No data was used for the research described in the article.

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