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### Understanding the human innate immune system

*In-silico studies*

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**Publication date**

2019

**Document Version**

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**License**

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**Citation for published version (APA):**

Presbitero, L. A. (2019). *Understanding the human innate immune system: In-silico studies*. [Thesis, fully internal, Universiteit van Amsterdam].

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# 3

## Detecting critical transitions in the human innate immune system

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*\*to be submitted*

# 3

## Detecting Critical Transitions in the Human Innate Immune System

### Abstract

**Background:** Coronary artery bypass grafting with cardiopulmonary bypass activates the human innate immune system and invokes a vigorous inflammatory response that is systemic. This massive inflammatory reaction can contribute to the development of postoperative complications that could topple the state of the system from the state of health to the state of disease or even death. The body, after all, is then in a state where the majority of its immune cell population is depleted, and sometimes needs days, or even longer, to recuperate. To gain deeper understanding on how the human innate immune system responds to complications after cardiac surgery, we perturb the immune system *in-silico* by adding another source of inflammation triggering moieties (ITMs) hours after surgery in various regimes.

**Results:** A critical transition occurs upon the addition of a critical concentration of ITMs when the insult is sustained for approximately 3 hours – a total concentration that in fact corresponds well to the fatal concentration of ITMs documented in literature.

**Conclusion:** By perturbing the human innate immune system model with additional sources of inflammation triggering moieties, we are able to

specify the conditions at which critical transitions occur in the human innate immune system. More importantly, we are able to pinpoint the critical concentration and duration of post-surgery insults that would drive the system into transitioning from the state of relative health to disease.

**Keywords:** human innate immune response, post-surgery complications, critical transitions, early warning signals

### 3.1. Introduction

Coronary artery bypass grafting (CABG) with cardiopulmonary bypass (CPB) provokes a systemic inflammatory response that activates the human innate immune system. The contact of the blood components with the artificial surface of the bypass circuit, thus inducing sheer stress on blood cells, ischemia-reperfusion injury due to the accumulated inflammation triggering moieties (ITMs) that have crossed the gut-barrier during hypoperfusion<sup>42</sup>, endotoxemia or the presence of endotoxins such as ITMs in the blood, and tissue damage caused by the surgical wound are all possible causes of systemic inflammatory response syndrome (SIRS). This massive inflammatory reaction may contribute to the development of postoperative complications such as myocardial dysfunction, respiratory failure, renal and neurologic dysfunction, bleeding disorders, altered liver function, and sequentially, multiple organ failure<sup>148</sup>. Postoperative respiratory failure has a mortality rate to 80% in of patients undergoing cardiac surgery<sup>149,150</sup>, taking into account that more than 800,000 patients per year undergo coronary artery bypass grafting (CABG) surgery worldwide while approximately 150,000 patients undergo valve surgery<sup>151,152</sup>. Myocardial dysfunction that escalates to symptomatic heart failure accounts for 50% of medical admissions to hospitals, and is associated with in-hospital mortality of 12% and a 1-year mortality of 20-35%<sup>153,154</sup>. More so, it was reported in the Society of Thoracic Surgeons National Database that 20% (22,000 patients) of “low-risk” patients developed postoperative complications.

Using the human innate immune response model that we have developed in an earlier work<sup>7</sup>, we show how the human innate immune system responds to complications after surgery by adding a source of ITMs (inflammation triggering moieties) *in-silico* hours after surgery. Inflammation triggering moieties may refer to any cell or enzyme that triggers the innate immune response, such as bacterial lipopolysaccharides (LPS) and extracellular nucleotides<sup>21-28</sup>. In case of a massive insult, the innate immune response

becomes amplified and dysregulated <sup>155</sup>, which leads to the imbalance between pro-inflammatory and anti-inflammatory cytokines <sup>156</sup>. By perturbing the system with different intensities of ITMs, we aim to test the resilience of the human innate immune system and assess at which point the system shifts between alternative regimes: from state of health to disease.

Various and diverse complex dynamical systems have been shown to exhibit transitions or so-called tipping points where there occurs an abrupt shift in stable states. In biological systems, such as the human body, this tipping point occurs as a rapid shift from state of health to disease <sup>157,158</sup>. In depression, fluctuations of emotions serve as indicators for tipping points from normal to the onset of a depressive state <sup>159</sup>. Other examples also include systemic market crashes observed in financial systems <sup>160,161</sup>, observed slowing down of fluctuations before a climate shift <sup>162-164</sup>, trends of a declining population prior to extinction <sup>165-167</sup>, blood parameters as indicators of tipping points in patients undergoing cardiac surgery <sup>168</sup>, and early warning systems in floods <sup>169-171</sup> and dams <sup>172</sup>.

Early warning signals (EWS) are hypothesized to serve as indicators of loss of system resilience prior to transitions between regimes. Subtle statistical properties of measurements in the system are assessed the presence of critical transitions <sup>173</sup>. Sometimes, these transitions are observed in changes in correlations, standard deviation, and skewness of system measurements through time <sup>15</sup>.

In this work, we perturb the human innate immune system by adding concentrations of ITMs hours after surgery *in-silico*. We show that the system shifts from the state of health to disease given a critical threshold of ITMs. We define healthy being the state where the human innate immune system is able to resolve all ITMs both in the bloodstream and tissue, while

deceased is when ITMs are not resolved within incubation time. We also assess whether EWSs are present prior to the shift from health to disease using the human innate immune system model. Finally, we also assess the capacity of early warning signals to detect critical transitions in the clinical trial data used to calibrate and validate the HIIIS model.

### 3.2. Metric and Model-Based Indicators

Early warning signals for detecting critical transitions in systems are divided into two categories: metric and model-based. Both methods aim to quantify the variations in correlation structure, and changes in variability in measurements prior to the system's transition between alternate regimes<sup>15</sup>.

Metric-based indicators aim to quantify changes in statistical properties of measurements without attempting to fit the measurements onto a model. We use *variance*, *skewness*, and *kurtosis* as metric-based indicators for transition from state of health to disease.

*Variance*. As the system approaches tipping point, it exhibits increasingly strong variations at measurements around the equilibrium. That is, the time it takes to return to equilibrium even after tiny perturbations strongly increases as the system approaches these points. This phenomenon is referred to as "critical slowing down," referring to how the system "slows down" going back to equilibrium<sup>174,175</sup>.

*Skewness*. Perturbations drive the state of the system to shift between alternate regimes. Critical slowing down, which refers to a decreasing return rate of the system towards equilibrium results in distribution asymmetry<sup>176</sup>. Hence skewness either increases or decreases depending on the direction of transition.

*Kurtosis*. Strong perturbations provokes the system to take on extreme values close to transition, increasing the occurrence of rare values in the measurements<sup>177</sup>. Therefore, an increase in *kurtosis*, or "bulging" is

observed in the measurements leading to a tipping point.

Model-based indicators quantify variations in measurements by fitting the data to a model. Autocorrelation is a simple method used to quantitatively describe slowing down in a system nearing a tipping point.

*Autocorrelation* is one of the simplest ways in measuring slowing down. Increasing autocorrelation implies that consecutive points in the time series have become increasingly similar <sup>178</sup>.

*Time-varying Autoregressive models (AR)* at time lag  $p$  is also one of the numerous methods used to estimate the local dynamics in measurements of a system <sup>179</sup>. The first step is calculating the inverse of the characteristic root ( $\lambda$ ), by estimating the autoregressive function. Values for  $\lambda$  that approaches 0 imply that the system quickly returns or stabilizes towards the mean. This is because we used a time lag equal to one, which indicates that the current value is based on the value immediately preceding it. Hence,  $\lambda$  would simply be the slope of change between two time points,  $y(t)$  and  $y(t - 1)$ . See the time-varying AR(1) model in equation (54). The smaller this slope is, the more similar the measurements are at time  $t - 1$  with  $t$ . Hence, it would be easier for the system to go back to equilibrium. On the other hand, when values for  $\lambda$  approach 1, measurements become increasingly varied hence implying instability.

$$y(t) = a(t)y(t - 1) + \varepsilon(t) \quad (54)$$

where  $a(t)$  corresponds to the autoregressive coefficient, and  $\varepsilon(t)$  corresponds to the environmental variability <sup>15</sup>.

### 3.3. Trend Detection

Any presence of increasing or decreasing trends, which should also be statistically significant, captured by early warning indicators can be evaluated using the Mann-Kendall trend test. The Mann-Kendall trend test is a non-parametric test that analyzes consistent increasing or decreasing

patterns in data series. The null hypothesis being that a monotonic trend does not exist, while the alternate hypothesis assumes the existence of a trend. These trends are tested to a significance level of 5%. We used a two-tail test. This means that we look into both positive and negative trends in values of early warning signals to be able to fully understand the system.

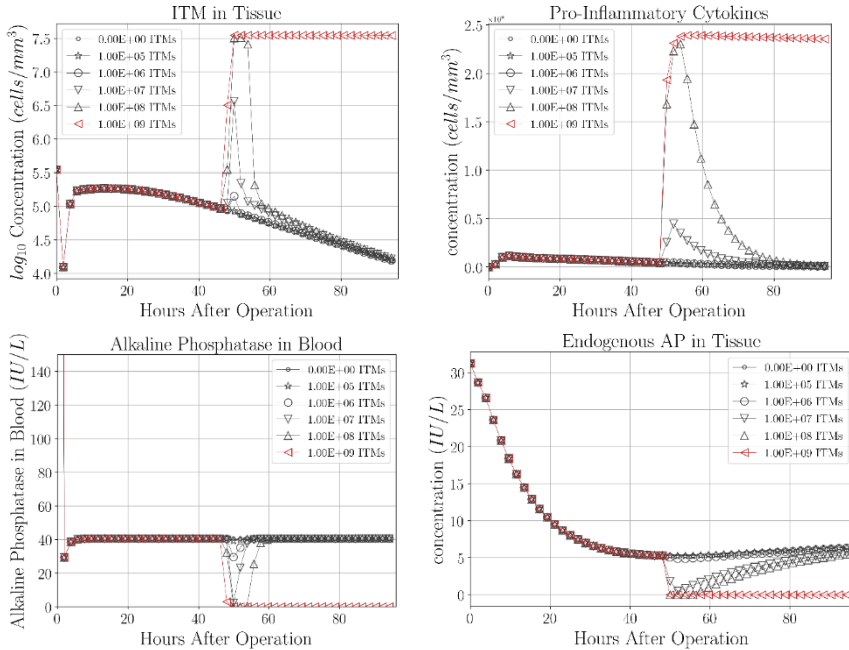
### **3.4. Effects of Adding Inflammation Triggering Moieties *In-Silico* to the Human Innate Immune System; 2 Hours After Surgery**

Complications sometimes happen 2 to 9 days after surgery<sup>180</sup>. In a study conducted by Hashemzadeh et al., the majority of complications, specifically postoperative atrial fibrillation, develop within the first 2 days after surgery<sup>181</sup>. Hence, in all our experiments, we add a source of ITMs that starts at 48 hours or 2 days after cardiac surgery. In this section, we present the results of various experiments that aim to understand how the human innate immune system shifts from a state of health to disease under various conditions.

Cardiac surgery with CABG activates the human innate immune system that invokes a vigorous response that most likely depletes the body's reservoir of immune cells, such as macrophages, neutrophils. Depending on the patient's conditions, it may take days, weeks or even months for immune cell levels to fully recuperate to normal levels. Nguyen et al., have shown that the activity of immune cells in cardiac surgery patients was impaired on the 3<sup>rd</sup> day post-surgery. These levels, however, returned to normal after a week after surgery<sup>182</sup>. Hence, the occurrence of complications post-operation becomes a serious threat as the body has not fully recovered yet.

In this section, we explore the effects of adding different concentration of ITMs *in-silico* 48 hours (2 days) after surgery for a duration of 3 hours. We picked 3 hours as this is the duration of insult that is typically observed in patients undergoing cardiac surgery before they stabilize back to normal

values, often 7 days after surgery<sup>182,183</sup>. Moreover, this duration of adding ITMs, as a matter of fact, tips the balance, pushing the state of the system from health to disease, which we will later show numerically in 3.8. These ITMs may come from complications from inflicted wound due to surgery, oxidative stress coming from various sources in the body, or external factors that invoke further production of ITMs. We summarize our results in Figure 27.



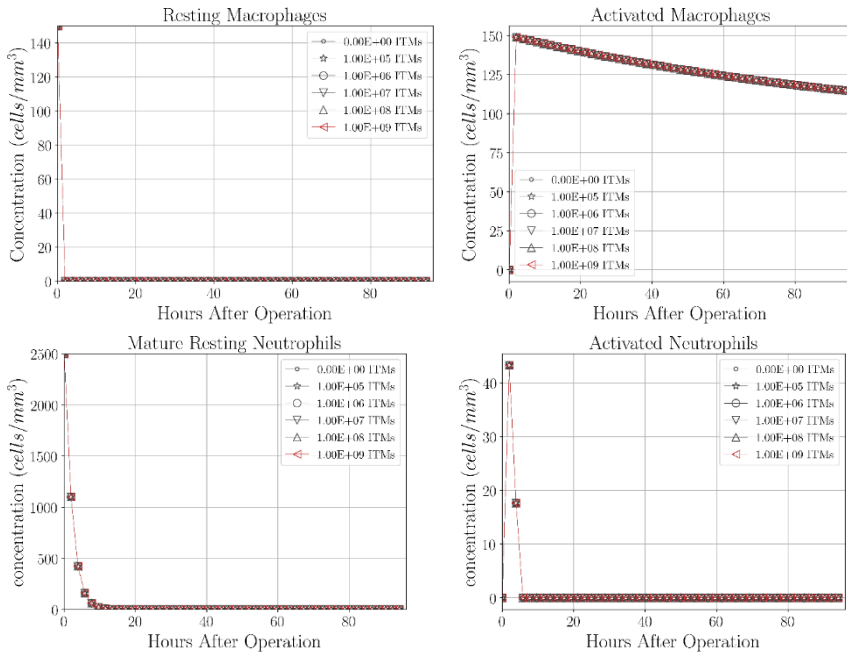
**Figure 27. Human Innate Immune Response to Post-Operative complications. Excess ITMs (inflammation triggering moieties) are continuously added for 3 hours in-silico at exactly 48 hours (3 days) after surgery.** Our results show that at an ITM concentration of  $1 \times 10^9 \text{ cells/mm}^3$ , the concentration of ITMs in the tissue remains unneutralized even after 96 hours of surgery. Compared to  $1 \times 10^8 \text{ cells/mm}^3$ , in this concentration the human innate immune system is able to completely neutralize the inflammation at 60 hours post-surgery. Pro-inflammatory cytokines, immune cells responsible for opening up the endothelial barrier to allow recruitment of more neutrophils from the bloodstream into the tissue, exhibit a saturation of concentration at added ITMs of  $1 \times 10^9 \text{ cells/mm}^3$ . Alkaline Phosphatase, enzymes known to neutralize ITMs, are depleted both in blood and tissue at added ITM concentration of  $1 \times 10^9 \text{ cells/mm}^3$ .

With additional ITM concentrations of  $1 \times 10^7 \text{ cells/mm}^3$ , we show in our

model that the remaining ITMs sharply decreases at 60 hours after surgery, implying that the body is still capable of neutralizing the additional amount of ITMs. However, we show a saturation of ITMs in the tissue when the added concentration of ITMs is at  $1 \times 10^8 \text{ cells/mm}^3$ . Pro-inflammatory cytokines are known for their function to open up the endothelial barrier, as a way to recruit new neutrophils into the site of inflammation. Here we show that the concentration of pro-inflammatory cytokines saturates to a steady level when the added concentration of ITMs is  $1 \times 10^8 \text{ cells/mm}^3$ . The same can be observed with concentrations of Alkaline Phosphatase, an enzyme known for its role in neutralizing ITMs. We show that Alkaline Phosphatase in blood and in tissue are depleted at such a high level of insult. However, when the concentration of added ITMs is  $1 \times 10^7 \text{ cells/mm}^3$ , pro-inflammatory cytokines level slides back to zero. This goes as well with Alkaline Phosphatase, which stabilizes back to normal levels of concentration roughly at 60 hours after surgery.

However, adding ITMs does not have any effect on the concentrations of resting and activated macrophages and neutrophils as shown in Figure 28. Even without adding another source of ITMs hours after surgery, resting macrophages and neutrophils have already been fully activated. Hence, adding another source of ITMs at a time when both types of immune cells have already been depleted will not significantly change the profiles of the immune cells as shown below.

Note that macrophages in our model also can go into homeostasis, but this rate is not fast enough to replenish macrophages even hours after surgery. Moreover, in the case of a massive insult such as that invoked by cardiac surgery, the bone marrow releases both mature and immature neutrophils into the bloodstream. This is the so-called “left shift,” which refers to the increase in the number of immature neutrophils in the bloodstream<sup>184</sup>. After which, it takes roughly a week for the bone marrow to release a new set of *mature* neutrophils into the bloodstream<sup>185–187</sup>.



**Figure 28. Resting and Activated Macrophages and Neutrophils' Response to Added Inflammation Triggering Moieties.** Even without additional ITMs, our model predicts the activation of all resting macrophages and neutrophils due to the scale of insult cardiac surgery with CABG invokes on the human innate immune system. Therefore, additional source of ITMs, especially when the immune cells are already depleted, will still invoke the maximum effect on macrophages and neutrophils.

### 3.5. Detecting the Critical Concentration of ITMs that Lead to the Shift of System State from Health to Disease

In the previous section, we have shown how the human innate immune system responds to added concentrations of ITMs hours after surgery. We also observed the saturation of concentrations of ITMs in tissue, pro-inflammatory cytokines and Alkaline Phosphatase in tissue and blood, where concentrations remain at a certain level that biologically does not correspond to stability or homeostasis.

In this section, we pinpoint the *critical* concentration of ITMs that is the

tipping point that shifts the state of the body from health to disease. The state of health simply corresponds to the situation where the body is capable of fully neutralizing the added ITMs post-surgery. The disease state, on the other hand, corresponds to when concentration of ITMs saturate to a high level of ITM value, and the body is no longer able to neutralize these ITMs fully.

We use the coefficient of variation as a simple metric to quantify how blood parameter concentrations vary as we increase the intensity of post-operative complications. Our results are summarized in Figure 29.

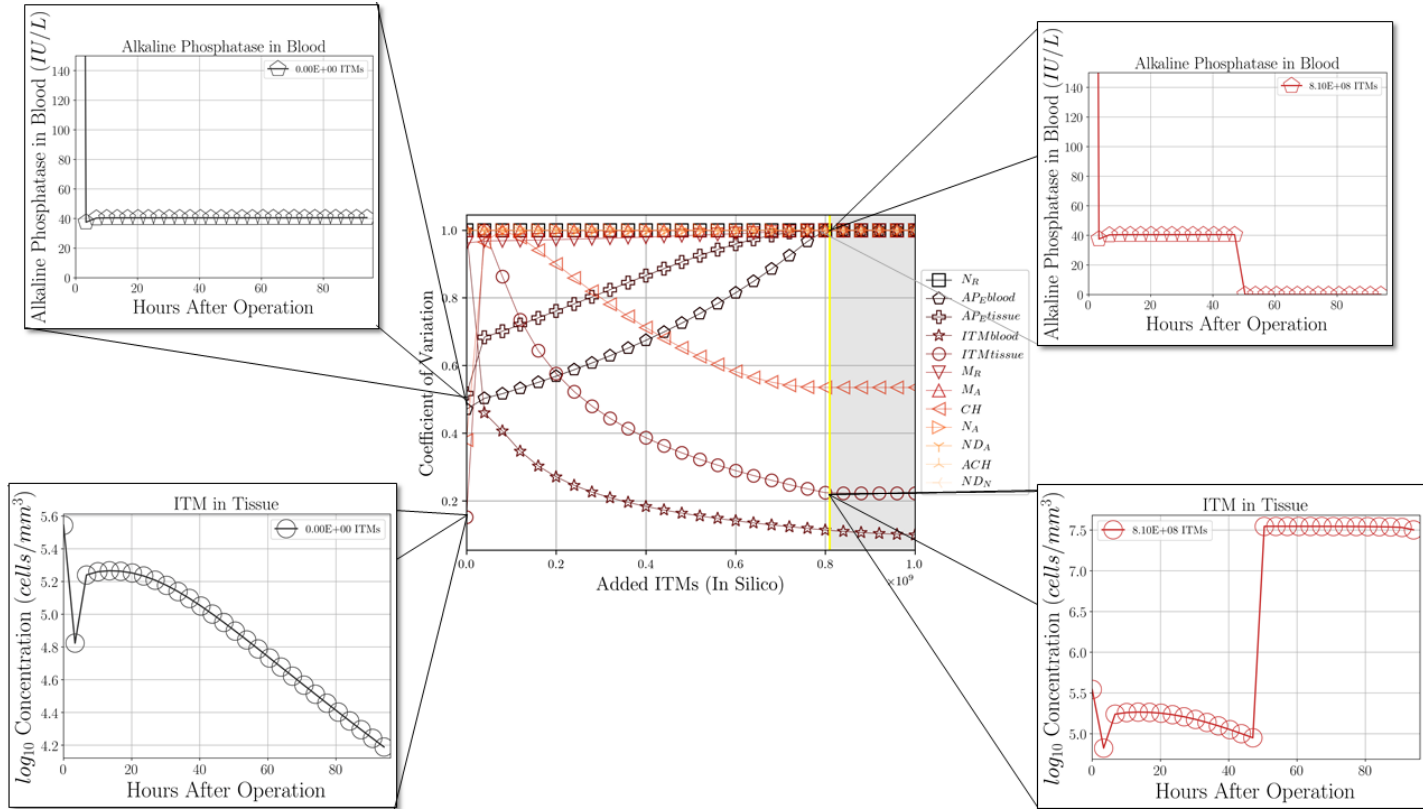


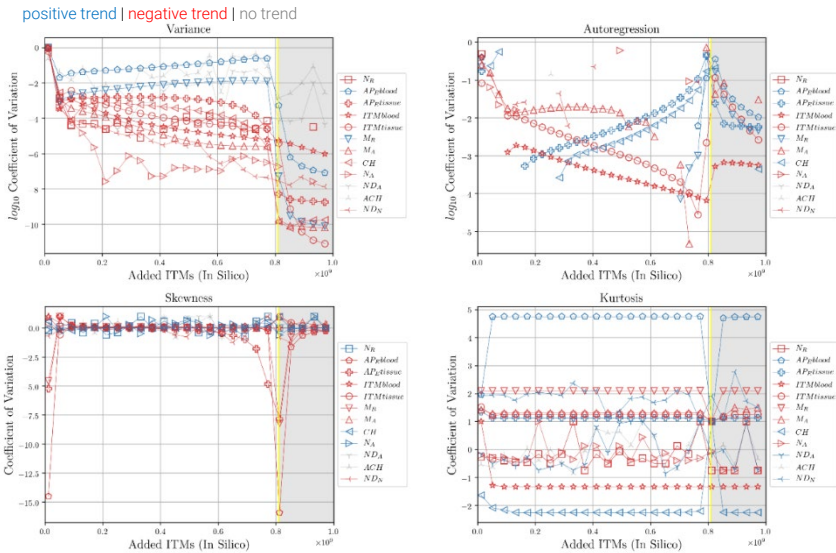
Figure 29. Normalized Coefficient of Variation for Blood Parameters in the Human Innate Immune System. Highlighted are concentrations for Alkaline Phosphatase in Blood and Inflammation Triggering Moieties in Tissue when the concentration of added ITMs are 0 (black plots on the left panel) and  $8.1 \times 10^8 \text{ cells/mm}^3$  (red plots on the right panel). Each point in the normalized coefficient of variation plot corresponds to the calculated coefficient of variation of a single time series.

The coefficient of variation is simply the ratio of the standard deviation with respect to the mean. This means that the higher the coefficient of variation is, the greater the level of dispersion is around the mean. Note that we have normalized the coefficient of variations in Figure 29 to be able to compare the trends across various blood parameters. Here we show that concentrations for pro-inflammatory cytokines ( $CH$ ), ITMs in tissue ( $ITM_{tissue}$ ) as well as in blood ( $ITM_{blood}$ ) exhibit decreasing coefficients of variation as the scale of post-operative complication gets more intense. This is because when the scale of insult post-surgery is not intense, hence unthreatening, the body is able to neutralize the ITMs that bring the concentrations of ITMs and pro-inflammatory cytokines back to stable values. The calculated mean therefore sticks close to the stable values despite the peak caused by the added insult. However, if the scale of insult post-surgery is too intense that the human innate immune system is not able to neutralize the threat, concentrations of ITMs in the blood and tissue as well as pro-inflammatory cytokines peaks at the onset of insult (2 hours after surgery) and saturates to this value. The mean, therefore, of these distributions are higher than those of non-threatening levels of added ITMs. Hence, we see lower values for coefficient of variation as the scale of insult intensifies.

What is interesting here is that it appears that the coefficient of variations for some blood parameters saturates at a stable value beyond  $8.1 \times 10^8 \text{ cells/mm}^3$ , as highlighted by the yellow vertical line in Figure 29. This means that the ratio between the standard deviation and mean of the blood parameters' distributions remain constant, hence the level of dispersion around the mean seems to stabilize beyond this point, showing that beyond  $8.1 \times 10^8 \text{ cells/mm}^3$ , the human innate immune system can no longer neutralize ITMs, hence the concentrations of blood parameters remain stable. Therefore, here lies the critical transition that separates the state of health from the state of disease.

We regard the measurements of coefficient of variation of each blood

parameter profile as measurements describing a system that can shift to alternate regimes with added ITMs as the driving force of the transition. We know, based on the results we presented in Figure 29, that the system undergoes a critical transition from health to demise at added ITM concentration of about  $8.1 \times 10^8 \text{ cells/mm}^3$ . In order to obtain a deeper understanding of the system and the changes the system goes through *before* and *after* a critical transition, we utilize early warning signals and assess their capability of predicting the onset of critical transition prior to shifting between alternate regimes. We summarize our results in Figure 30.



**Figure 30. Detecting Critical Transitions on Coefficient of Variation.** Early warning signals are able to detect increasing (shown in blue) and decreasing (shown in red) to a significance level of 5% using the Mann-Kendall two-tailed trend test for almost all blood parameters except for apoptotic neutrophils ( $ND_A$ ) and anti-inflammatory cytokines ( $ACH$ ).

Here we show that almost *all* blood parameters exhibit a significant increase (blue) or decrease (red) in trends for early warning signals of autoregression, skewness, and variance, except for concentrations of apoptotic neutrophils ( $ND_A$ ) and anti-inflammatory cytokines ( $ACH$ ). Due to the massive scale of insult, adding more ITMs into the system leads to majority, if not all, of the

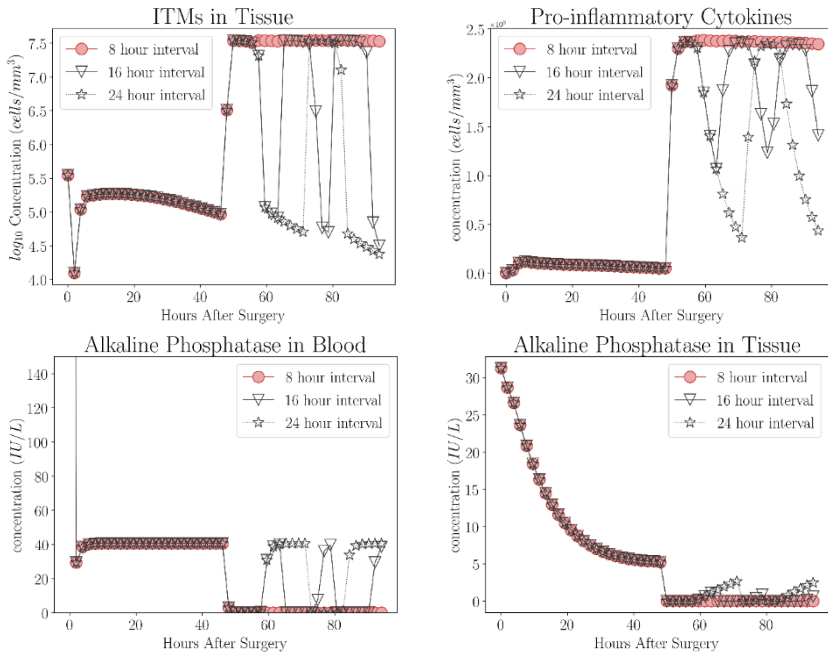
activated neutrophils going into necrosis<sup>6</sup>, instead of apoptosis. This was also shown in an early work where we modeled the emergence of necrosis and apoptosis through various levels of insult using game theory<sup>89</sup>. Consequently, since pro-inflammatory cytokines are released by activated macrophages ( $M_A$ ) upon removal of apoptotic neutrophils, pro-inflammatory cytokines also exhibit the same profile as apoptotic neutrophils.

### **3.6. How does the Human Innate Immune System Respond to Persistent and Recurrent Episodes of Post-Surgery Complications**

What we have shown so far is one possible scenario of when the innate immune system transitions from a state of health demise. In this section, we further explore how the human innate immune system responds to complications that are either persistent or recurring.

### **3.7. Effects of Adding Inflammation Triggering Moieties *In-Silico* at Different Time Intervals**

In order to obtain a deeper understanding on how the human innate immune system responds to post-surgery complications, more so, in response to complications that are persistent and recurrent, we introduce an additional source of ITMs at various regimes or intervals: 8 hour, 16 hours, and 24 hours intervals. The concentration of ITMs is continuously added for 30 minutes to mimic those complications that are persistent.



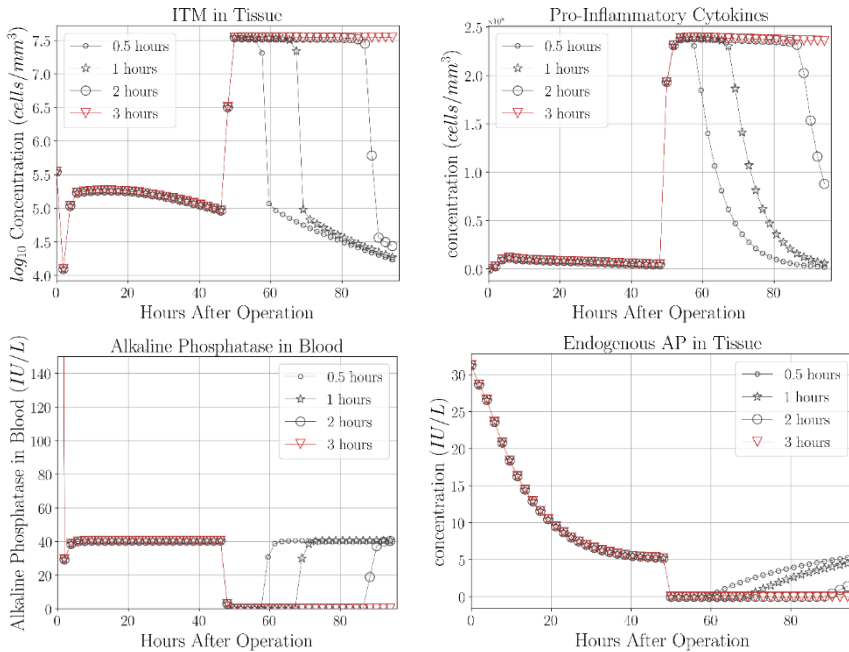
**Figure 31. Human Innate Immune Response to Additional Sources of Inflammation Triggering moieties at Varying Time Intervals.** A non-fatal concentration of  $1 \times 10^9$  cells/mm<sup>3</sup> ITMs<sup>53</sup> was added at different time intervals starting at 2 days (48 hours) after surgery continuously for 30 minutes to mimic a persistent and recurring post-surgery complication. Our results show that when the interval between each episode decreases to 8 hours, the system undergoes a transition where it is no longer able to neutralize the ITMs effectively. Hence, we see that the ITMs in the tissue remain at a stable concentration because the remaining population of immune cells are no longer able to neutralize the ITMs. Moreover, more pro-inflammatory cytokines are induced due to the intense scale of insult.

Our results show that recurrent episodes of post-surgery complications can lead to disease, or in worse cases, death, when the intervals between episodes are as close as 8 hours and when the insult is sustained for 30 minutes.

### 3.8. Effects of Adding Inflammation Triggering Moieties *In-Silico* at Different Time Range

In order to mimic post-surgery complications that are persistent, we added ITMs in various durations starting from 30 minutes of continuous infusion,

to 1 hour, 2 hours and finally 3 hours. Our results are summarized in Figure 32.



**Figure 32. Human Innate Immune Response to Added ITMs at Different Time Durations.** In order to mimic a persistent type of post-operation complication, we extend the duration at which ITMs are added from 0.5 hours, 1 hour, 2 hours and 3 hours. Our results show that at 3 hours, the system is no longer able to neutralize the inflammation. That is, concentrations of ITMs in the tissue as well as concentration of pro-inflammatory cytokines remain at high value. Alkaline phosphatase in blood and tissue, however, are depleted.

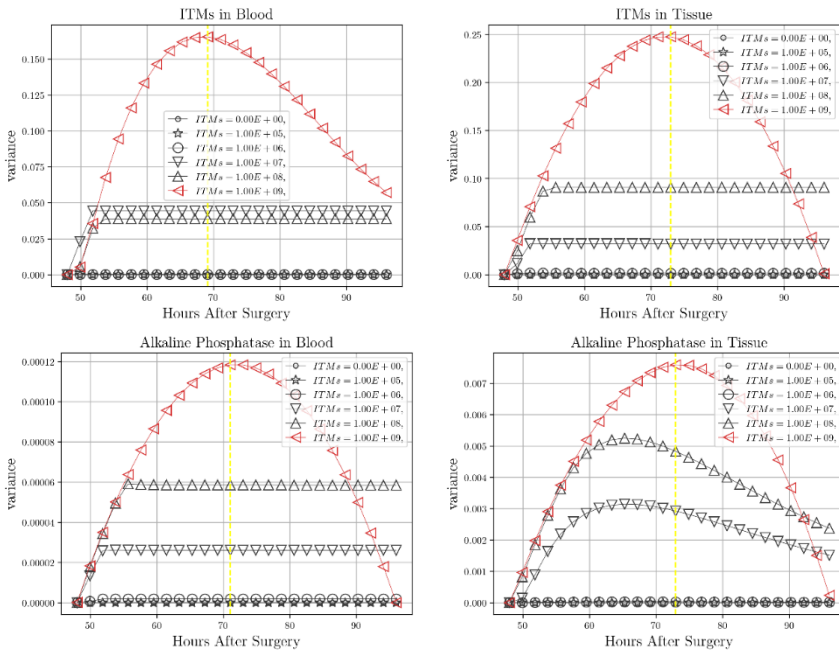
Prolonging the duration of added ITMs in the system has prominent effects on the ITMs in tissue, pro-inflammatory cytokines, and alkaline phosphatase concentrations in blood and in tissue. As the body recuperates after cardiac surgery, there comes a point when the system can no longer neutralize the inflammation. We have shown in the previous section that recurrent episodes of post-surgery complications could tip the balance between health and disease when the time interval reaches 8 hours apart. In this section, we show that this critical transition happens when the post-surgery complication is persistent and lasts for 3 hours. This is in fact consistent

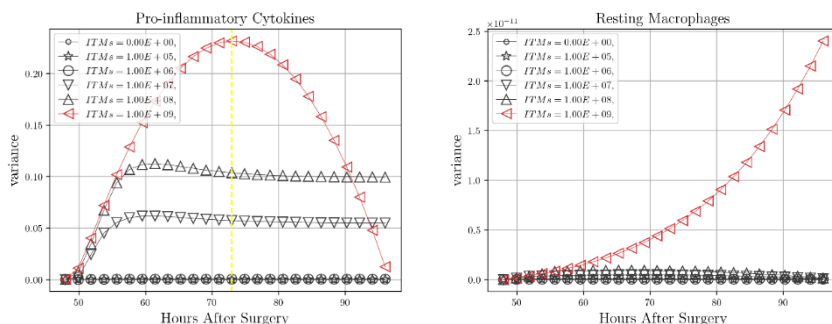
with the findings of Damas et al., where the overall concentration of ITMs within this 3-hour duration corresponds to the fatal concentration of ITMs in humans<sup>53</sup>.

### 3.9. Do Blood Parameters Exhibit Characteristics of Early Warning Signals Before a Critical Transition?

#### *Variance as an Early Warning Signal*

Variance aims to measure whether the system is critically slowing down. More specifically, a rising variance is known to indicate a critical transition. By using a rolling window of half the size of the measurements (48 hours). We summarize our results in Figure 33.





**Figure 33. Variance as an Early Warning Signal to Detect Critical Transitions in the Human Innate Immune System.** The vertical yellow lines designate the highest points of the variance where supposedly, the critical transition happens.

Highlighted in red are the measurements for variance when the added ITMs is  $1 \times 10^9 \text{ cells/mm}^3$ . Profiles for ITMs, Alkaline Phosphatase in blood and in tissue, as well as pro-inflammatory cytokines and resting macrophages show remarkably different profiles from lower concentration of ITMs that we have added in-silico. The differences between the effects of adding increasing concentrations of ITMs on blood parameters is apparent at approximately time point 55 hours after surgery and peaks at around 70 hours after surgery.

As critical transition can be observed in as early as within a 4 hour-time frame (4 hours after the onset of a post-surgery complication) in ITMs, 7 hour-time frame pro-inflammatory cytokines and a 10-hur timeframe in alkaline phosphatase and resting macrophages. These results imply the urgency of monitoring closely the change in concentrations of indicated blood parameters (ITMs in blood and tissue, alkaline phosphatase in blood and tissue, pro-inflammatory cytokines and resting macrophages) within these timeframes because within these hours, a critical transition is most likely to occur especially when dealing with post-surgery complication that is intense.

## Autoregression as an Early Warning Signal

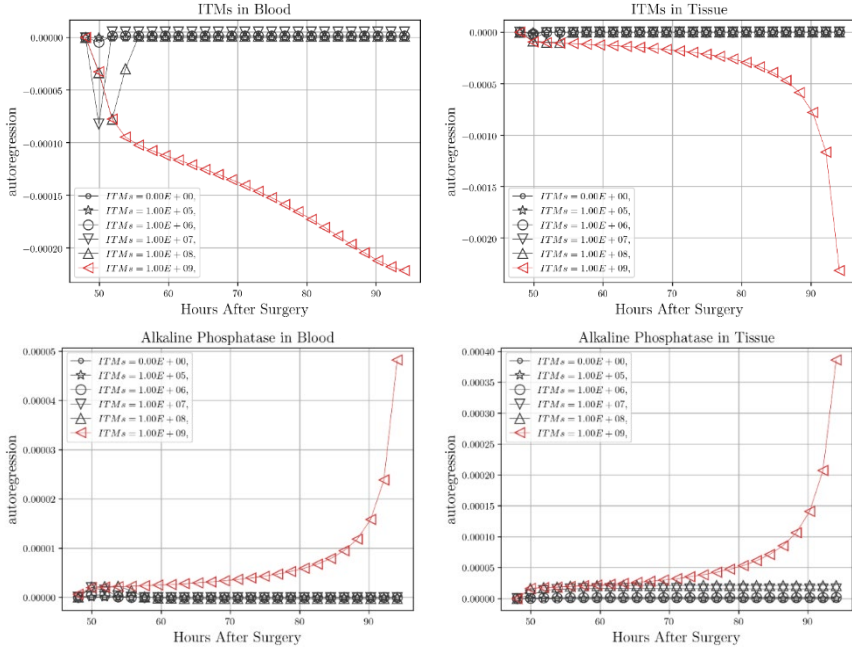


Figure 34. Autoregression as an Early Warning Signal for Detecting Critical Transitions in the Human Innate Immune Response.

By using autoregression, on the other hand, we are also able to detect critical transition for as early as within the 4-hour timeframe after the onset of post-surgery complication in ITMs in blood. However, autoregression shows a critical timeframe that is much later than what is observed when using variance as an indicator of critical transition.

Our results suggest that in order to prevent the possible fatal complication in patients undergoing cardiac surgery, medical practitioners would need to inspect the trends in blood parameters, with a special focus on ITMs and alkaline phosphatase in blood and tissue in as early as within a 4, 7 or 10-hour timeframe after an observed onset of post-surgery complication depending on the blood parameter under scrutiny.

We note that although early warning signals provide ways in detecting the onset of critical transitions occurring in various systems even before this transition happens, this is not the case for the human innate immune system model. The dynamics of the blood parameters, meaning how in some cases the concentrations of blood parameters go back to stable values, can be observed by just looking at the plots themselves. However, using early warning signals make it possible to enhance the main difference in the profiles of blood parameters that exhibit critical transitions and those do not. In this way, it is easier for medical practitioners to spot the critical timeframes at which the system shifts from health to disease.

### **3.10. Critical Transitions in Blood Parameter Timeseries of Patients Undergoing Cardiac Surgery**

The clinical trials data used to calibrate and validate the human innate immune system model in Chapter 1 revealed that 3 out of 53 patients who have undergone cardiac surgery died after surgery. In this line of work, we utilize early warning signals to segregate critical patients, or patients who died after surgery, from non-critical patients with the assumption that critical patients exhibit changes in patterns in their time series when a critical transition from health to diseases occurs. However, we do not detect how much time these transitions occur in advance, and this will be the future direction of this research because the most important, and useful motivation of using early warning signals is its potential of real-time use as early warning for increased risk of patient death, with eventually the goal of improved prevention. This subchapter is based on an earlier version of a work that was presented in a conference <sup>168</sup>. The results presented are based on a preliminary work and still needs further refinements to perhaps, incorporate, the functionality of being able to detect how early in the time series the critical transitions occur. What will be presented below is a re-assessment of the original time series of clinical trials data.

### **3.11. Data Preparation and Analysis**

The raw data is composed of concentrations of 43 various blood parameters sampled from 53 patients who are undergoing cardiac surgery with bypass filter. The time stamps at which the samples were taken were also recorded and indicated in the data. Patients who are involved in the clinical trials are either males or non-pregnant and non-lactating females regardless of race with ages above 18. The data was collected from two separate hospitals: Catharina Hospital Eindhoven (The Netherlands), and Zuid Oost-Limburg Hospital (Belgium). The conditions at which the patients have undergone, methods used to obtain the blood parameter samples, as well as time intervals for the data collection were standardized between the hospitals. A more detailed description of the population of patients can be found in **Chapter 1 A.1**.

The blood parameter concentrations were collected within an interval of 24 hours prior to surgery up until 30 days after surgery. More samples of blood parameter concentrations were done within the first 24 hours after surgery as an attempt to closely monitor the patients. This is because complications are most likely to occur within this 24-hour period post-surgery.

The raw data contains a huge amount of missing data points (58.7 %) because not all blood parameters are sampled. For instance, at the onset of surgery, patient 35 was only sampled for Alkaline Phosphatase and not for basophils. Missing values are inevitable in clinical trial data, so it is necessary that the methods used are capable of handling this type of situation. Numerous techniques deal with missing values. But what is important is that whatever these techniques may be, they should not significantly increase the rate at which false negatives are being detected. That is, labeling critical patients as healthy. In this case, the signal is rendered noisy and makes it impractical for medical practitioners to act upon.

### **Data Preparation**

Missing values are dealt with by using a simple technique called *bootstrapping*. The basic idea behind bootstrapping involves a repeated random sampling with replacement from the original data to come up with random samples (or bootstrap samples) that have the same size as the original data. Each measurement can be sampled more than once. We resampled 100 times to ensure variability in the bootstrap samples.

### **Evaluation of Metric and Model-Based Indicators**

We assess the performance of early warning signals in detecting critical and non-critical patients by calculating the F1 score based on outcomes of the detection based on the definitions summarized in Table 5.

Table 5. Definition of terms used for assigning critical and non-critical patients.

<i>Symbol</i>	<i>Interpretation</i>	<i>Definition</i>
$T_P$	True Positive	assigning critical patients as critical
$F_P$	False Positive	assigning non-critical patients as critical
$F_N$	False Negative	assigning critical patients as non-critical
$T_N$	True Negative	assigning non-critical patients as non-critical

The F1 score is calculated based on equation (55):

$$F_1 = 2 \frac{P \cdot R}{P + R} \quad (55)$$

Where  $P$  corresponds to precision and provides a measure or percentage of the results that are relevant. It is formally defined as in equation (56).

$$P = \frac{T_P}{T_P + F_P} \quad (56)$$

While  $R$  is the so-called Recall that measures the fraction of relevant instances retrieved or what percentage of the actual number of critical

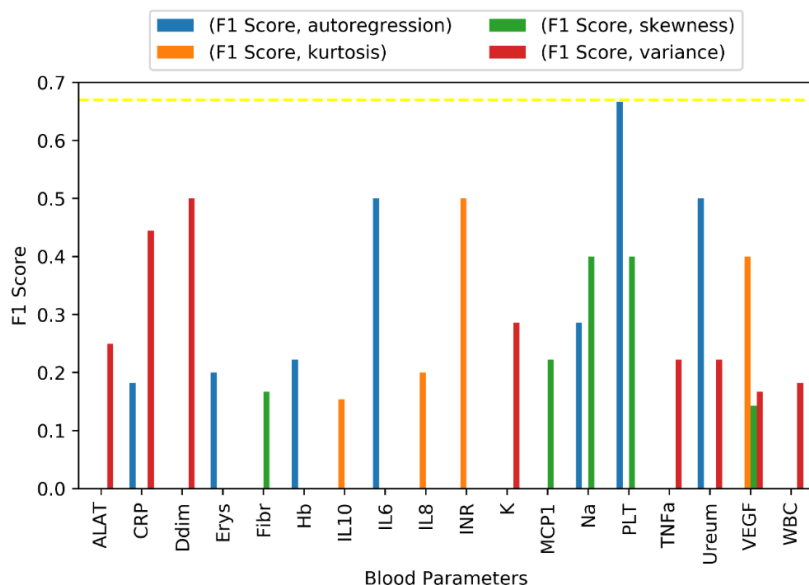
patients are correctly identified by the methods.

$$R = \frac{T_P}{T_P + F_N} \quad (57)$$

Having high precision implies the unlikely occurrence of labeling non-critical patients as critical. High recall, on the other hand, refers to the unlikely occurrence of labeling critical patients as non-critical. A system that has high precision but low recall reports less number of critical patients, but most of these critical patients are labeled correctly. A system having low precision but high recall, on the other hand, reports more critical patients, but most of the detected critical patients are wrongly identified because most of them are, in fact, non-critical.

### **3.12. Using Early Warning Signals to Pinpoint Blood Parameter Markers of Death**

Each time series corresponding to a timely record of a patient's concentrations of blood parameter is assessed on whether a critical transition is detected or not using early warning signals. This is done by using a rolling window of half the size of the data for each methodology for early warning signals. The Mann Kendall trend test is then used to test the presence of a significant increasing or decreasing trend. The results are evaluated by calculating for the F1 scores per blood parameter. The motivation here is to pinpoint blood parameters that may be the best option for medical practitioners to focus on, as opposed to doing an extensive scan on all blood parameters that in fact do not reveal signs of critical transitions in patients at all. In this way, resources as well as time are wisely conserved and patients, who are prone to criticalities, can readily be given the immediate treatment they need. We processed both bootstrapped and original data, but the results of our simulations are similar for both. These results are summarized in Figure 35.



**Figure 35. F1 Score of model output after using early warning signals in detecting critical and non-critical patients.** The highest F1 score corresponds to PLT or platelet counts when using the autoregression as an early warning signal. This is followed by Ddim (D-dimer for assessing the presence of blood clots), IL6 (pro-inflammatory cytokine), INR (used to determine the clotting of blood), and Uream (amount of urea nitrogen found in blood).

The blood parameter with the highest F1 score corresponds to PLT or the number of platelets in the blood using autoregression. This is followed by Ddim (D-dimer for assessing the presence of blood clots), IL6 (pro-inflammatory cytokine), INR (used to determine the level of clotting of blood), and Uream (amount of urea nitrogen found in blood) that corresponds to either autoregression, variance or kurtosis.

PLT, D-dimer, and INR are parameters used to evaluate the risk of bleeding in patients. Post-operative bleeding poses risks in patients as it remains a big problem after cardiopulmonary bypass. As a matter of fact, Tettet et al. have shown the platelet counts are significant predictors of post-operative bleeding<sup>16</sup>. Our results reveal that platelet count could be the most

significant marker in detecting criticalities or death in patients undergoing cardiac surgery. More so, an abundant amount of IL6, or pro-inflammatory cytokines, in the blood could reveal the magnitude of inflammation the patient is undergoing as IL6 is a potentially useful marker of the human innate immune system activation. An elevated urea in the blood could mean that the kidneys or liver are not functioning well.

### 3.13. Summary and Conclusion

Using the model of the human innate immune response for patients undergoing cardiac surgery, we show how the human innate immune system responds to complications that occur post-surgery by adding inflammation triggering moieties *in-silico* at 48 hours (2 days) after surgery. We show that there exists a critical transition from health to disease when the added concentration of ITMs is at  $8.1 \times 10^8 \text{ cells/mm}^3$ . This transition between alternate regimes can be detected in 10 out of 12 key players in the human innate immune system using early warning signals namely variance, autoregression, skewness, and kurtosis. We are able to pinpoint important blood parameters that exhibit critical transitions, implying that medical practitioners would need only to focus on the dynamics of *inflammation triggering moieties* and *alkaline phosphatase in blood and tissue* within a 4, 7 and 10-hour timeframe from the onset of post-clinical complication. We also show that a continuous insult of  $1 \times 10^9 \text{ cells/mm}^3$  ITMs that last for 3 hours leads to the shifting of the system between alternate states, which corresponds to the fatal concentration of ITMs shown in literature.

We also used early warning signals to detect the presence or absence of critical transitions in clinical trials data of patients undergoing cardiac surgery. By using early warning signals, we are able to pinpoint blood parameter markers that reveal significant presence of critical transitions, where the most significant is the platelet count.