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Finding Intervention Points in the Pathogenesis of Dengue Viral Infection

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***Abstract*- We use probabilistic boolean networks to simulate the pathogenesis of Dengue Hemorraghic Fever (DHF). Based on Chaturvedi's work, the strength of cytokine influences are modeled stochastically as inducement probabilities. We use an aggregated function approach to derive the DHF Infection Model. Two basins of attractors are observed with synchronous updating; the Null Infection cycle attractor shows an expected cross-regulation of Th1 and Th2 cytokines corresponding to the homeostasis of an uninfected person, while the DHF Infection attractor shows the onset of DHF. With asynchronous updating, our model remains valid with clinical comparisons against qualitative changes in signal durations. In order to find intervention points that could prevent DHF, we design a genetic algorithm to shift the DHF attractor to the DF attractor basin by using the DF final state as the fitness measure. Our simulation results identify TGF-β, IL-8 and IL-13 as the intervention points which are consistent with known clinical results to prevent DHF from occurring.**

I. Introduction

The dengue virus is transmitted through the bite of the aedes aegypti/albopictus mosquito, which is also a natural host for the dengue virus, apart from humans. Today, almost 2.5 billion people are under threat from the dengue virus and 100 million people are said to be infected with the Dengue Virus annually. Infection with the dengue virus may lead to Dengue Fever (DF) or the potentially fatal Dengue Hemorrhagic Fever (DHF). DHF may be accompanied with shocks, which is termed as Dengue Shock Syndrome (DSS). DHF proves fatal in 5% of cases while DSS is fatal in an alarming 40% of its reported cases. Patients with DHF have increased vascular permeability and abnormal hemostasis. This can cause the individual to lose blood volume, result in hypotension, go into shock and die. Researchers [1][2] have hypothesized that the synergistic effect of the cytokine cascade which shifts a Th1 to a Th2 type immune response causes the said inflammatory characteristics of DHF. Understanding how and when the dynamics of this cytokine cascade will progress to DHF is vital if we are to find a way to defeat the pathogen [3]. In recent years, researchers have used computer models [4-6] to simulate how the immune system responds to viral infections, both at the intra- and extra-host population levels. Originally developed by Stuart Kauffman [7], the Boolean Network is a mathematical tool that is popularly used for modeling genetic regulatory networks. Much research [8-10] describes the mathematical implications as well as the dynamics that it exhibits.

This paper is organized as follows. Section 2 will explain the pathogenesis of dengue viral infection and introduce the use of random boolean network models. Section 3 presents the simulation toolkit developed to simulate the dynamics of the DHF cytokine cascade and the effects of synchronous and asynchronous updating. Section 4 presents a novel probabilistic boolean network model which uses function aggregation in its representation. Section 5 verifies our Null and DHF Infection Models with simulation and clinical results. Section 6 presents a Genetic Algorithm to find intervention points and results are given in Section 7.

II. Characterizing Dengue Viral Infection with Boolean Networks

We use a Probabilistic Boolean Network [11][12] (PBN) model of the cytokine cascade and regulation in the disease progression of dengue viral infection. Cytokines are a group of molecules produced by leucocytes (white blood cells) for signalling between cells of the immune system [5]. The levels of such signals change over the course of infection. Each of these cytokine subtypes has a regulatory effect on each other, causing an increase or decrease in chemical production. While certain specific inhibitors or catalysts have been identified through biological testing, the collective effect of these cytokines interacting together is still not fully understood; hence, a model to simulate these interactions to find emergent patterns would be instructive.

A. What causes Dengue Hemorrhagic Fever?

The main difference between cytokine regulatory networks and genetic regulatory networks (where boolean network models are typically used) is that the latter models intra-cell interactions while the former models inter-cell interactions. Most immunological ODE-based models focus on cell population levels and assume at best, homogenous signaling pathways for intercellular communication. While this may be sufficient to explain some stages in viral infections such as HIV, it is in contrast the hypothesized underlying mechanism that is believed to cause DHF.

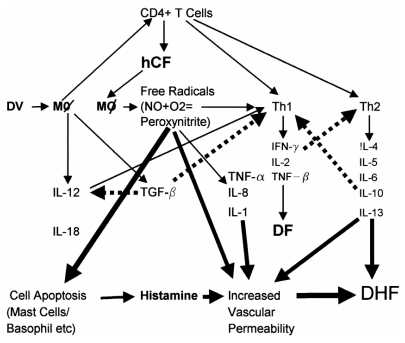


Figure 2.1 DHF Cytokine Cascade (adopted from [1])

The main feature of DHF is the early generation of a unique cytokine called human cytotoxic factor (hCF) that initiates a series of events leading to a shift from Th1-type response to Th2-type response. The dengue virus induces the activated T-helper (Th) cells to produce hCF that in turn induces macrophages (M∅) to produce free radicals such as nitrate, reactive oxygen and peroxynitrite. These free radicals will kill target cells by apoptosis and at the same time upregulate the production of pro-inflammatory cytokines IL-1β, TNF-α, IL-8 and hydrogen peroxide in macrophages. Changes in IL-12 and TGF-β will push a Th-1 dominant response to a Th-2 biased response, resulting in exacerbation of the dengue disease. The increased vascular permeability results due to the combined effect of histamine, free radicals and pro-inflammatory cytokines (in secondary infections, the presence of activated memory Th cells actually aids in the cause of DHF). Figure 2.1 illustrates these interactions. The thin lines describe positive induction, dashed lines as inhibition and thick lines as damaging effect. We will subsequently refer to Figure 2.1 as the **DHF Cytokine Cascade**.

B. Modeling with Boolean Networks

We define a boolean network G, where the set of nodes N(G) represents individual cytokines that are known to be significant in the DHF Cytokine Cascade. The edge set E(G) represents directed chemical influences that cytokines have on each other via some intermediate cell or cells (as some cytokines may be produced by more than one type of cell), which we omit from N(G) so as to obtain a pure cytokine-based communication network. Each edge $x \rightarrow y$ in G represents a causal relationship between cytokine x and y, and a boolean function for y will compute its expected likelihood of being assigned a state of 1 or 0 (an ON or OFF state) given all the incident nodes (including reflexive effects). Figure 2.2 gives the resulting **DHF Infection Model** constructed based on the DHF Cytokine Cascade in Figure 2.1.

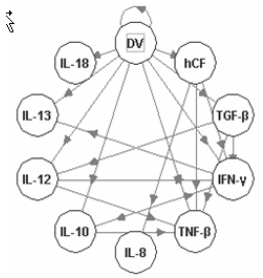


Figure 2.2 DHF Infection Model

In order to verify that the DHF Infection Model does correspond to a homeostasis in the absence of DV, we would require simulation results from a null version, and compare these with clinical data. If these results are accurate, then the subsequent introduction of DV would produce justifiable results. The null version of the DHF Infection Model is obtained through the self-loop at the node DV. This reflexive influence (the only incoming edge) can be either 0 or 1, allowing us to selectively simulate either the **DHF Null Model** or the DHF Infection Model.

III. BNet - A Boolean Network Simulation Tool

We have developed a Java-based toolkit called **BNet** to simulate and visualize the dynamics arising from prescribed boolean network models. Using BNet, we can encode cytokine cascades with various configurations of boolean networks and explore the resulting dynamics. Examples include the Classical Random Boolean Network (CRBN) [13][7] and the Asychronous Random Boolean Network (ARBN) [14]. Users are able to create Boolean networks of arbitrary order, size and node functionalities and view its graph representation, its runtime trajectory and basin diagrams. In the subsections that follow, we will describe the various configurations provided and the implications of using each with regards to the simulation of the DHF cytokine cascade.

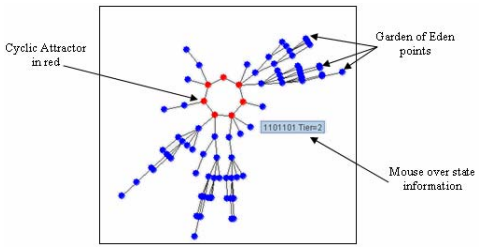


Figure 3.1 Example of a Basin Diagram for a 7-Node Boolean Network using Bnet

There are three modules that comprise the BNet toolkit. The **Single State Module** handles the creation of a boolean network and simulation of the trajectory path that it takes. The **Attractor Module** analyzes the network's basin and the attractors by which states will converge to. This convergence represents the biological system's stable states and hence a representation of the disease pathogenesis. An example of a BNet basin diagram is shown in Figure 3.1. The **Evolution Module** evolves boolean network instances so that a pre-specified attractor can be found. It allows the users to analyse the evolution process and examine the resultant network.

A. Synchronous versus Asynchronous Updating

In CRBNs, the updating scheme used was synchronous. A synchronous RBN is a boolean network in which all the nodes are updated at once at each discrete time-step. This results in deterministic state pathways that give rise to attractors and basins. However, this does not accurately model genetic [15] nor cytokine regulatory pathways. As homeostatic regulation is adaptive in nature, there is an

element of stochasticity in the local interactions. Genes do not simply march together in step and neither does an adaptive immune response follow an unchanging recipe for defending against known and unknown infections.

```
Network.step()
     If Synchronous {
        For all nodes i{
            Find all influencing nodes values of node i
            Use function to derive new value of node i
            Store new value of node i into a temporary
                    array of node values
        }
        Update all nodes with the temporary array
     } else if Asynchronous {
          Node i =
          (int)(Math.random()*1000)%(number of nodes)
          Find all influencing nodes values of node i
          Use function to derive new value of node i
          Update node i value
     }
```

Figure 3.2 Algorithm for Updating the Boolean Network

For an ARBN, only *one* random node is updated at once at each discrete time-step. This method depicts the randomness of any genetic signal that may be changed at any one time. Essentially, the ARBN ignores the time between interactions and assumes that no two signals change at the same time. This randomness in the updating process results in a non-deterministic boolean network model whose basin size and attractors are not stable but change dynamically. Point attractors can however be present and they do have loose attractors [15] where some nodes are temporarily stable. In this work, we use synchronous updating to verify that the DHF Infection Model produces clinically valid Null Infection homeostasis and clinically valid DF and DHF final states. We then use asynchronous updating to verify that our DHF Infection Model does indeed approximate clinical markers for characterizing the onset of DHF or DF under realistic conditions. Figure 3.2 gives the updating algorithm that BNet uses.

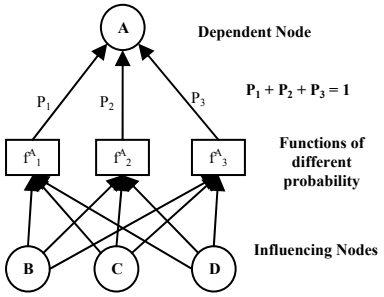


Figure 4.1 Example of a Shmulevich PBN block

IV. The Probabilistic Boolean Network

Originally introduced by Shmulevich [11][12], the advantage of a PBN is that it models the stochasticity of biological pathways. The classical RBN is rigid in that its functions are predetermined at the start. This assumes that the environment has no uncertainty. PBN assumes that there are some latent variables and inherent stochasticity in genes and cellular signals and accounts for it with a probabilistic function. The function, unlike for the classical RBN, is defined for a specific network. As a result, the inferences made with the model moves away from general local behaviour to detailed generic properties of a specific structure. This approach can potentially give more insight to the understanding of disease progression and the associated immune regulatory pathways.

Figure 4.1 shows a possible structure for a node in Shmulevich's PBN. Instead of one deterministic boolean function, there are many distinct ones, each having a different probability by which the dependent node can take. A function is selected and the **dependent node** value is determined from the **influencing nodes** and the selected boolean function. Inhibition and induction of each cytokine defines the edges in the PBN. An inducing influence will increase the probability of node transition to an ON state while an inhibiting influence decreases it. More precisely, suppose Node A is the dependent node which has four influencing nodes that changes the production level of node A. Node B and C are inhibiting while D and E are inducing nodes. If B and C are ON but D and E are OFF, the overall resulting influence is set to inhibiting. In general, for a boolean network G, let f: N(G) $\rightarrow$ Z compute the **degree of influence** for a given node x in N(G). Let $Inf_i(x)$ be the boolean value of the i^{th} influencing node on x, and $S(x)$ be a boolean function that gives 1 if x is inducing and 0 otherwise. We define $f(x)$ as

$$f(x) = \sum_i Inf_i(x)(2S(x) - 1)$$

We say that there is an **inducing influence** on x if $f(x) > 0$, an **inhibiting influence** if $f(x) < 0$ and **null influence** if $f(x) = 0$. The **strength of influence** (either inducing or inhibiting) is given naturally by $|f(x)|$.

A. Aggregating Functions

A problem with Shmulevich's PBN is the number of function types to be stored. From Figure 4.1, suppose $|N(G)| = n$ for a given boolean network G, then there are $2^n - 2$ distinct function types for any given node x in G (including reflexive influences except for the trivial case of the node as the only influence to itself). We assume that with hashing, the selection of which boolean function to use can be computed in $\Theta(1)$ time. This implies that in the worst-case, we have $\Theta(n2^n)$ functions to store. This is a large number irregardless of the storage facility being used and the structure is also not amenable to evolutionary computation (also noted by Shmulevich). We propose a method of functional expression that not only reduces the space complexity but is also amenable to chromosome encoding.

In our construction, each dependent node will have only 1 probabilistic function (instead of $2^n - 2$). Since the influencing node values are the same for any selected function, we can combine the function with its probability to form a single Probability Karnaugh-Map (which we

refer to as a PK-Map). In a normal K-Map, the boolean function for a dependent node is denoted by a matrix with dimensions for all possible boolean values of the influencing nodes. In the example given in Figure 4.2, there are two possible K-Maps, f_1 and f_2. f_1 has a probability of 0.4 while f_2 has a probability of 0.6. As shown for a possible permutation of the influencing nodes (BCDE), we compute the expected value for the dependent node's PK-Map. This gives a probability value in the new map of 0.6. 0.6 is the probability which A will take a value of 1 if BCDE was 0000. We now have $\Theta(1)$ space requirements for each node and hence $\Theta(n)$ functions for the whole network.

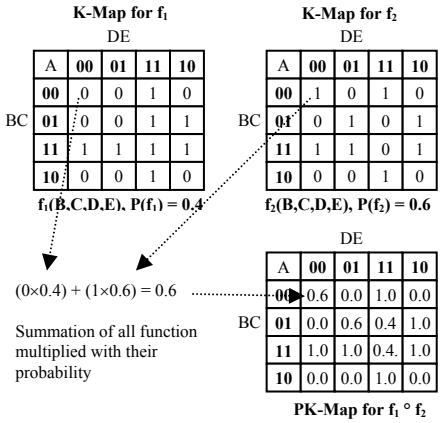


Figure 4.2 Aggregation of Shmulevich PBN block

B. Specifying Inducement Probabilities

It is commonly believed that boolean states are insufficient to model most nontrivial biological systems. In the case of cytokine cascades, we need to also consider the strength of influence. We use the term signal and cytokine interchangeably in this paper. Suppose there are two inducing signals, how do we account for the increase in production? We propose to map the strength of influence to the probability node transition to the ON state. In the example in Figure 4.3, node A has inducing nodes B and C. Using a K-Map alone cannot represent the marked increased production of A when *both* B and C are available. In a PK-Map however, a 0 would denote the certainty of being switched OFF while a 1 denotes the certainty of being ON. For inhibiting influences, the probability is close to but not equal to 0. This is because there is some small chance that the dependent node will be ON. Hence if the overall influencing nodes are inhibiting, the probability of the dependent node being ON will be between 0 and 0.5. The stronger the inhibition, the closer the probability is to 0. If p is the probability of transitioning to 0 (inhibition) then (1-p) would be the probability of transitioning to 1 (inducement). The cells in the PK-Map contain **Inducement Probability** (IP) values.

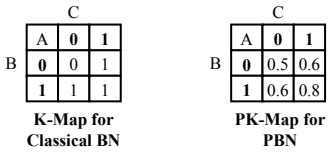


Figure 4.3 Using Probabilities to model Strength of Inducement

TABLE 4.2 MAPPING INFLUENCE STRENGTH TO INDUCEMENT PROBABILITIES

STRENGTH OF INFLUENCE $f(x)$	INDUCEMENT PROBABILITY
3 Inducers	0.9
2 inducers	0.8
1 Inducer	0.7
Balanced	0.6
1 Inhibitor	0.4
2 Inhibitor	0.3
3 Inhibitor	0.2

We manually map (in Table 4.2) influence strengths to probabilities for each PK-Map function of each of the PBN nodes (to complete our construction of the DHF Infection Model in Figure 2.2). Note that when there is an equal number of inducers and inhibitors, we assign a 0.6 IP instead of 0.5 because we assume that the body will more likely produce the signal than not. We are not increasing the magnitude of the influence, just the likelihood that the signal will be induced. Hence the IP is increased. The resulting value of the dependent node still remains boolean.

V. THE DHF INFECTION MODEL

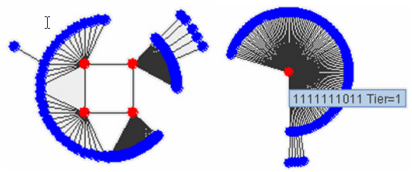
We simulate our completed DHF Infection Model (with assigned probabilities) using synchronous updating to verify that stable attractors correspond to clinical predictions. There are two distinct attractors observed. The first attractor develops when DV is not introduced while the second, develops when DV is introduced. These correspond respectively to a Null Infection Model of a healthy uninfected person and the final state of DHF.

A. A Null Attractor for Homeostasis Verification

When DV is not introduced, we see that our PBN quickly progresses into a cycle attractor. This cycle attractor corresponds to a negative feedback loop that regulates between Th1 and Th2 type immune responses; which is a commonly known homeostasis in immunology. In Figure 5.1(a), we see very few tiers in the progression towards the attractor. G-density is high in this basin and we can interpret this as an ordered regime which corresponds to a healthy patient with no abnormalities in the body.

The states of the cycle attractor in the Null Infection Model is given in Table 5.1. We see that when DV is not introduced, the Th1 and Th2 signals toggle between ON and OFF states in the cycle. This cross-regulation of Th1 and Th2 immune responses according to Fishman [16] is achieved mainly by IL-10 and IFN-γ. Yates, Bergman,

Hemmen, Stark and Callard [17] also gives a mathematical model to support the hypothesis that this regulation is done in an oscillatory manner, in that secreted levels of Th1 and Th2 cytokines often swing from a high to low value to regulate each other. This is exactly predicted in our model through the Th1 cytokines: IFN-γ and TNF-β, and Th2 cytokines: IL-10 and IL-13.



(a) (b)

Figure 5.1 Attractor Basins of the (a) Null and (b) DHF Infection Models

B. The DHF Attractor

The attractor when DV is introduced is one that corresponds to the DHF final state (see Figure 5.1(b)). This deterministic model shows a short and stable pathogenesis. The cyclic attractor is no longer present. This means that the cross-regulation of Th1 and Th2 cytokines is now absent. Without this cross-regulation, the Th2 cells will increase significantly, which Chaturvedi [1] describes as causing increased vascular permeability leading to DHF.

TABLE 5.1 CYCLE ATTRACTOR IN THE NULL INFECTION MODEL

	Cycle State Attractor 1	Cycle State Attractor 2	Cycle State Attractor 3	Cycle State Attractor 4
DV	0	0	0	0
hCF	0	0	0	0
TGF-β	0	0	0	0
IFN-γ	**1**	**0**	**0**	**1**
TNF-β	**1**	**0**	**0**	**1**
IL-8	0	0	0	0
IL-10	**0**	**0**	**1**	**1**
IL-12	1	1	1	1
IL-13	**0**	**0**	**1**	**1**
IL-18	0	0	0	0

As mentioned, asynchronous updating of nodes, although more biologically realistic, does not identify stable attractors. However, by computing the duration that a node remains in the ON state, we can use relative differences in this duration distribution (for the network) in the Null and DHF Infection Models and compare them with reported strengths of cytokine signals found in patients with DV infection. In effect, without a point attractor (like that in Figure 5.1(b)) to indicate the onset of DHF, we have to rely on an **average duration-based attractor** to approximate expected quantities of cytokines. The duration that each node is ON is computed for a simulation period of 1000 steps taken from all possible start states. The values in column 2 and 3 from Table 5.2 are computed based on the algorithm given in Figure 5.2.

TABLE 5.2 COMPARISON BETWEEN BNET RESULTS AND CLINICAL RESULTS ON THE DHF INFECTION MODEL

	Null Model (%)	DHF Model (%)	Difference (%)	Difference* (Increase / Decrease)	DHF Clinical Results* [1]
hCF	30.04	70.00	39.96	↑↑	↑↑
TGF-β	30.05	69.94	39.89	↑↑	↑↑
IFN-γ	62.03	62.63	0.6	↑	↑
IL-8	41.99	58.08	16.09	↑↑	↑↑
IL-10	41.45	63.64	22.19	↑↑	↑↑
IL-12	50.97	48.93	-2.04	↓	↓
IL-13	41.28	63.67	22.39	↑↑	↑↑
IL-18	30.09	70.00	39.91	↑↑	↑↑

*↑ Increase, ↑↑ Marked Increase, ↓ Decrease, ↓↓ Marked Decrease

```
Node.Probabilities ()
     new float Prob[number of nodes] = 0.0
     For all possible states i {
          Initialise Boolean Network with i
          For step 1 to 1000 {
               Network.step() // see Figure 3.3
               For all nodes j
                    Prob[Network.getCurrrentNode[j]]++ }
     }
     For all possible node i
          Prob[i] /= (1000 * number of possible states)
```

Figure 5.2 Algorithm for Computing Node Probabilities

Column 4 and 5 of Table 5.2 shows the quantitative and qualitative differences in cytokine production durations between the Null and the DHF Infection Models when using asynchronous updating. We observe that hCF increases significantly, consistent with Chaturvedi's hypothesis on hCF being the main cause of DF. Also, while Th1 cytokines increased only slightly (<1%), Th2 cytokines, IL-10 and IL-13, increased significantly from 41.45% and 41.28% to 63.64% and 63.67% respectively. These results are consistent with increased Th2 cytokine levels reported to be the direct causes of DHF. Overall, Table 6.2 shows that our simulation results matches cytokine level changes reported in [1] for DHF. Unfortunately, the report [1] gave only the qualitative changes. We use a change of 15% points or more as a Marked Increase or Marked Decrease. The data on TNF-β was not given in [1], hence we omit a comparison with this cytokine.

VI. Finding Intervention Points

We postulate that the pathogenesis of dengue infection has certain undesirable 'traits' that lead from DF to the more severe form of DHF (or DSS). In general, suppose we can find a minimum set of changes to the network to cause the attractor to move from an infection (undesirable) basin towards a healthy (desirable) basin. This would allow us to find the best possible (in terms of simplest) *intervention point* to prevent a viral infection. This simplest intervention point must not change the structure of the network and yet produce a significant change in attractor basins. In our context, we wish to find an intervention point that prevents DHF from arising for a

patient having DF. To do this, we use a Genetic Algorithm (GA) [18] to find changes in the DHF Infection Model that can shift the DHF attractor basin to a DF basin; in effect, identifying changes in signal values that can shift more states from an unhealthy attractor to a healthy or non-fatal one.

A. Increasing the Solution Space for Intervention Points

Shmulevich [12] utilized a GA to find intervention points by maintaining a constant number of probabilistic functions (or K-Maps) for each node. Therefore, discovered intervention points did not give new functions but only changes in K-Map cell values for existing ones. With PK-Maps, we can infer new functions if necessary, through linear regression. Another limitation in Shmulevich's method for evolving boolean networks is the fixed probabilities of the K-Maps. During genetic search, only the boolean values in each function (of the K-Map) changes and not the function probabilities. PK-Maps on the other hand, allow evolutionary change in IP values; allowing the discovery of new inducement probabilities.

B. Designing the Genetic Algorithm

Each GA chromosome represents a concatenated string of all the aggregated functions of the DHF Infection Model (see Figure 2.2). The IP values of each dependent node's PK-Map are alleles within the chromosome. In Figure 6.1, we see an example of how the rows of a PK-Map for a particular node are transcribed onto a chromosome string.

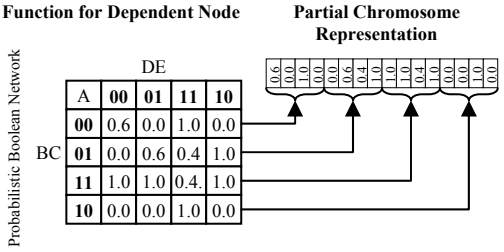


Figure 6.1 Encoding a PK-Map onto a Chromosome

C. Fitness Measurement

Using asynchronous updating will give a nondeterministic state to state transition. Hence, finding a fixed attractor or stationary point is difficult. Without identification of attractors, the asynchronous updating scheme prevents us from measuring the fitness level of a given chromosome (i.e. encoded PK-Map functions). How do we know if the current simulation of functions gives us an accurate description of the biological system? We can verify this by determining a particular state's frequency of occurrence within a certain period (for example, 1000 time steps). Loose attractors can then be found this way. We consider the frequency of certain loose attractors (phenotypic behaviour) as the fitness measure of the genotypic trait; the higher it is, the more likely is its occurrence within the dynamics of DHF infection. This is intuitive as the longer a node is ON, the more likely that cytokine signal will be produced. Our final state values of DF and DHF (in Table 6.1) comprises the digitization of increased and decreased levels of cytokines from Chaturvedi's [1] results (sampled from dengue patients). We have already seen the DHF final state point attractor in our earlier simulations. An obvious limitation of our boolean state representation is the under-sampling of cytokine levels, where a Marked Increase and a regular Increase are both given state values of 1. Resolving this problem is nontrivial as it may require a theoretical reconstruction of boolean networks as multimodal networks.

TABLE 6.1 DERIVING FINAL STATES FOR DF AND DHF BASED ON CHATURVEDI [2]

	Dengue Fever		Dengue Hemorrhagic Fever	
	Cytokine Level	Boolean State	Cytokine Level	Boolean State
DV	↑	1	↑↑	1
hCF	↑	1	↑↑	1
TGF-β	↓	0	↑↑	1
IFN-γ	↑↑	1	↑	1
TNF-β	↑↑	1	↑↑	1
IL-8	↓	0	↑↑	1
IL-10	↓	0	↑↑	1
IL-12	↑↑	1	↓	0
IL-13	↓	0	↑↑	1
IL-18	↑	1	↑↑	1

*↑ Increase, ↑↑ Marked Increase, ↓ Decrease, ↓↓ Marked Decrease

VII. How to Prevent DHF

We wish to find intervention points that decreases the basin of attraction of DHF by increasing the basin of attraction of DF. Using the final states of DF (column 3 in Table 6.1) as a fitness measure and individuals as mutated instances of the DHF Infection Model, we conduct experiments with 100 generations, at 0.1 mutation rate and 0.01 crossover rate (obtained through experimentation). For each allele, there is a 0.1 chance of mutation and a 0.5 chance of either a positive increase or a negative decrease by 0.1. We use single-point crossover from two parent chromosomes with 0.01 chance of occurrence. Population size was maintained at 30 with Tournament selection and replacement of 10 individuals. The resulting PBN obtained is the **DF Infection Model**.

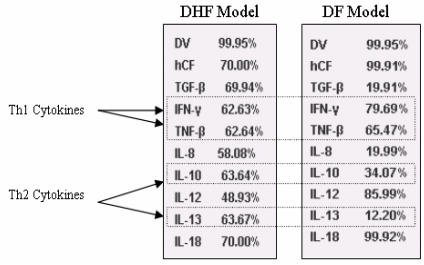


Figure 6.2 Comparison of cytokine activation levels in the simulation of DHF and DF Infection Models

We then identify significant differences between the evolved DF Infection Model and the DHF Infection Model. By comparing IP values between PK-Maps for corresponding nodes, we find intervention points in the network. These changes in cytokine influences imply the impact of certain cytokines that causes the shift from a DHF attractor to a DF attractor. By simulating (with asynchronous updating) the evolved DF Infection Model, we verify that the DF final state is commonly occurring. A comparison of the DF Infection Model with the DHF Infection Model show changes that correlates with Chaturvedi's [1] hypothesis that DHF is caused by the increased levels of Th2 cytokines while DF reaction is by increased levels of Th1 cytokines (see Figure 6.2). A comparison of the DF Infection Model with the Null Infection Model in Table 6.2 shows a change in activation levels (hence strength) that correlates with clinical results [1]. However, the magnitude of increase differs from the clinical results in hCF, IL-8, IL-13 and IL-18 as our GA only uses the final states as the basis of measuring the fitness level. Overall, we identify three intervention points; namely, **TGF-β**, **IL-8** and **IL-13**. TGF-β is found to have significant decrease in probability of production (from 0.7 to 0.1) in the presence of DV. The change shows that TGF-β is an important factor in the development of DHF. This corresponds to clinical results [1] that maximum levels of TGF-β were detected in patients with DHF grade IV implying a correlation between TGF-β and the severity of DHF. To decrease the severity of DHF, our result suggests a need to decrease the influence of DV on TGF-β.

TABLE 6.2 COMPARING NULL AND DF MODELS

	Null Model (%)	DF Model (%)	Difference (%)	Difference* (Increase / Decrease)	DF Clinical Results * [33]
hCF	30.08	99.91	69.83	↑↑	↑
TGF-β	30.15	19.91	-10.24	↓	↓
IFN-γ	61.87	79.69	17.82	↑↑	↑↑
IL-8	42.02	19.99	-22.03	↓↓	↓
IL-10	41.37	34.07	-7.3	↓	↓
IL-12	50.92	85.99	35.07	↑↑	↑↑
IL-13	41.46	12.20	-29.26	↓↓	↓
IL-18	30.08	99.92	69.84	↑↑	↑

*↑ Increase, ↑↑ Marked Increase, ↓ Decrease, ↓↓ Marked Decrease

In the presence of DV, IL-8 also has a significant change (from 0.7 to 0.0) in probability of production. This change supports the hypothesis that increased vascular permeability with leakage of plasma in DHF is associated to the presence of high levels of IL-8. To decrease the severity of DHF, our result suggests a need to decrease the influence which hCF has on IL-8 production. Finally, IL-13 levels also decreases significantly when DV is present (by 0.7 and 0.6 on two influencing node values of IFN-γ). As IFN-γ is a Th2 inhibitor, an increase in the former will reduce levels of IL-13 (see Figure 2.1). In Mustafa, Elibishbishi, Agarwal and Chaturvedi [20], IL-13 is recognized as an important signal in the development of DHF. To decrease the severity of DHF, our result suggests that IL-13 should not be induced irrespective of the presence of IFN-γ.

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