The Future of Dengue Infection Management by Integrating Hematology EIPs for Better Outcomes

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Dengue Infection

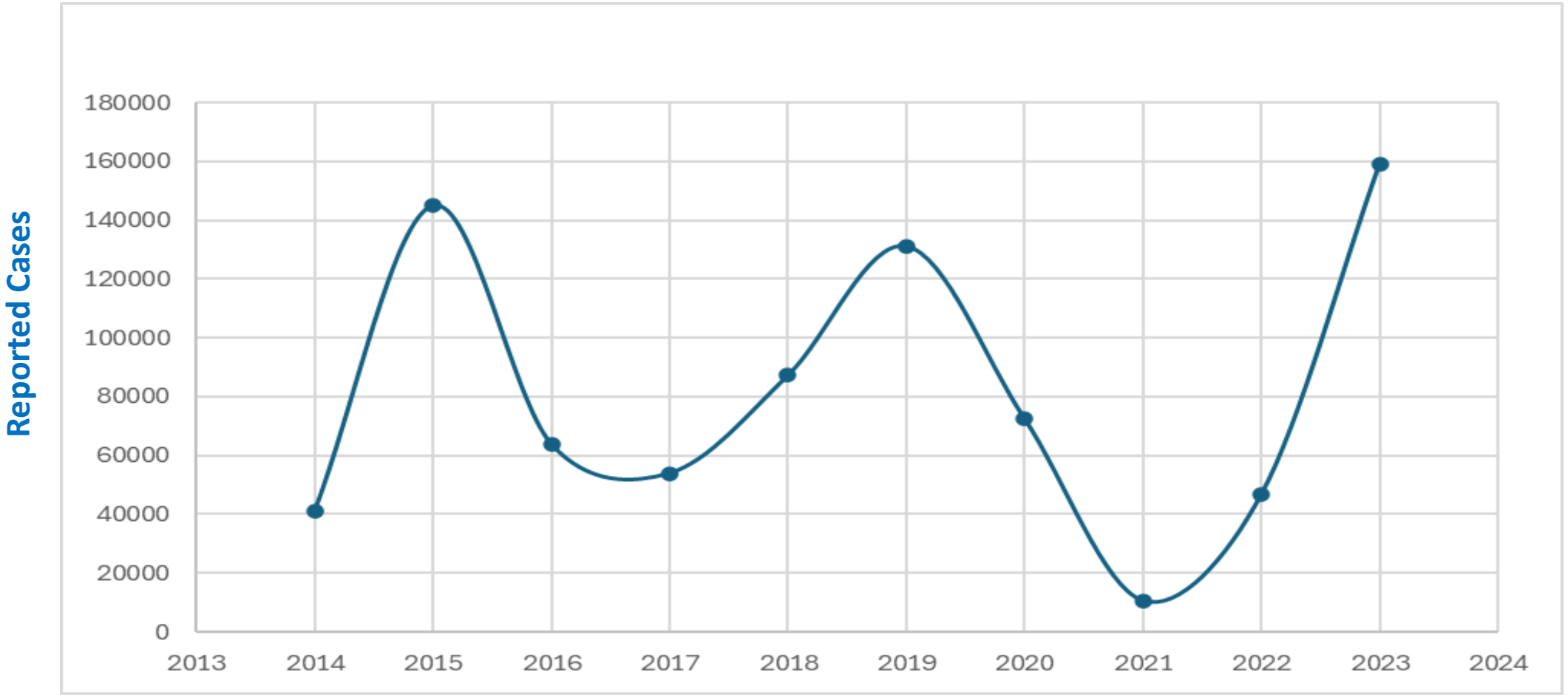
- Still the most common mosquito-borne viral diseases of public health significance

Flaviviridae (RNA virus)

Five serotypes: Den 1-5 (Den 1-4 in human)
Mostly Den 1 and Den 2 in Thailand

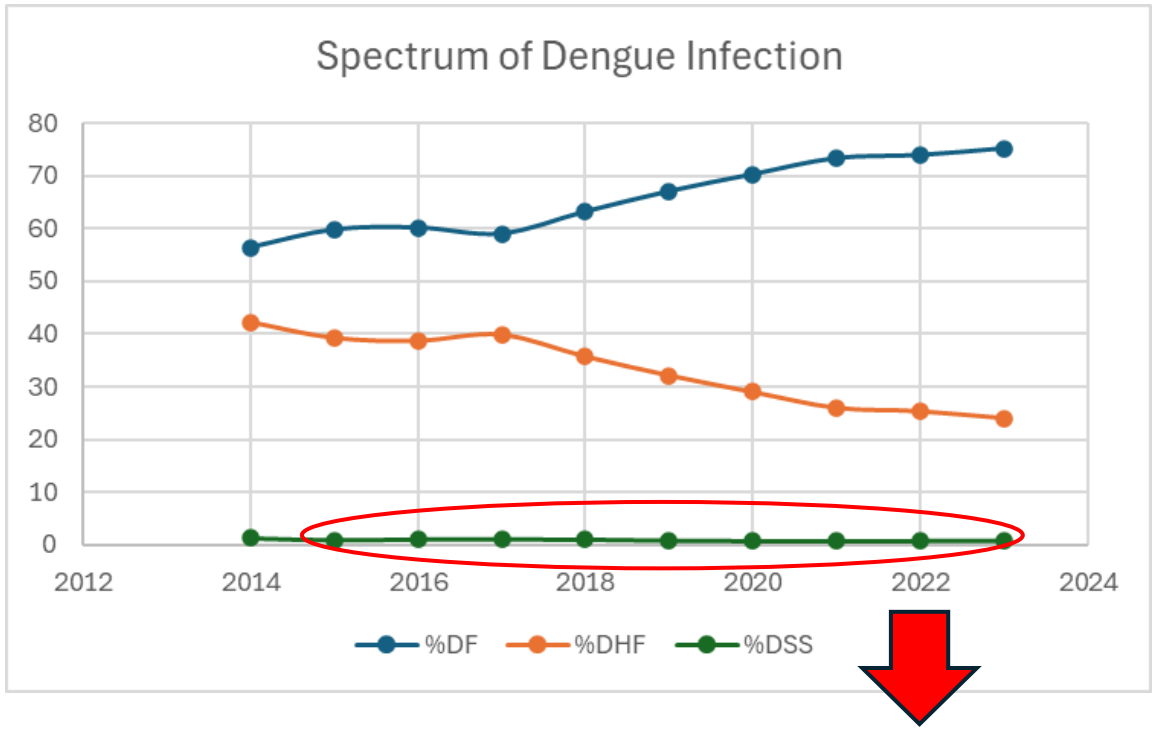
Transmission
Female *Aedes spp*.

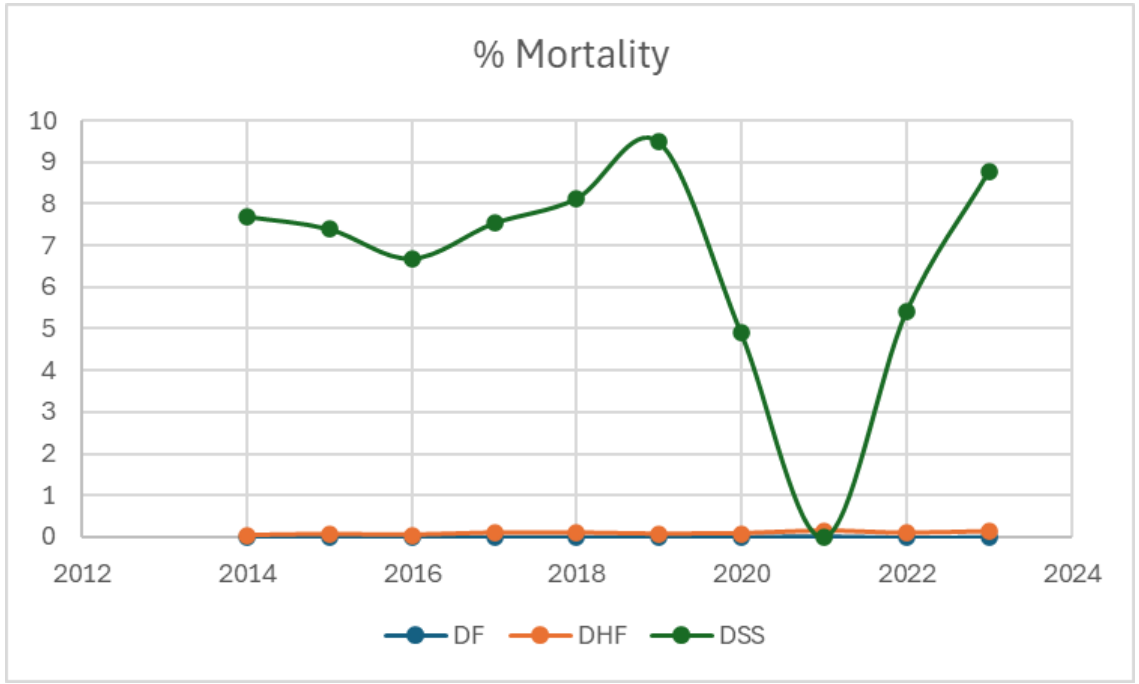
Current Situation in Thailand



Department of Disease Control , Ministry of Public Health

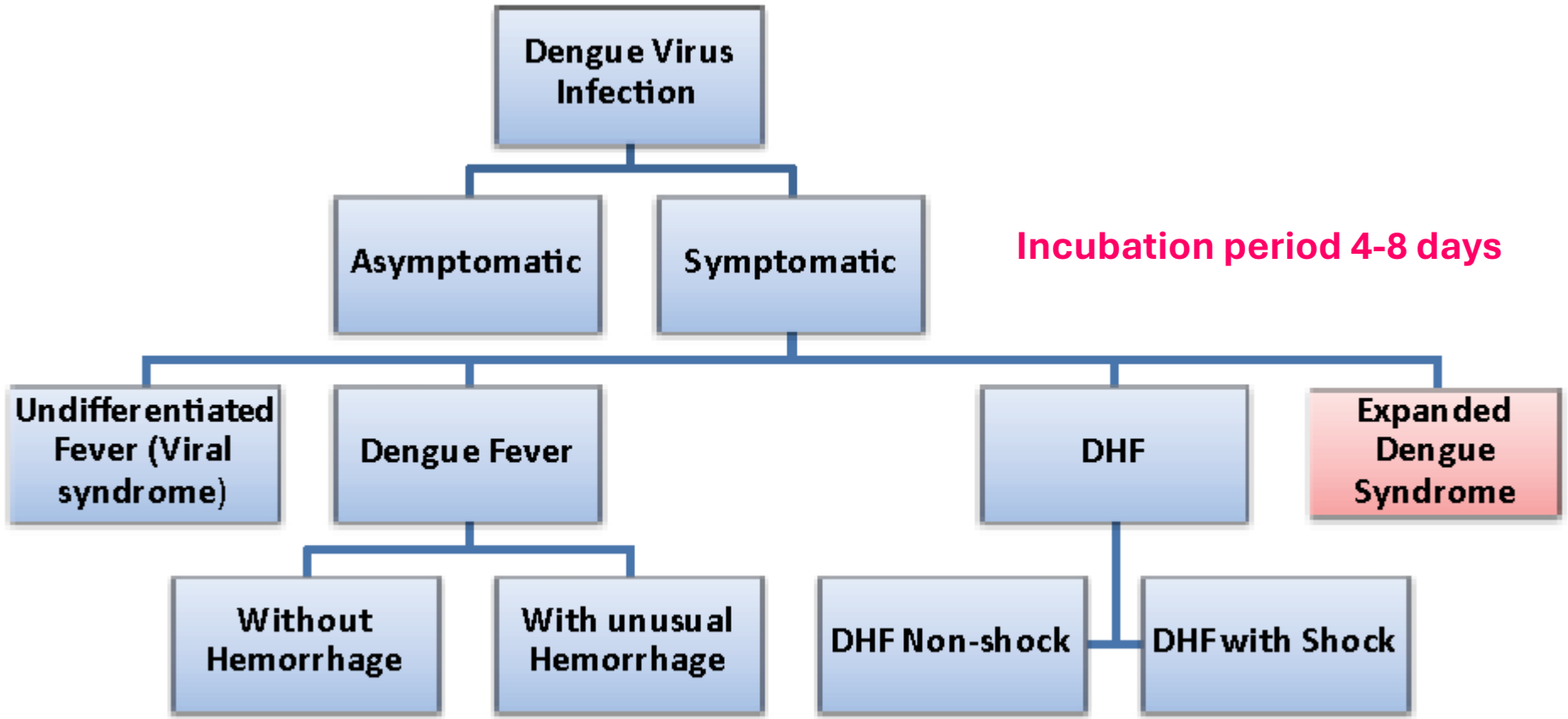
Current Situation in Thailand





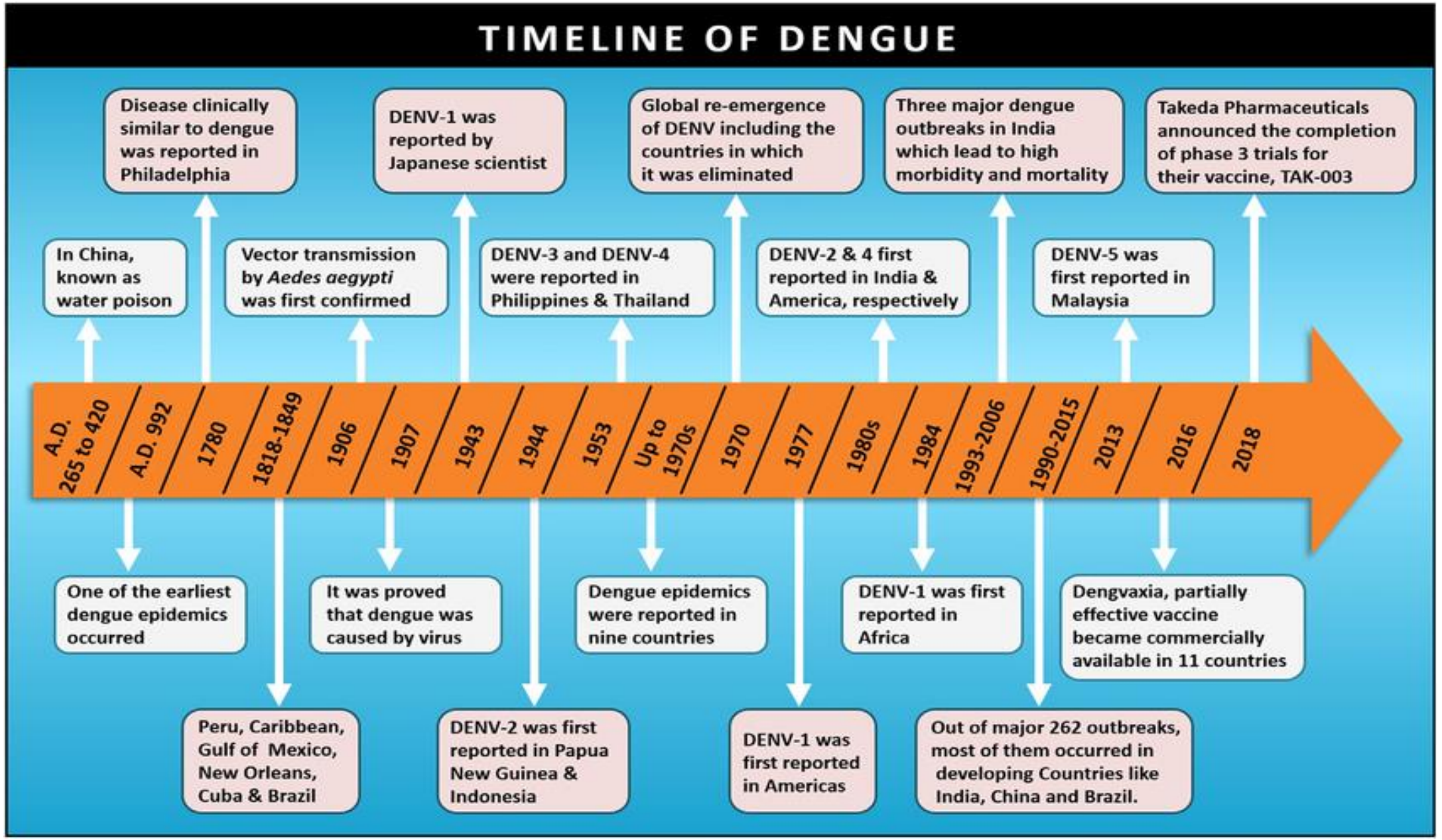
DSS < 1%

Spectrum of Dengue Infection



DSS = Dengue Shock Syndrome; DHF = Dengue Hemorrhagic Fever

Kadam DB,et al. J Assoc Physicians India. 2016;64:59



Sharma N, et al. Infect Med. 2023;2:167

Tools for Fighting against Dengue

- **Still no active anti-viral drugs**
- **Advancing in diagnostic tests**
  - **Early diagnosis**
  - **Early complication detection**
- Evolving supportive care
- Mosquito control
- Vaccination

Factors Affecting Clinical Outcomes

- Age
- Host immune response
  - Main pathogenesis
- Genetic factors
- Viral serotypes

Dengue Infection/Hallmarks

Fragility of capillary vessels and coagulopathy

- **Leakage of intravascular volume to interstitial space** e.g. pleural cavity, peritoneal cavity
  - Leading to hypovolemic shock
- Bleeding
- Multiple organ failure

Clinical Course of Dengue Infection

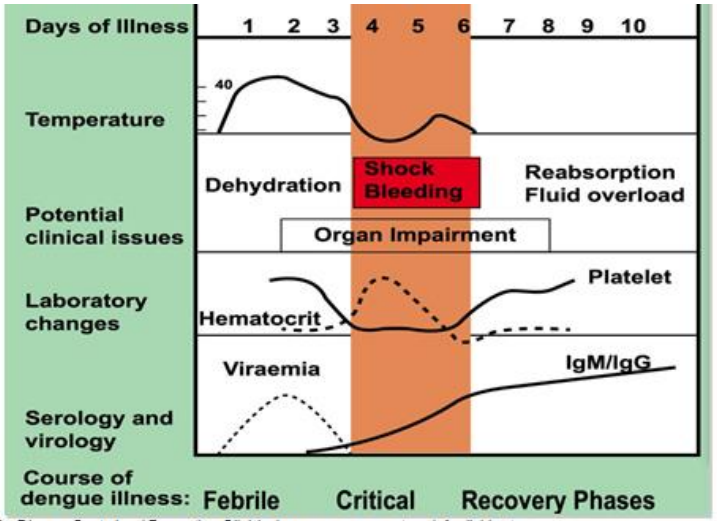
BLEEDING

High grade fever
Bone pain
Loss of appetite
Nausea/vomiting
Rash

Leakage of plasma
Into interstitial
space

Shock

Gain of appetite
Bradycardia
Convalescent rash
Diuresis



Center for Disease Control and Prevention. Clinician's case management card. Available at: http://www.cdc.gov/Dengue/resources/Dengue%20Case%20Management_card_125085_12x6_Zcard_Dengue.pdf Accessed July 21, 2011.

Febrile stage 2-7 days Critical stage 1-2 days Convalescent stage

LEAKAGE

DAY OF DEFERVESCENCE (Day 0)

Dengue Infection

- Any parameters to predict DHF that may lead to leakage
  - Dengue fever: not dangerous
  - Dengue hemorrhagic fever: able to progress to shock

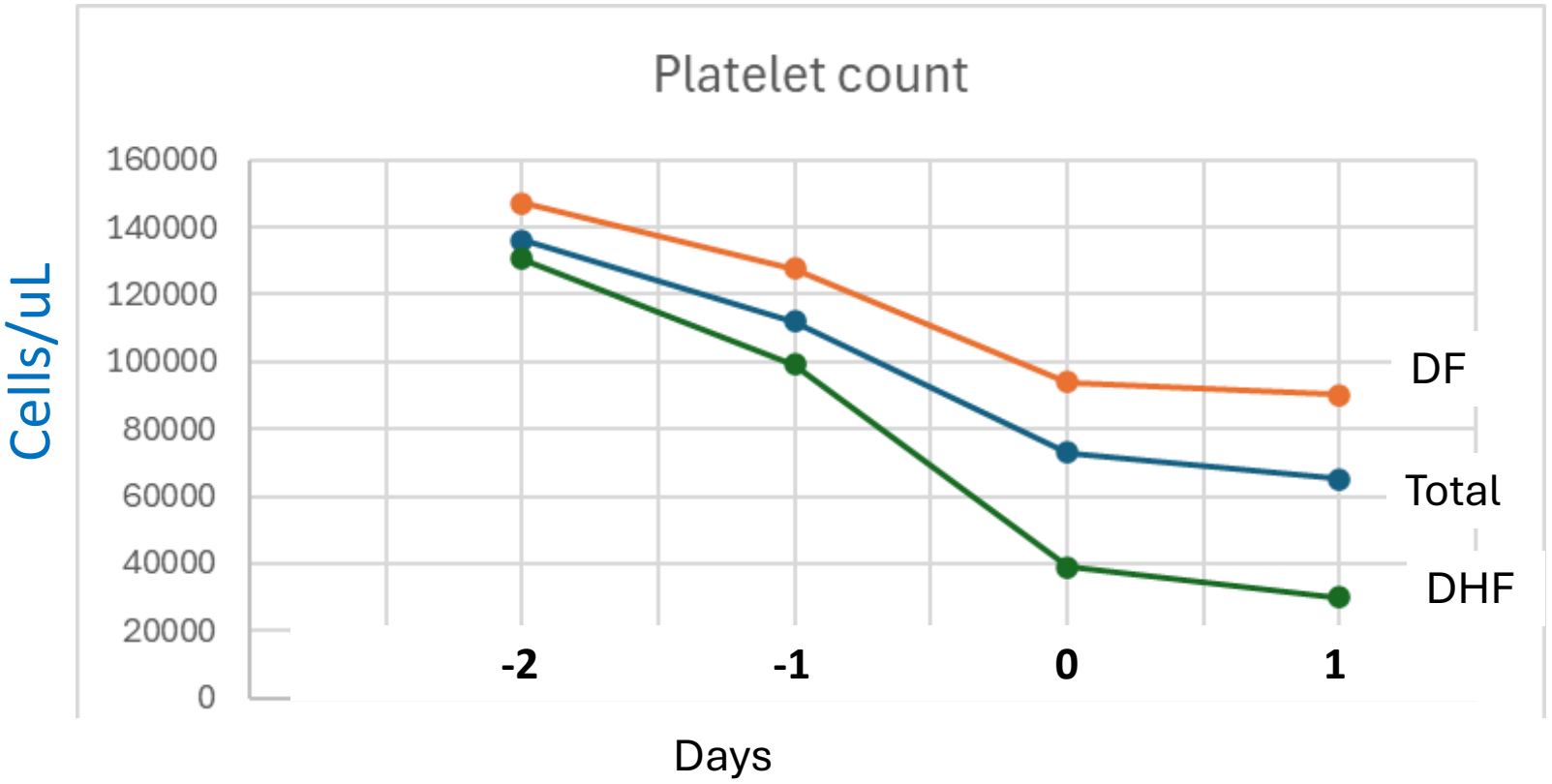
- **Close monitoring of patients who may develop DHF**

- **Current routine monitoring: CBC**

What's in CBC and Beyond?

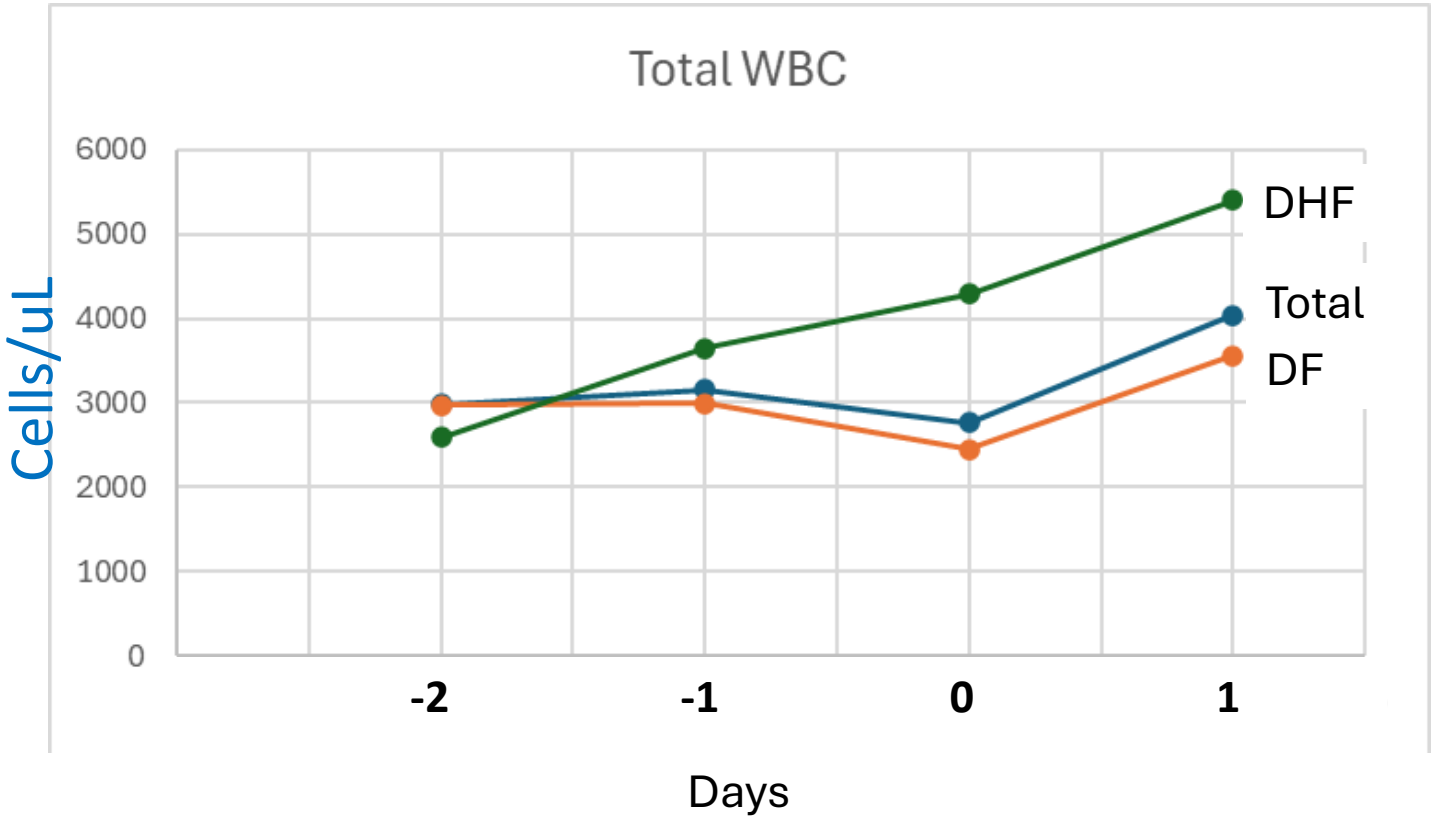
- Providing information about the cellular components of blood, including **red blood cells, white blood cells, and platelets**
- Easy and quick diagnostic tool for clinical decision-making and prompt treatment
- Useful in monitoring for further management

Platelet Count in Dengue



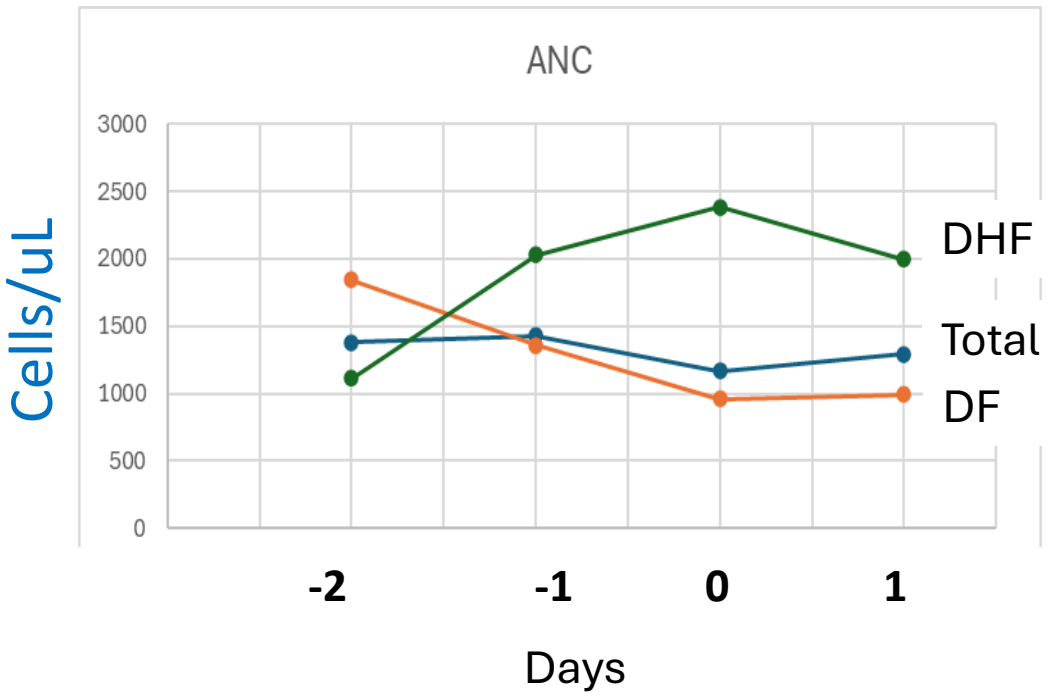
All cases of DHF: plt < 100,000/uL
Some cases of DF: plt< 100,000/uL

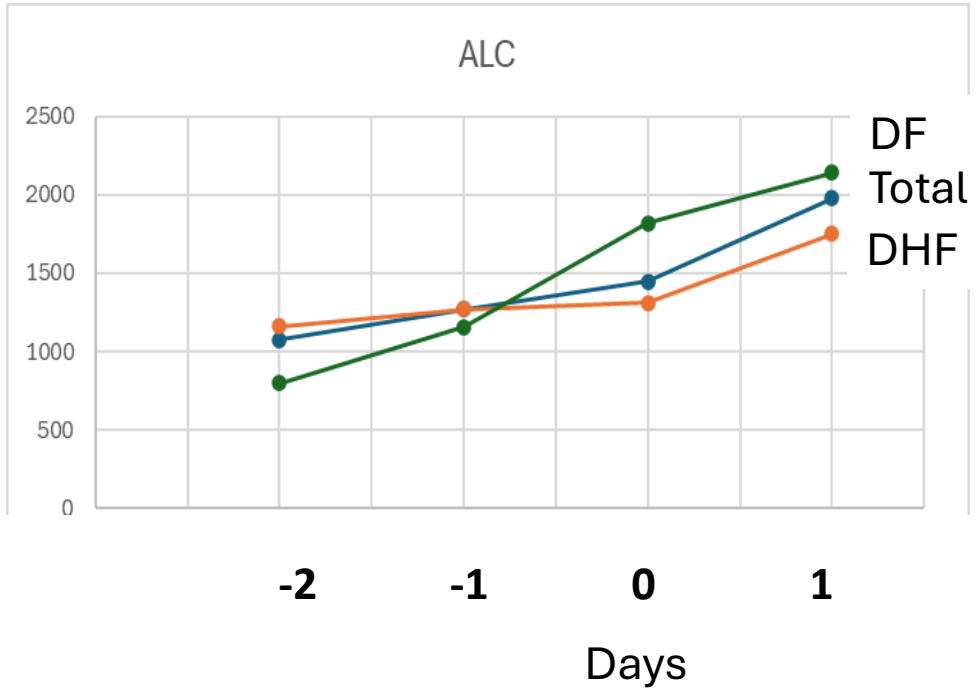
Total WBC in Dengue Patients



Day 0: Day of defervescence

Neutrophil/Lymphocyte in Dengue Patients





ANC: absolute neutrophil count

ALC: absolute lymphocyte count

Laboratory tests to differentiate DHF and DF

Clinical Variables	DF	DHF	*p*-value
Age (years)	10.0 [6.0,13.0] (81)	12.0 [8.0,13.5] (60)	0.035
Maximal Hct rising (%)	8.8 [5.1,12.2] (81)	15.7 [9.9,23.0] (60)	<0.001
WBC (x10^3 cells/mm^3) at day 0			
Total WBC	3.5 [2.5,4.8] (75)	4.2 [2.7,6.6] (57)	0.062
ANC	1.0 [0.7,1.5] (75)	1.7 [0.9,3.3] (57)	0.001
ALC	1.8 [1.3,2.5] (75)	1.7 [1.1,2.4] (57)	0.185
AMC	0.2 [0.2,0.3] (75)	0.3 [0.2,0.6] (57)	0.131
Platelet count (x10^3 cells/mm^3) at day 0	105.0 [62.0,141.0]	53.0 [32.0,87.0]	<0.001
Serum albumin (g/dL) at day 0	3.8 [3.6,4.0] (40)	3.3 [3.0,3.5] (50)	< 0.001

Jarutunyaluk P, et al. Manuscript in preparation

Laboratory tests to differentiate DHF and DF

- **DHF vs DF**
  - **Increased AST**
  - Increased IL-8
  - Decreased serum albumin
  - **Increased CRP**

- **Severe dengue vs non-severe dengue**
  - Increased vascular adhesion protein-1
  - Increased syndecan-1
  - **Increased CRP**
  - **Increased AST**

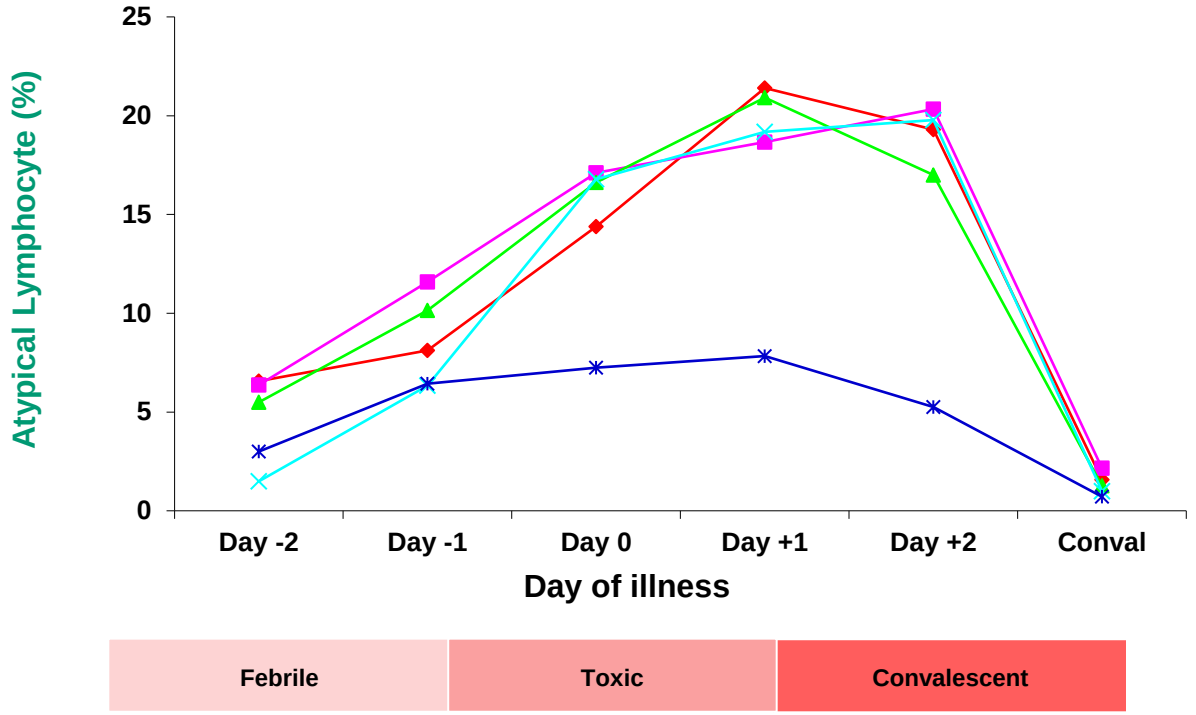
Moallemi S, et al. Scientific Reports. 2023;13:17485

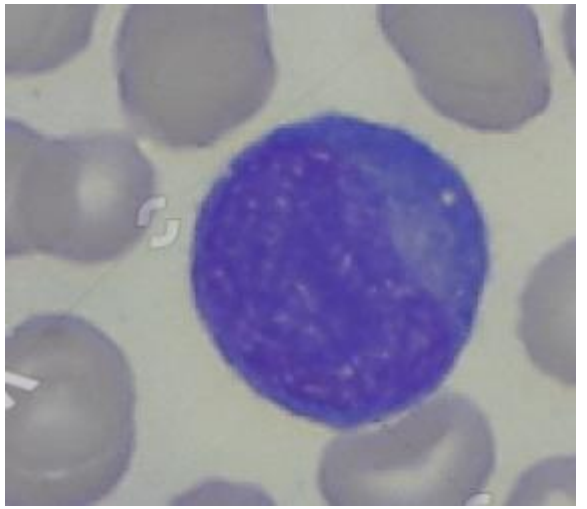
Laboratory tests to differentiate DHF and DF

- Predictors of DSS
  - Hct rising > 25%
  - Platelet count < 40,000/uL
  - APTT > 44 sec
  - PT > 14 sec
  - TT > 16 sec
  - VWF Ag > 210%

Chuansumrit A, et al. Southeast Asian J Trop Med Public Health 2010;41:326

Atypical Lymphocytes

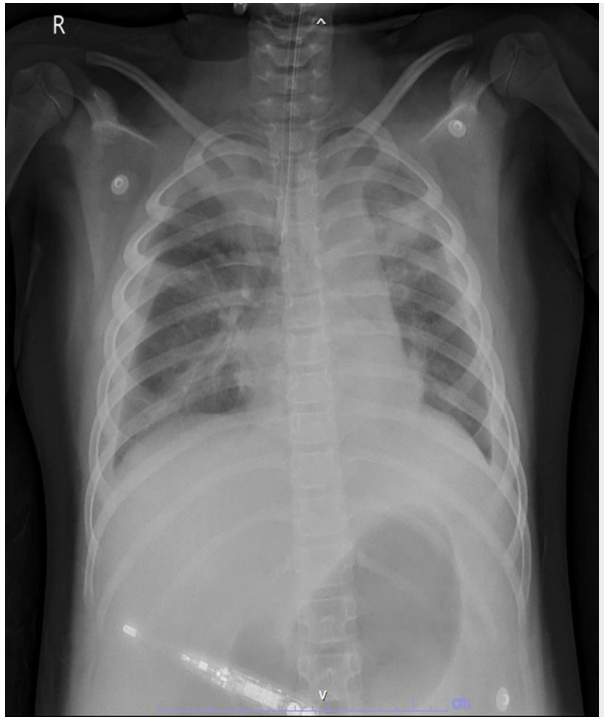


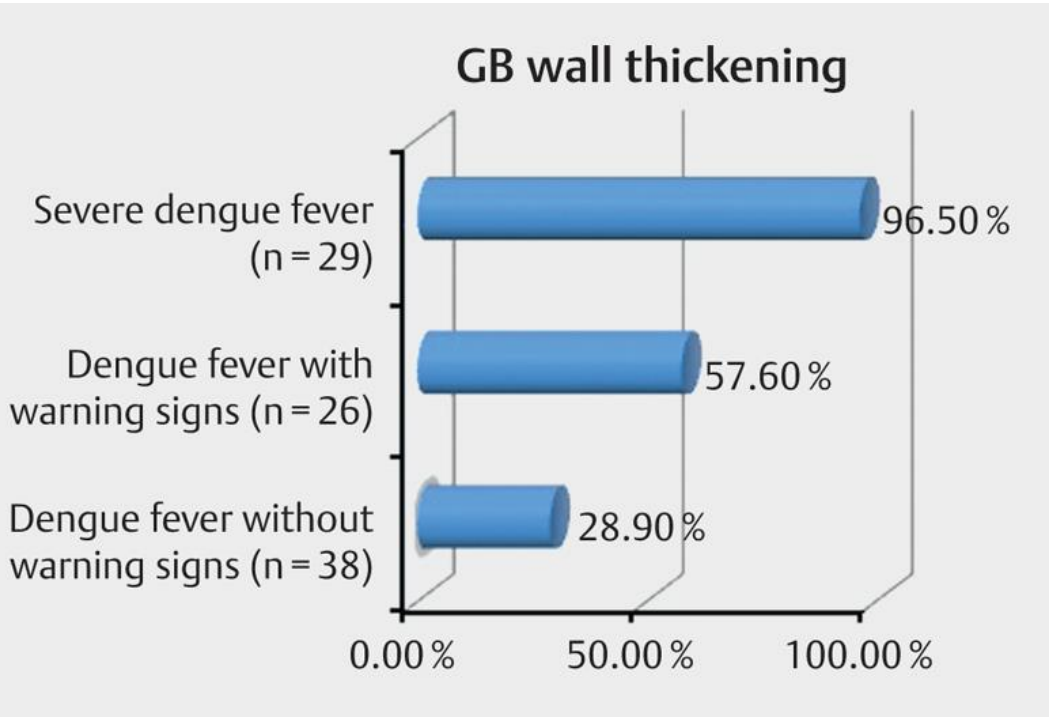


Plasmacytoid

Abundant in the convalescent phase

Role of Imaging

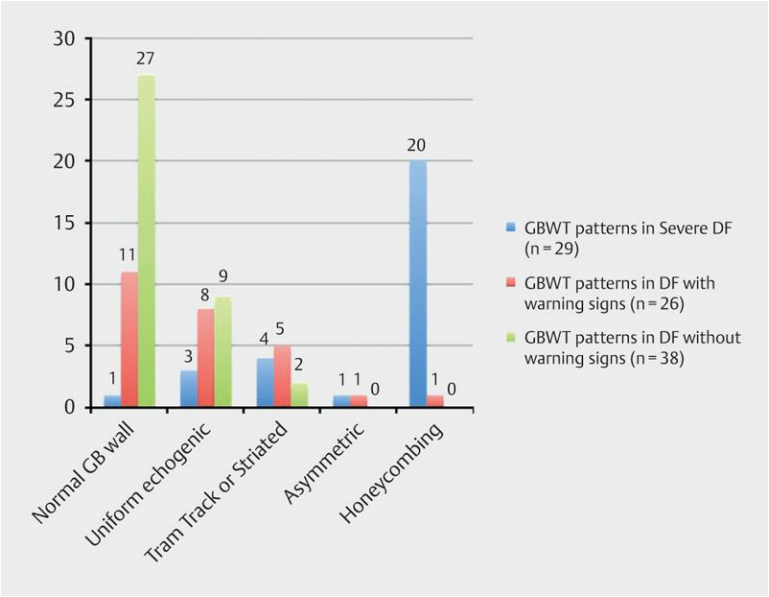




Right pleural effusion

Parmar JP et al. Ultrasound Int Open 2017; 3: E76–E81

Role of Ultrasound of Gall Bladder



Parmar JP et al. Ultrasound Int Open 2017; 3: E76–E81

ORIGINAL ARTICLE

Gallbladder Wall Thickening for Early Detection of Plasma Leakage in Dengue Infected Adult Patients

Leonard Nainggolan, Candra Wiguna, Irsan Hasan, Esthika Dewiasty

Departement of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

SS=65%, SP=70%

ORIGINAL STUDIES

Natural History of Plasma Leakage in Dengue Hemorrhagic Fever

A Serial Ultrasonographic Study

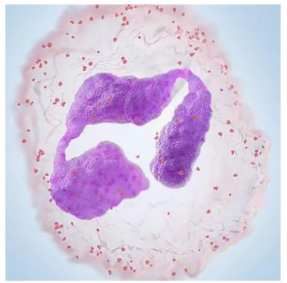
Srikiatkhachorn, Anon MD*; Krautrachue, Anchalee MD†; Ratanaprakarn, Warangkana MD†; Wongtapradit, Lawan MD†; Nithipanya, Narong MD†; Kalayanarooj, Siripen MD†; Nisalak, Ananda MD‡; Thomas, Stephen J. MD‡; Gibbons, Robert V. MD‡; Mammen, Mammen P. Jr MD‡; Libraty, Daniel H. MD*; Ennis, Francis A. MD*; Rothman, Alan L. MD*; Green, Sharone MD*

12/17 DHF cases without hemoconcentration be diagnosed

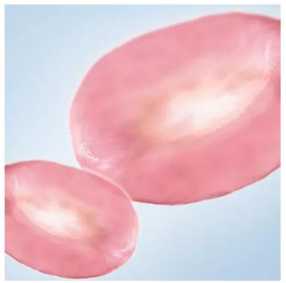
What's in CBC and Beyond?

- Providing information about the cellular components of blood, including red blood cells, white blood cells, and platelets
  - **Having additional parameters very helpful**
- Easy and quick diagnostic tool for clinical decision making and prompt treatment
- Useful in monitoring for further management

Extended Inflammation Parameters (EIPs)

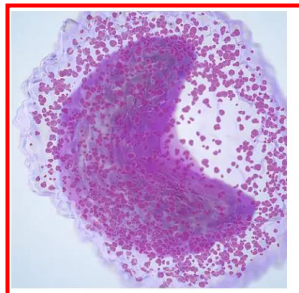


NEUT-GI
NEUT-RI

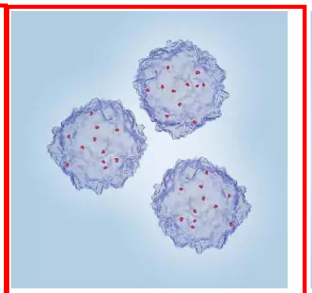


MICRO R
MACRO R

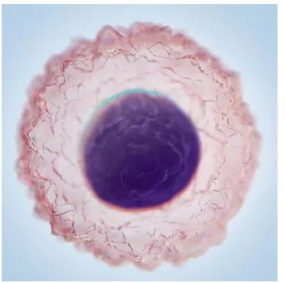
HYPO HE
HYPER HE



IG Count

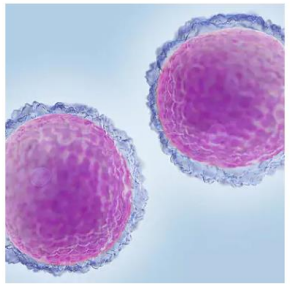


IPF# and %

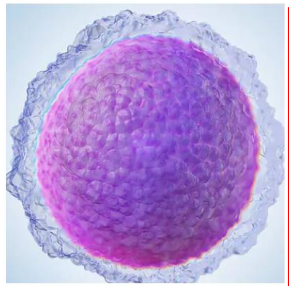


NRBC

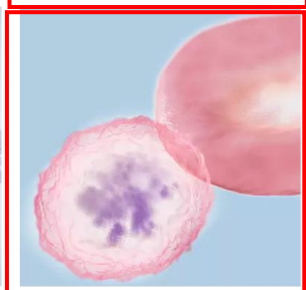
RET-HE



RE-LYMP
AS-LYMP



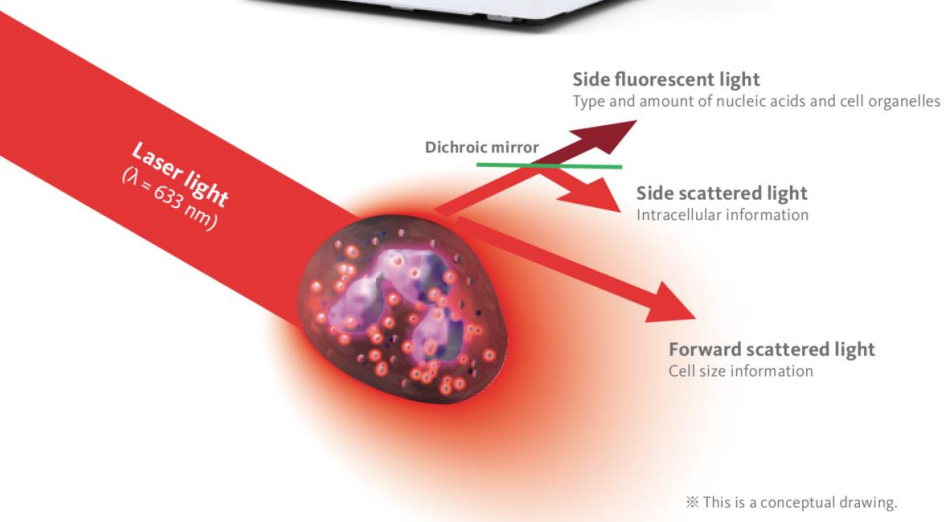
HPC



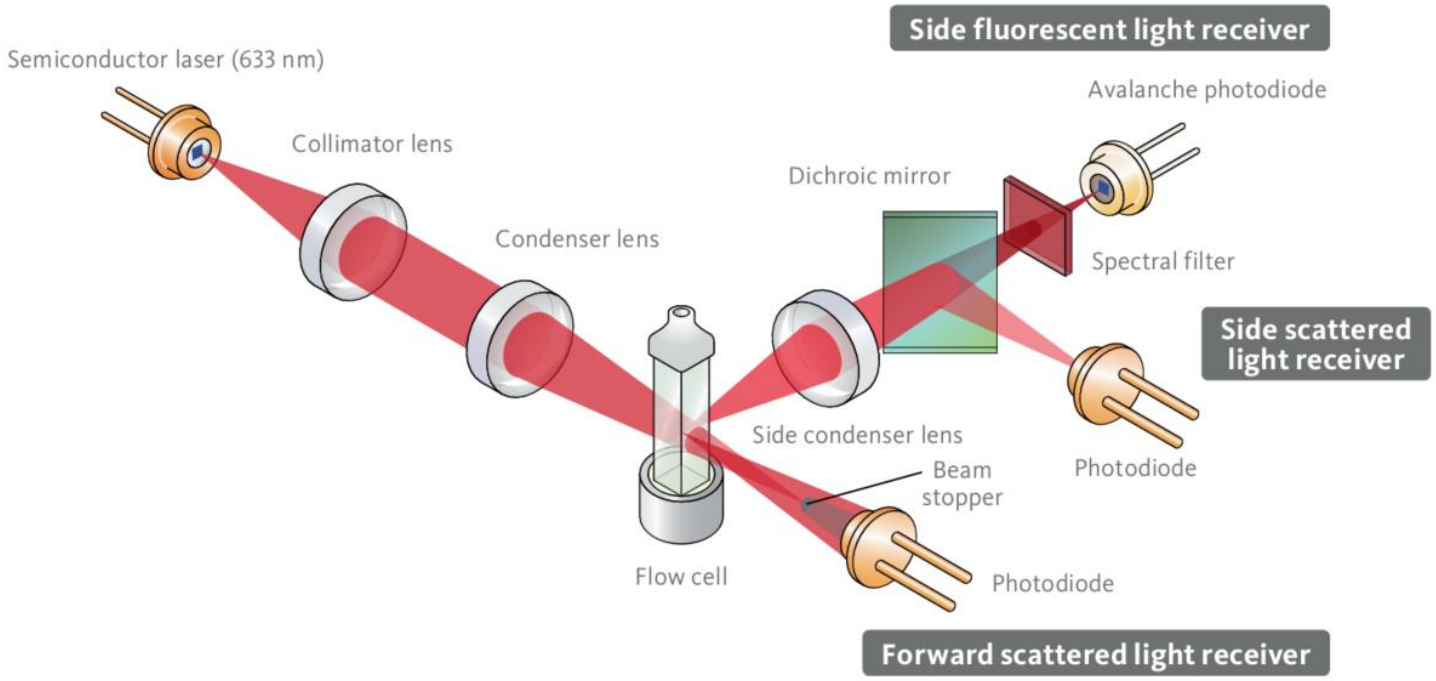
DELTA-HE

Online (1 May 2024) https://www.sysmex-europe.com/academy/knowledge-centre/sysmex-parameters/

Advanced Clinical Hematology Parameters



※ This is a conceptual drawing.



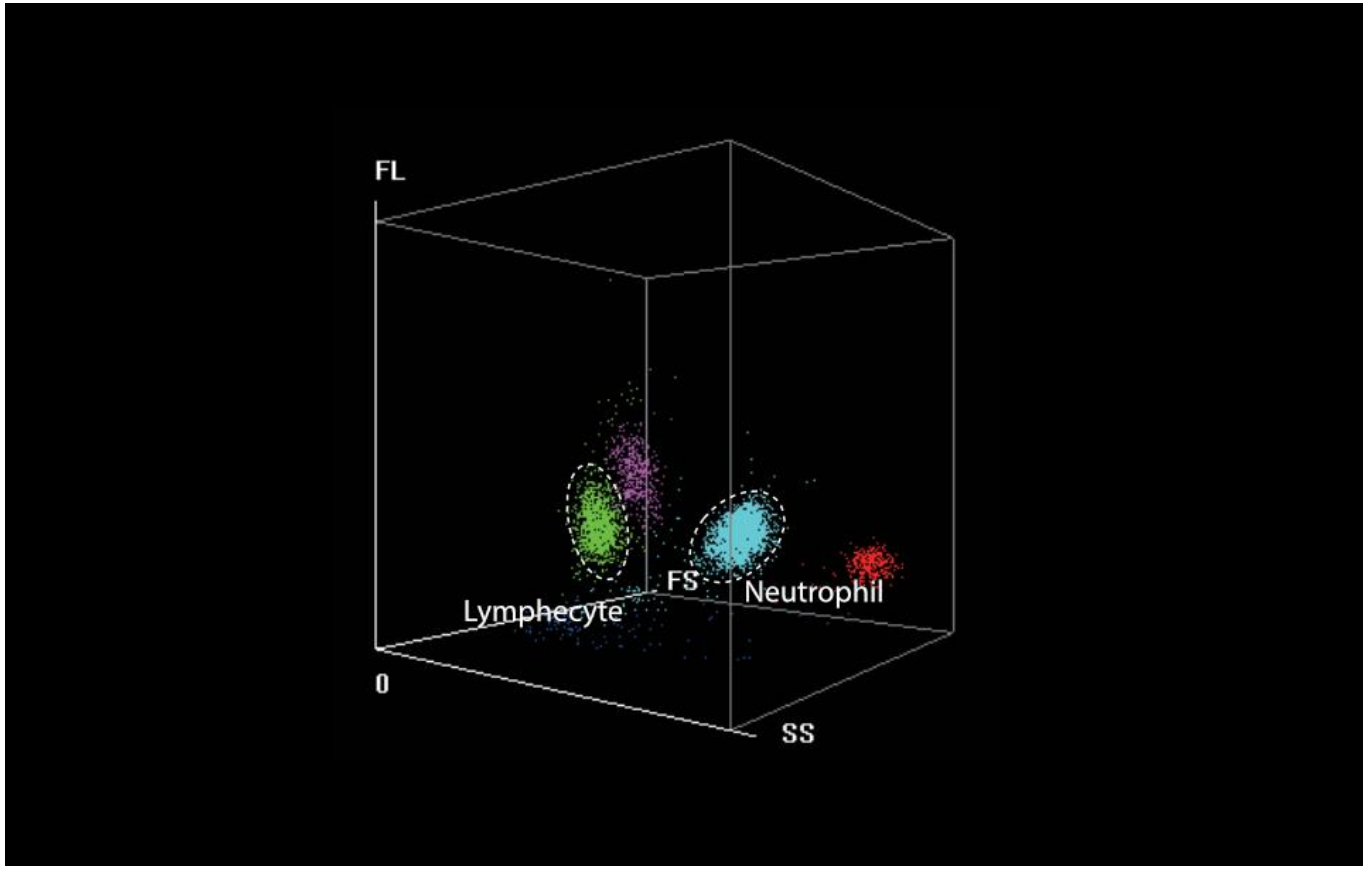
Advanced Clinical Hematology Parameters

- NRBC – Fluorescent Nucleated Red Blood Cell count
- PLT-O – Fluorescent optical platelet count and traditional impedance PLT counting
- Retic – Fluorescent reticulocyte count
- RET-He* – Reticulocyte Hemoglobin Content (incorporation of iron into red cell)
- IPF* – Immature platelet fraction (measurement of reticulated platelets)
- HPC* – Quantitative hematopoietic progenitor cell count as a screen for the presence of hematopoietic progenitor cells in peripheral blood and cord blood samples

White Blood Cell Population Data

- Detailed granularity/size/activity of WBC's
  - Permeation of cell membrane with maintenance of cell integrity
  - Staining intracellular structure with special dye (polymethine); activity of cells

Scattergram form Analyzer/3 Dimension

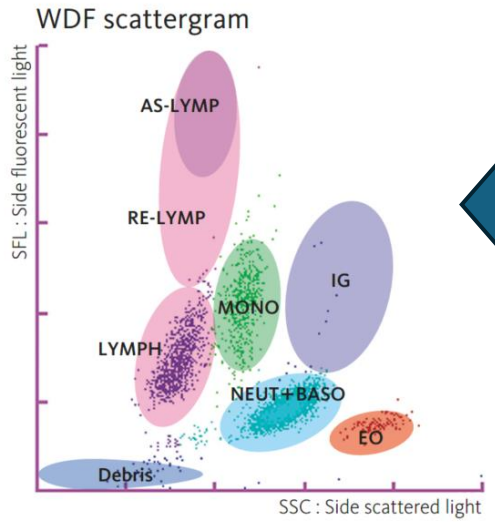


X-axis: SSC, cell complexity/granularity

Y-axis: SFL, cell metabolic activity

Z-axis: FSC, cell size

Advanced Clinical Hematology Parameters



	Hemolysis	Staining	Side fluorescent light (SFL)	Side scattered light (SSC)
Lymphocytes	▶	▶	Medium	Weak
Monocytes	▶	▶	Medium	Weak
Neutrophils	▶	▶	Weak	Medium
Eosinophils	▶	▶	Weak	Strong
Atypical Lymphocytes (AS-LYMP, RE-LYMP)	▶	▶	AS-LYMP: Strong RE-LYMP: Medium - Strong	Weak
Immature granulocytes	▶	▶	Medium–Strong	Medium

*This is a conceptual drawing.

Neutrophils

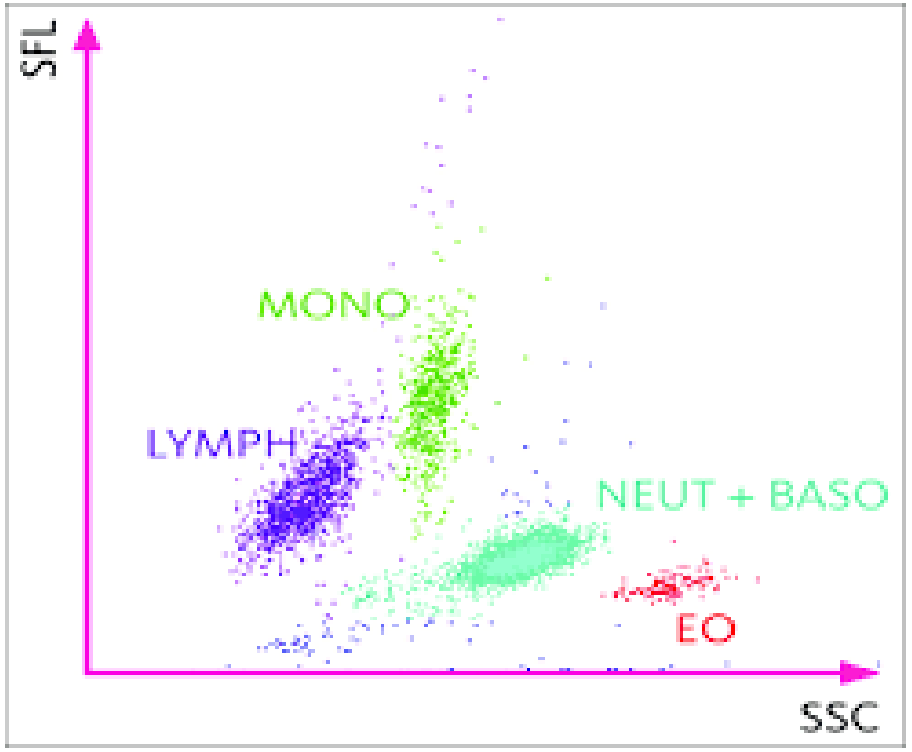
- Neutrophil granularity (x-axis on the scattergram)
  - complexity of neutrophils; nucleus lobularity, granulations
- Neutrophil reactivity (y-axis on the scattergram)
  - expressed in fluorescence intensity, related to the activation or immaturity of the population
- Neutrophil size (z-axis on the scattergram)
  - size of the cells

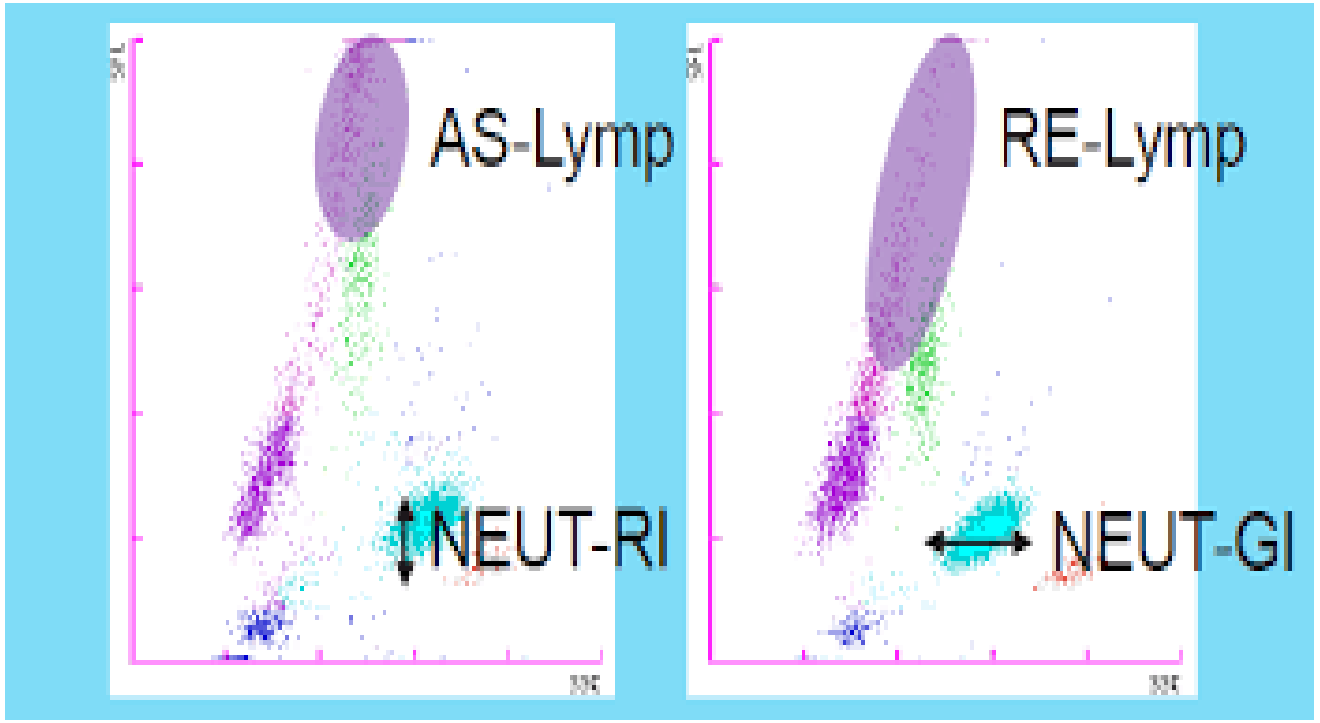
Neutrophils

- Immature granulocytes (IG)
  - Response to infection or stress reflecting active bone marrow response
  - Represented as %IG or total number of IG

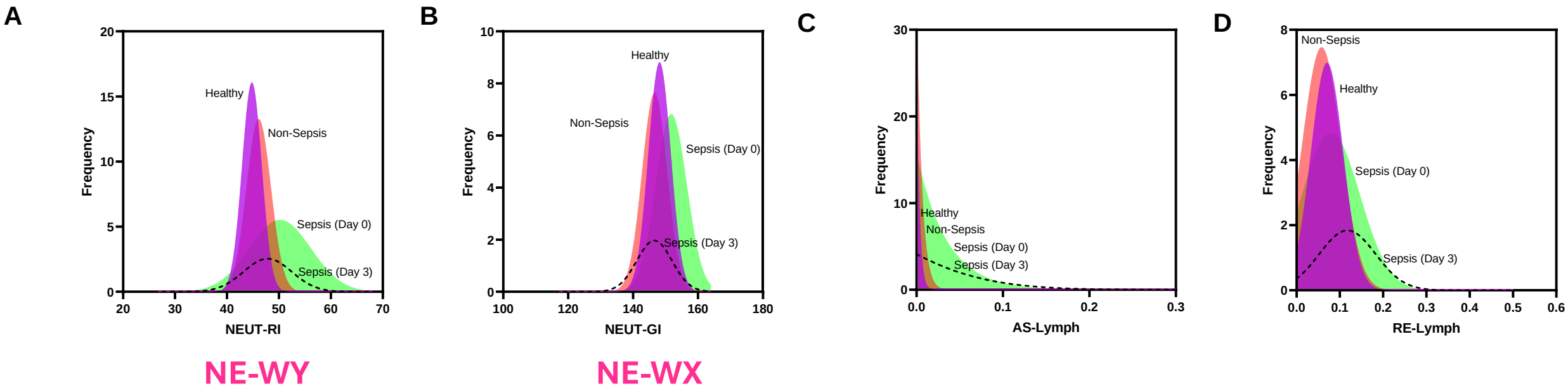
- Dispersion width
  - Representing dispersion of neutrophil distribution
    - ➢Cell heterogeneity

Scattergram from Analyzer





Dispersion/Heterogeneity of Cell Activity



NE-WY NE-WX

- Septic cases exhibit wider dispersion in cell activity results: more heterogeneity

Manuscript in Press

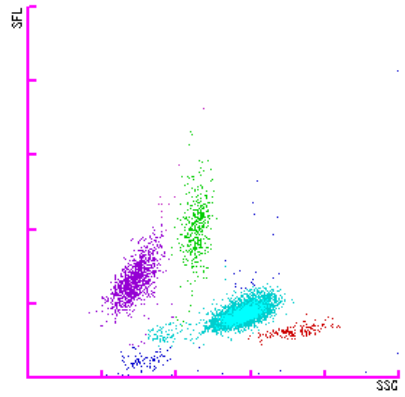
Lymphocytes

- Reactive lymphocytes
  - immune-stimulated lymphocytes with upregulated synthesis of inflammatory mediators, and/or immunoglobulins (antibodies), or both

- Antibody-secreting lymphocytes
  - antibody-secreting cells (plasma cells)

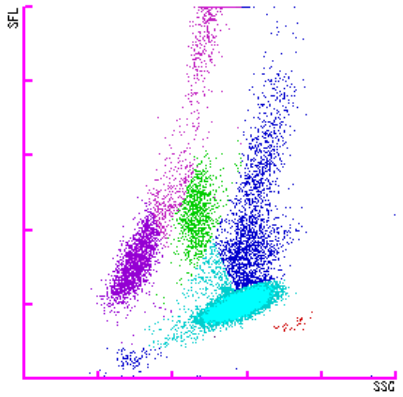
Infection Patterns

Normal



No infection

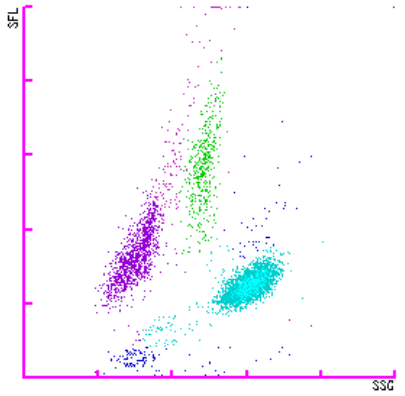
Extracellular bacteria



- Neutrophilia
- Immature granulocytes
- Humeral Lymph response
  T-cell independent

Leptospirosis

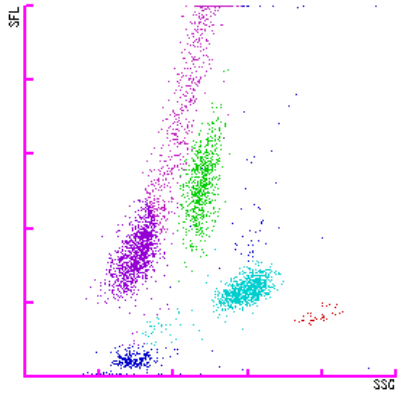
Intracellular bacteria



- Activation monocytes
- Cellular Lymph response
- No neutrophilia
- Activation neutrophils

Salmonella enterica Typhi

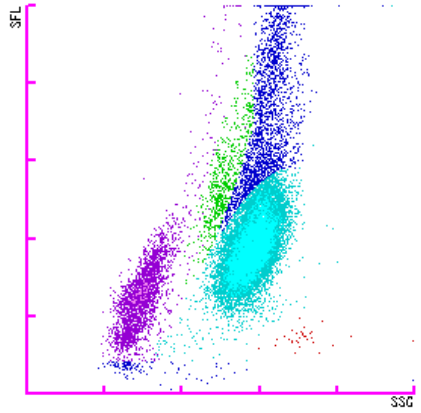
Virus infection



- Activation monocytes
- Humeral Lymph response
- No neutrophilia

Dengue

Bacterial Sepsis



- Neutrophilia
- Activation neutrophils
- Immature granulocytes
- Negative delta He

E-coli sepsis

Reticulocyte

- Ret-hemoglobin
  - Hemoglobin content of reticulocytes
  - Detection of iron status changes (more rapid than in mature red blood cells)

➢Detection of iron-deficient erythropoiesis and are useful markers of iron deficiency

Reticulocyte Hemoglobin Level

- Delta-Ret : difference between Hb level in reticulocyte and in mature red blood cells
  - a marker of an impaired hemoglobinization of newly formed reticulocytes occurring during inflammatory processes
    - representing impaired iron uptake in reticulocyte during inflammatory process

Prospective Cohort of Dengue Infection

- Pediatric patients aged < 18 years old
- Admitted at the pediatric wards
- CBC followed up daily with EIPs until convalescence
- Defining Day 0 as the day of defervescencd

Prospective Cohort of Dengue Infection

- Dengue infection
  - Positive NS1
  - Positive IgM with clinically compatible

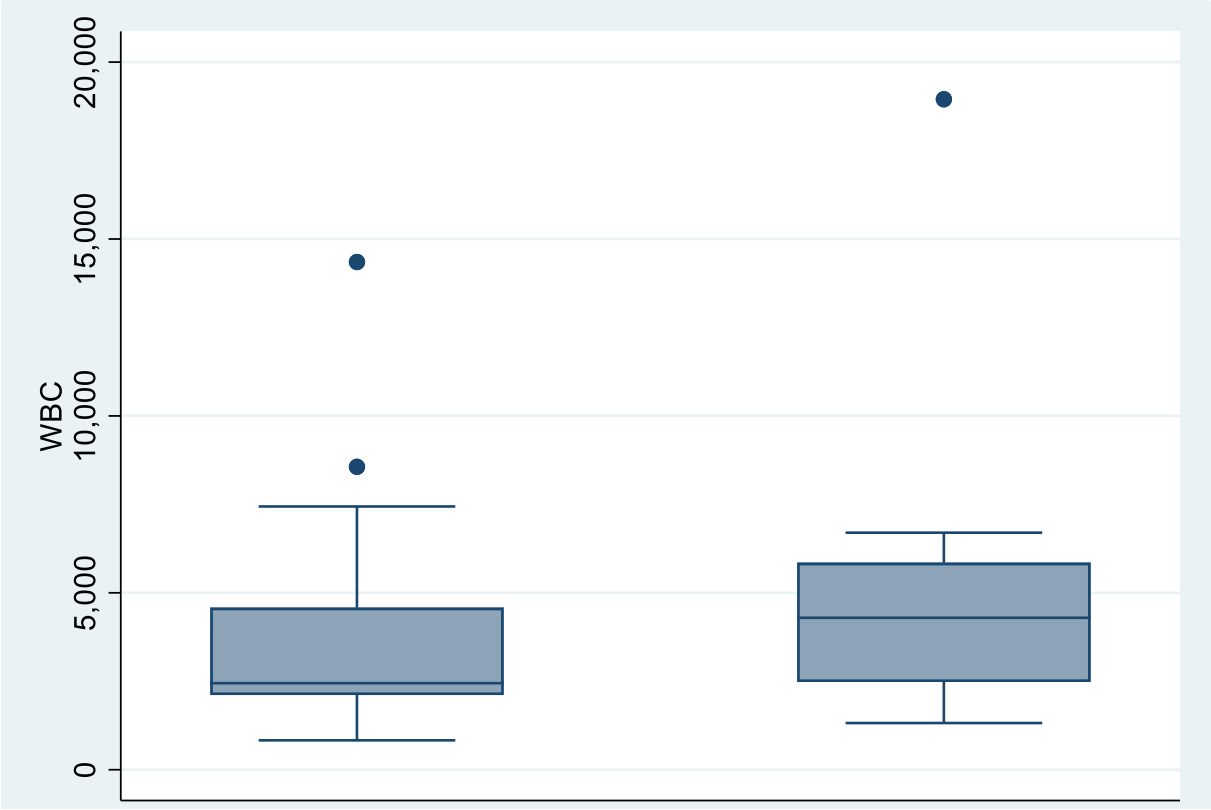
- Two groups of patients
  - Dengue fever
  - Dengue hemorrhagic fever: Dengue fever with leakage

Demographic Data

	N
Total Patients	53
Sex	
Male	24
Female	29
Age (years)	9.76 + 4.44
Diagnosis	
DF	31
DHF grade I	7
DHF grade II	7
DHF grade III	6
DHF grade IV	2

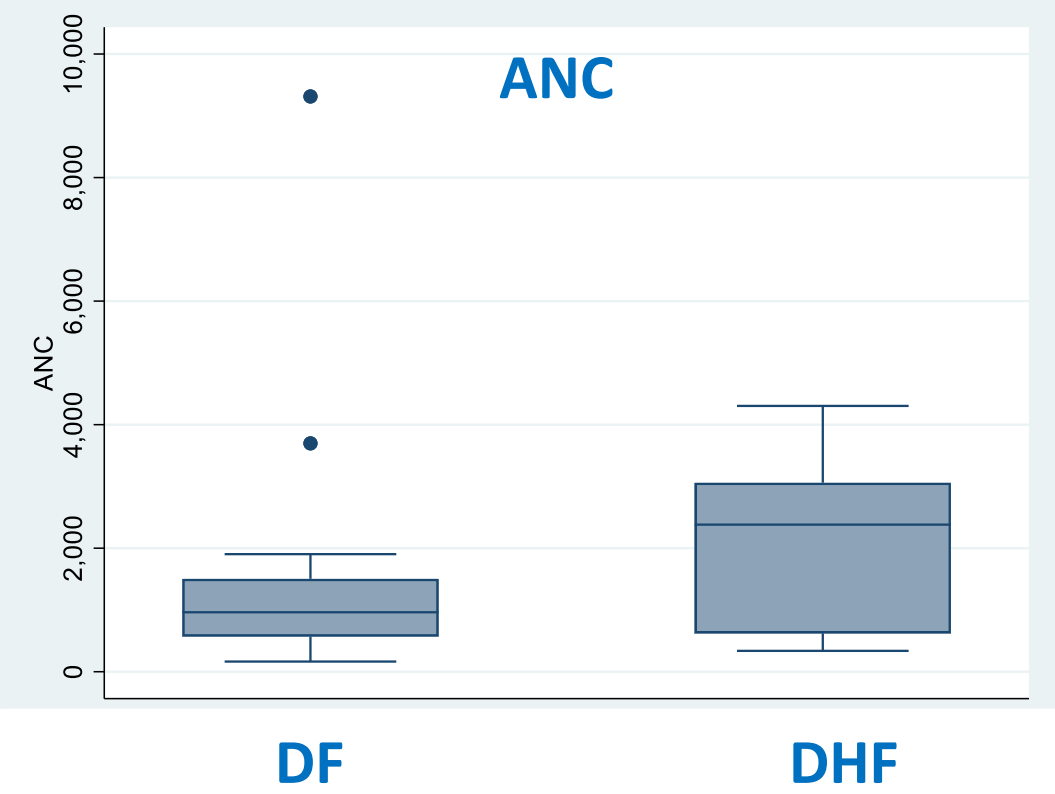
DF: dengue fever
DHF: dengue hemorrhagic fever

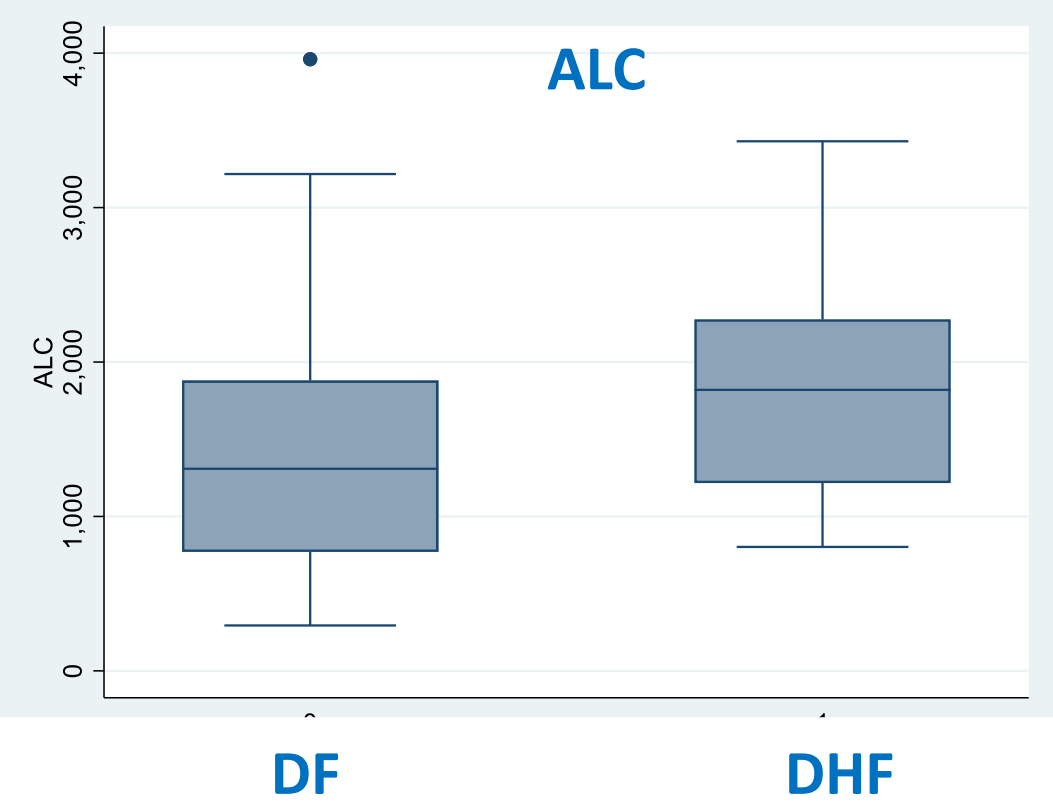
Hematological parameters in Dengue



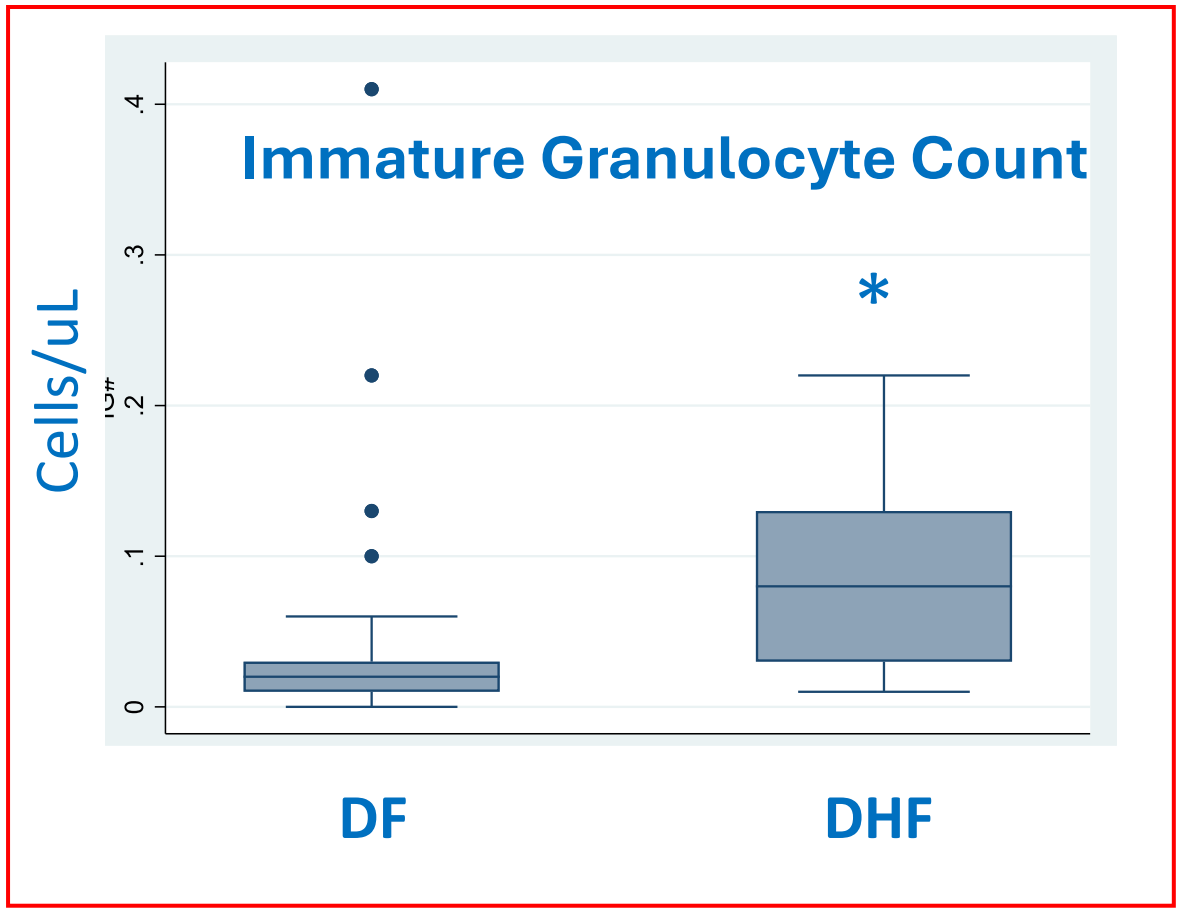
WBC at day 0
Defervescent day

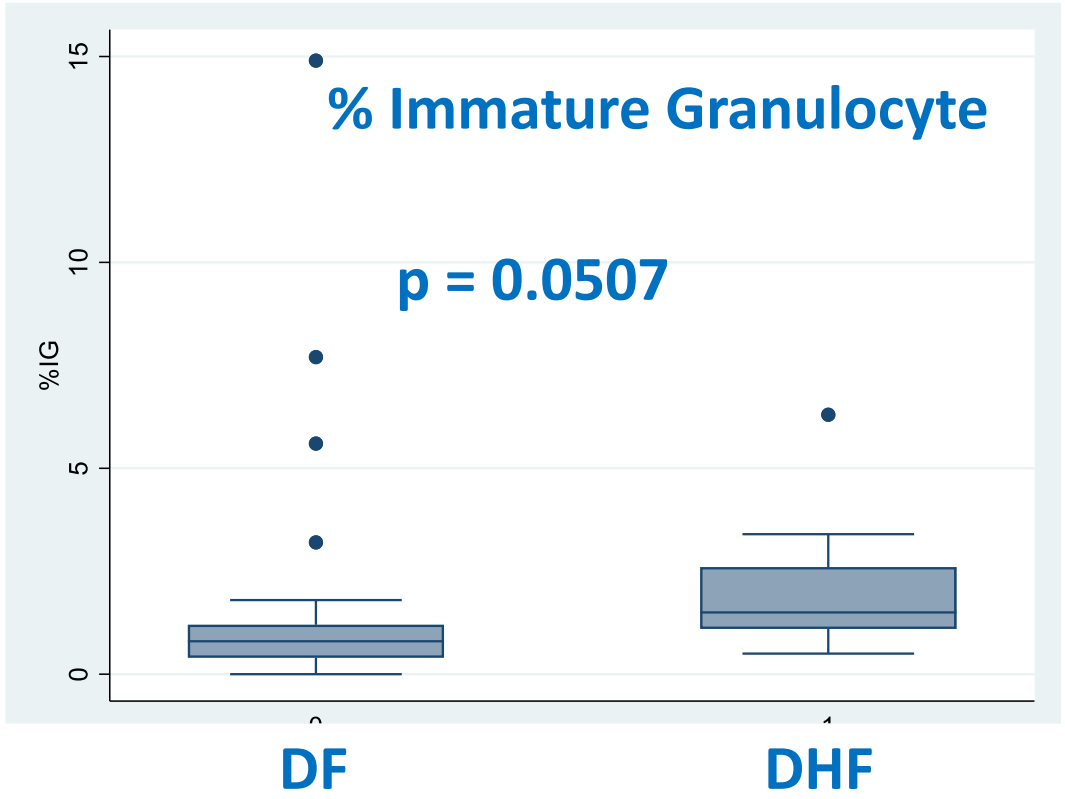
Hematological Parameters



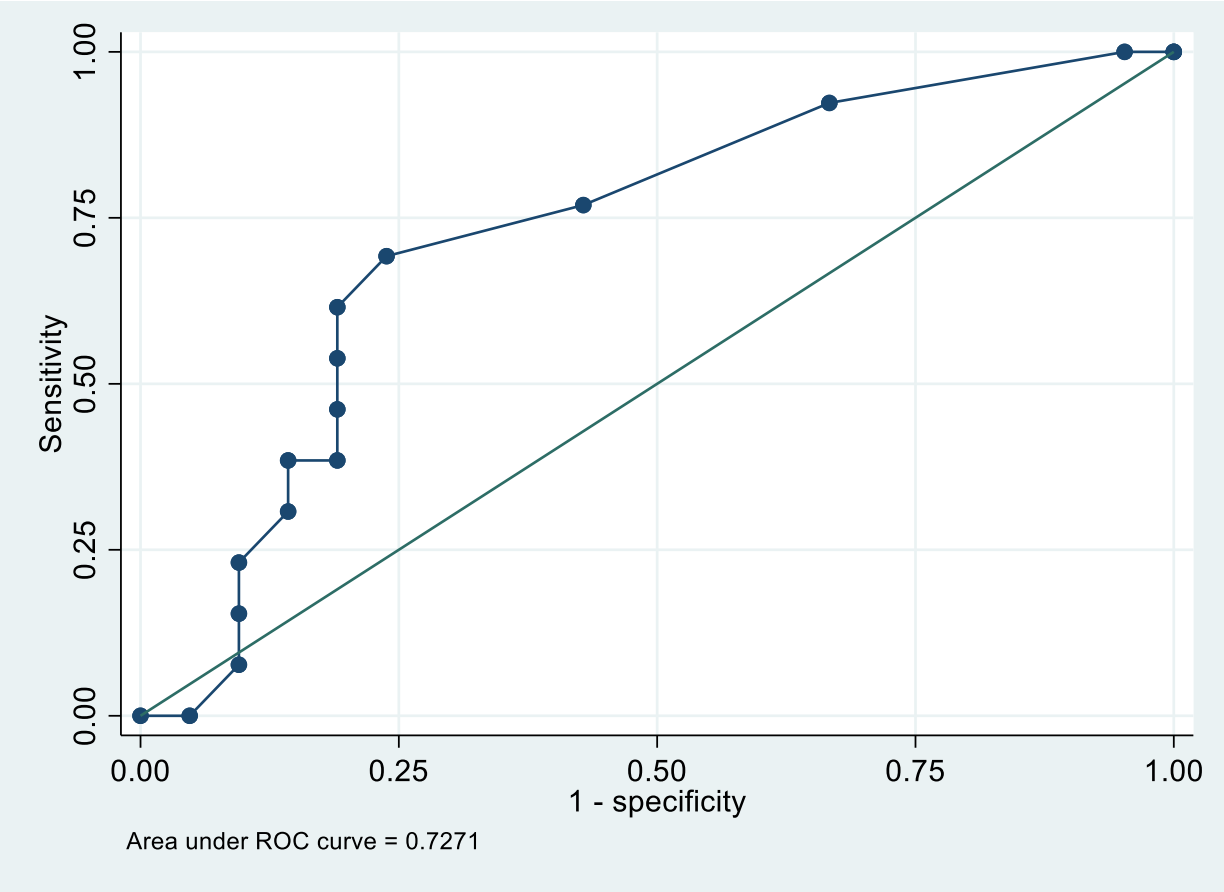


Hematological Parameters at Day 0





Hematological Parameters at Day 0

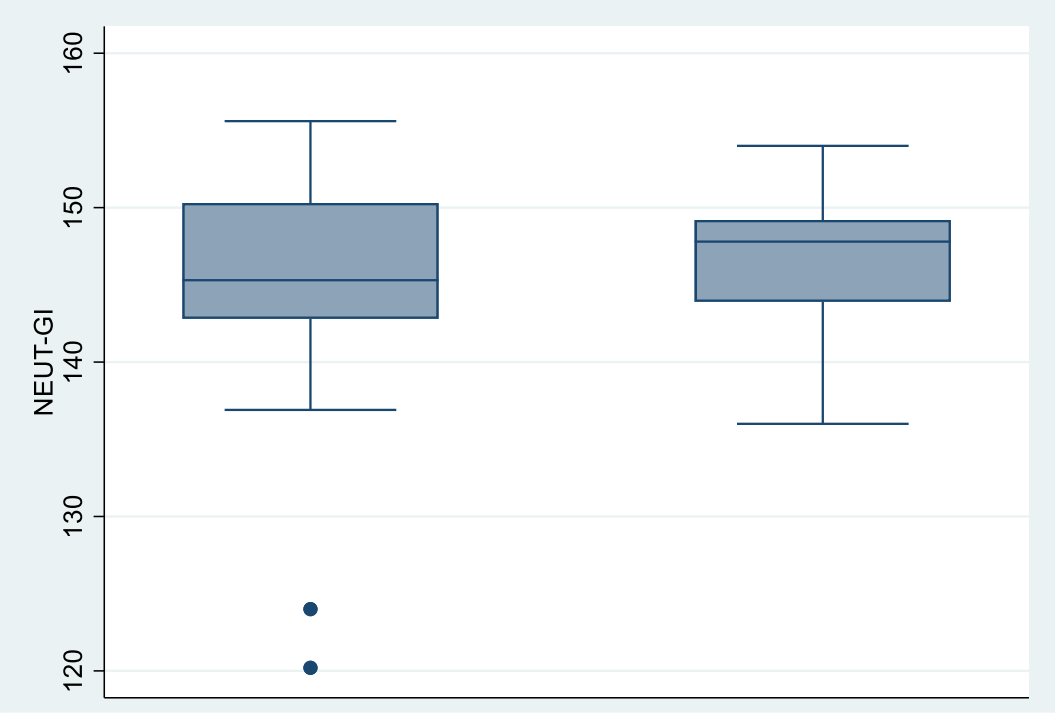


Immature Granulocyte Count
> 0.06 cells/uL

Sensitivity 70%
Specificity 75%

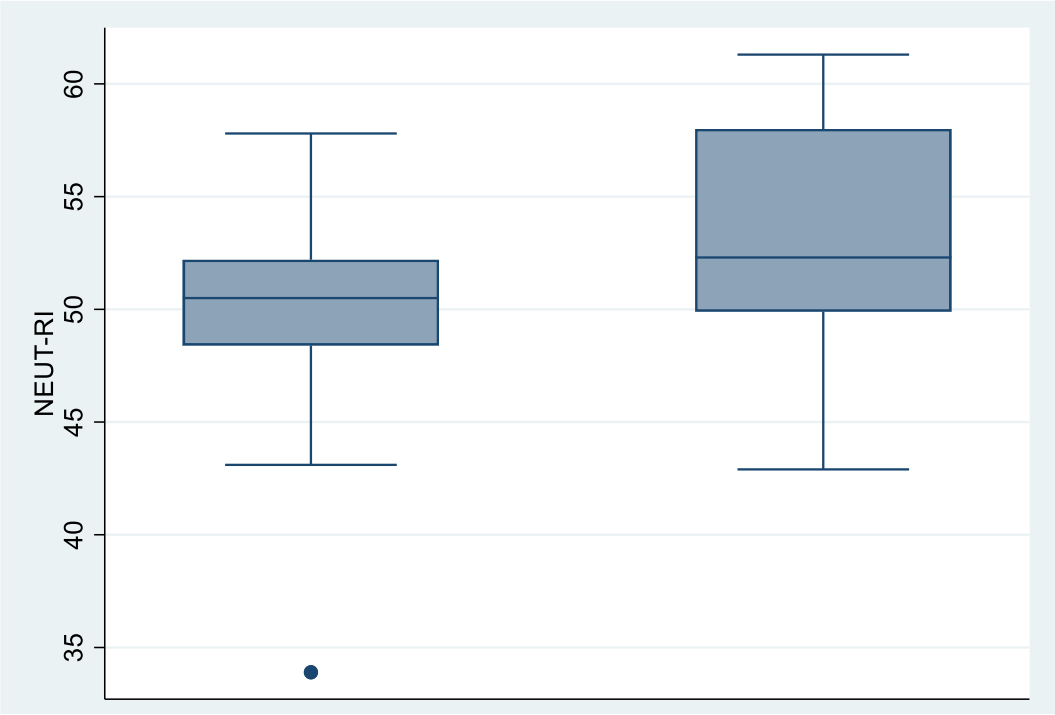
Hematological Parameters at Day 0

NEUTGI



DF DHF

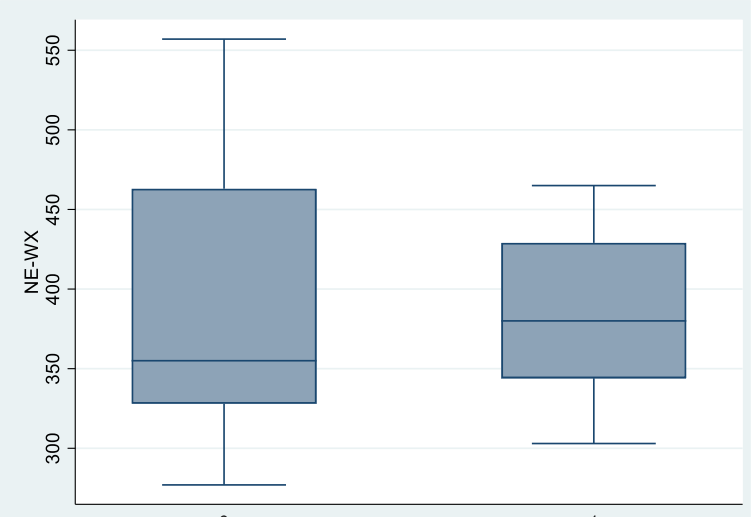
NEUTRI



DF DHF

Hematological Parameters at Day 0

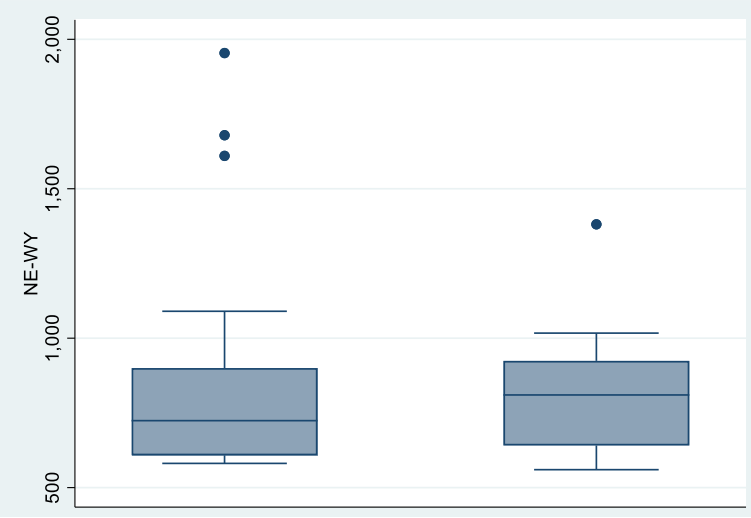
NEWX



DF DHF

Heterogeneity of granularity

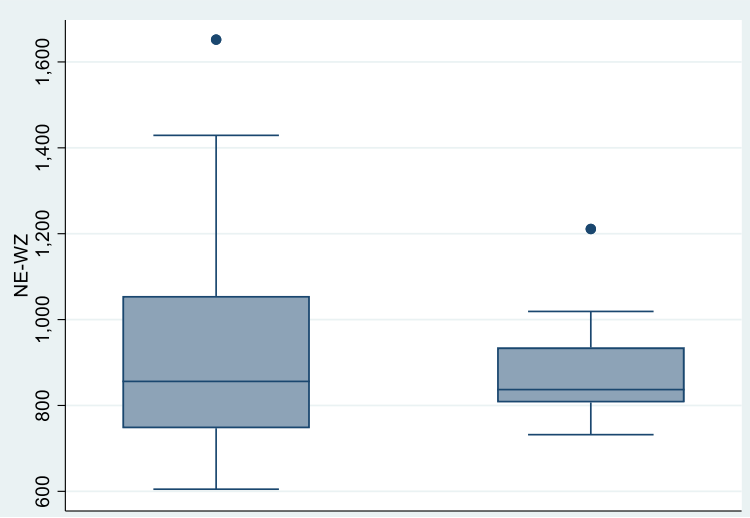
NEWY



DF DHF

Heterogeneity of fluorescence
(Metabolic activity)

NEWZ

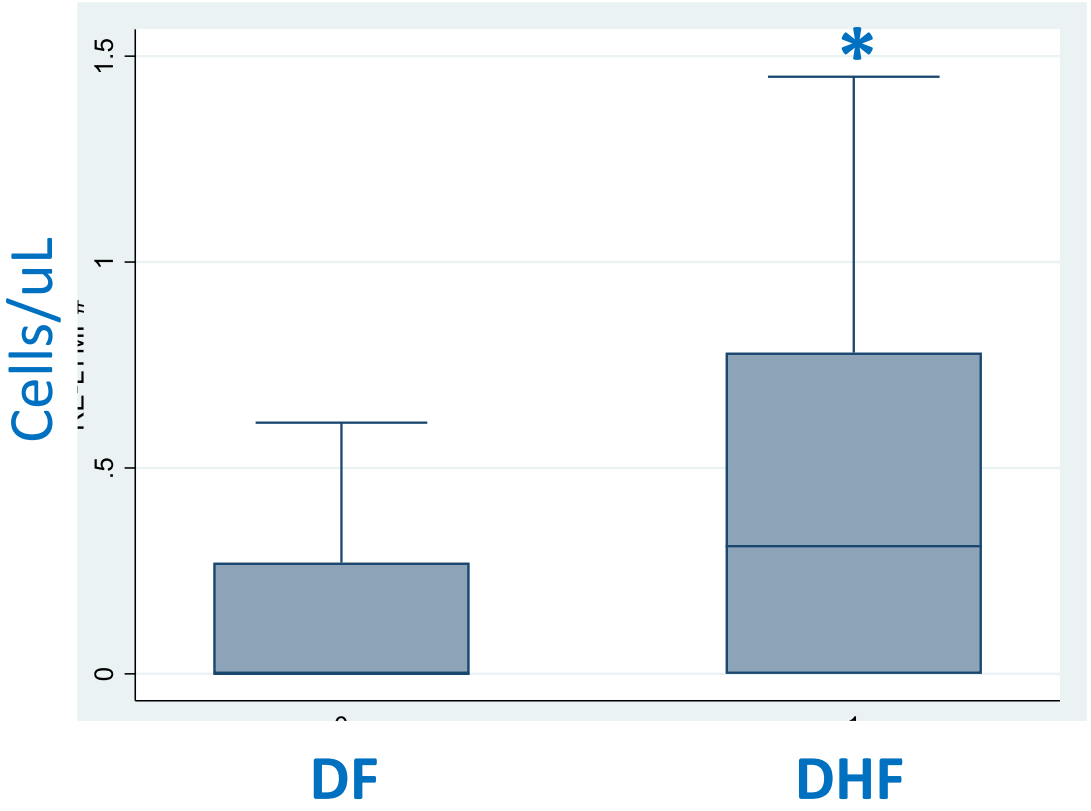


DF DHF

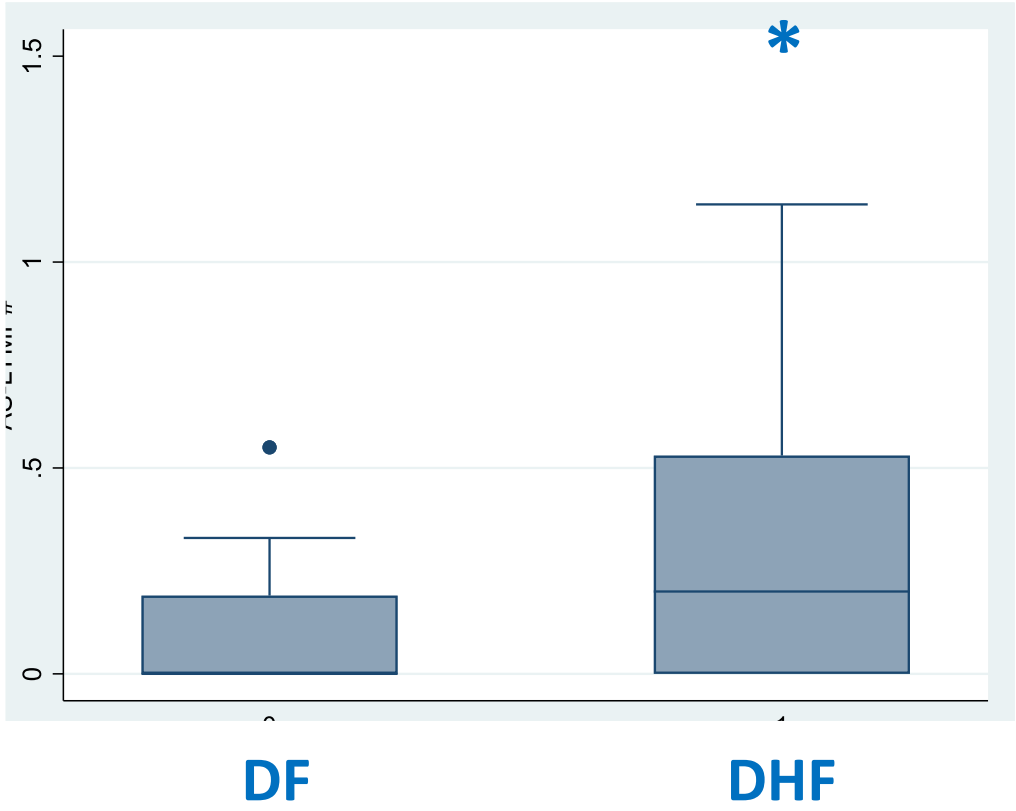
Heterogeneity of cell size

Hematological Parameters at Day 0

Reactive Lymphocyte

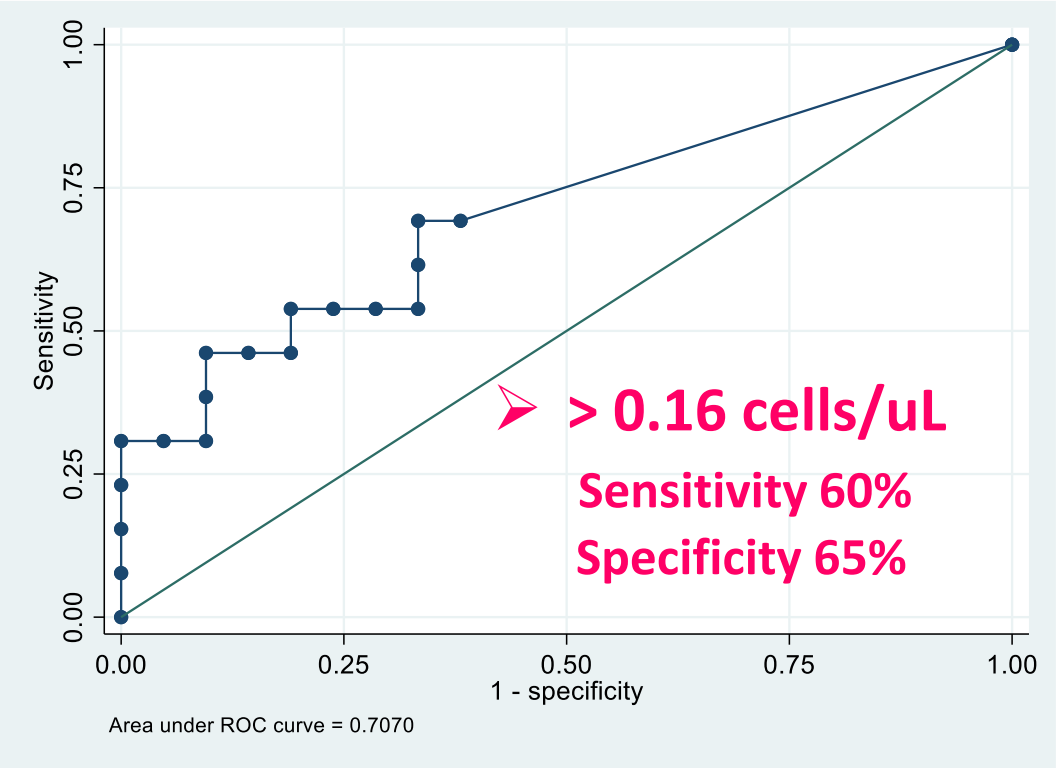


Antibody Secreting Lymphocyte

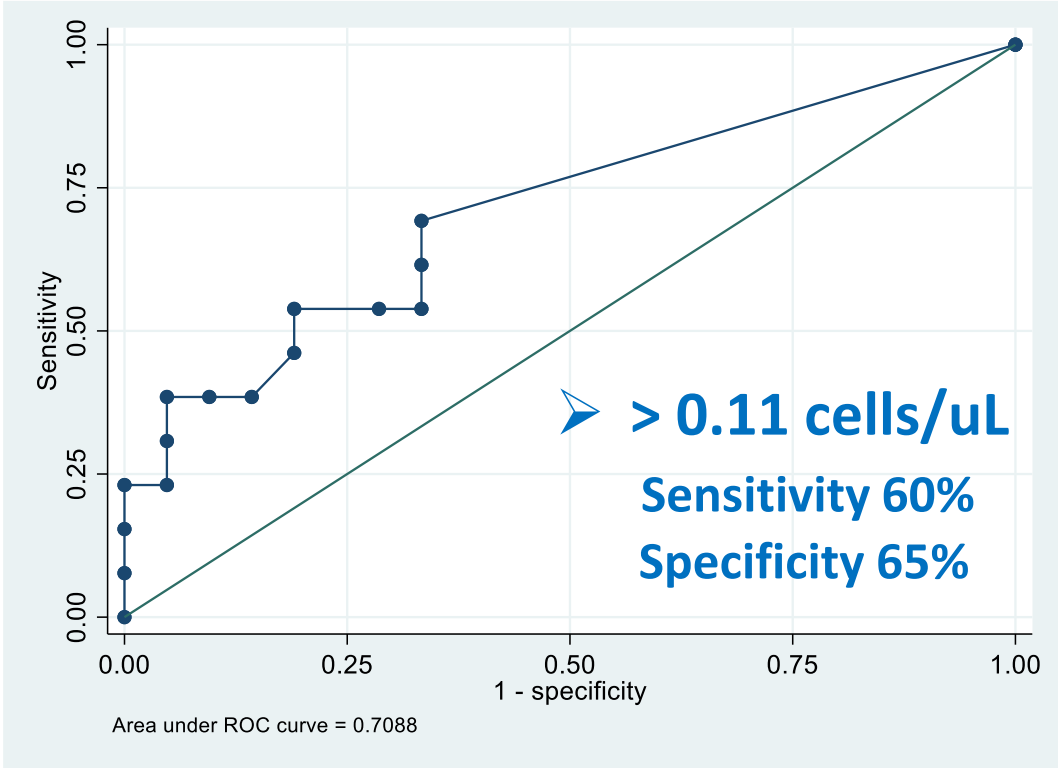


Hematological Parameters at Day 0

Reactive Lymphocyte

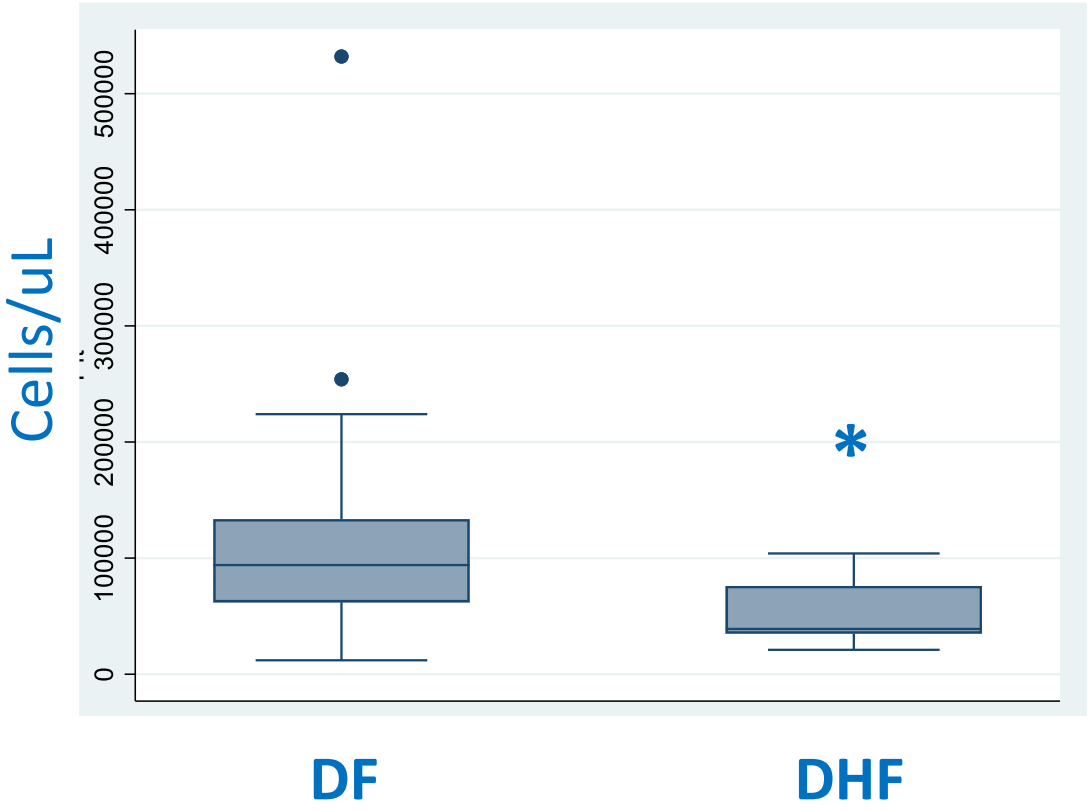


Antibody Secreting Lymphocyte

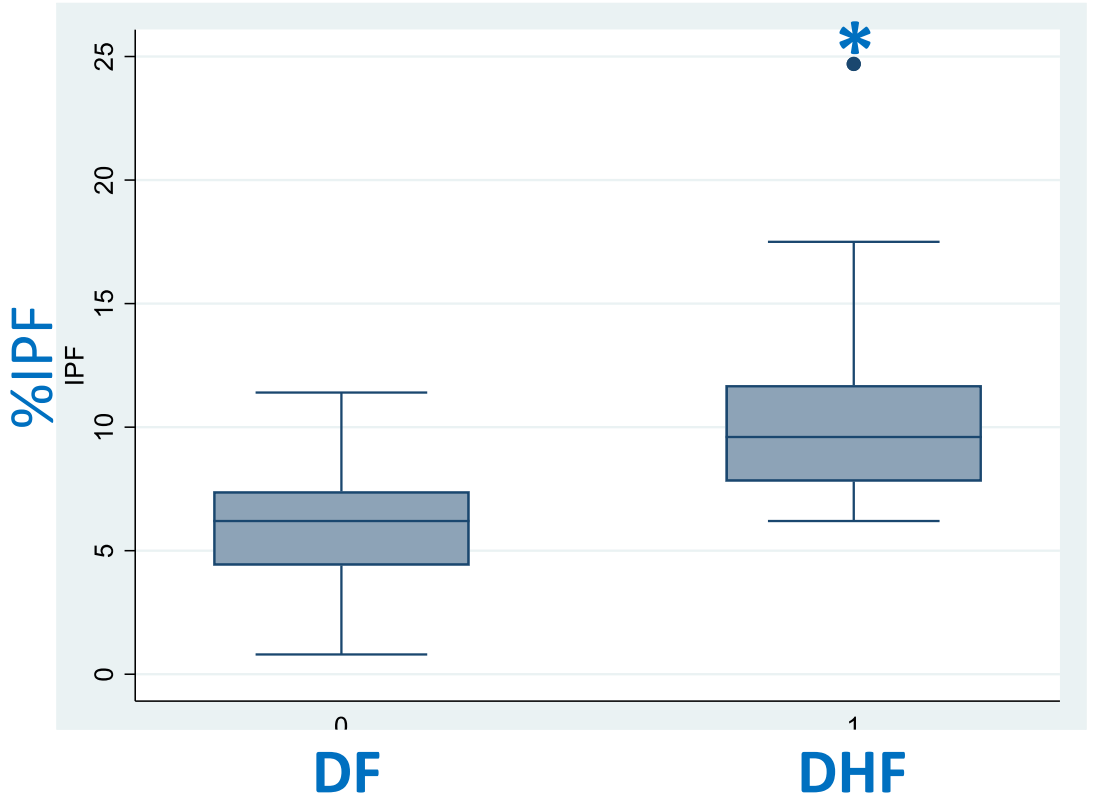


Hematological Parameters at Day 0

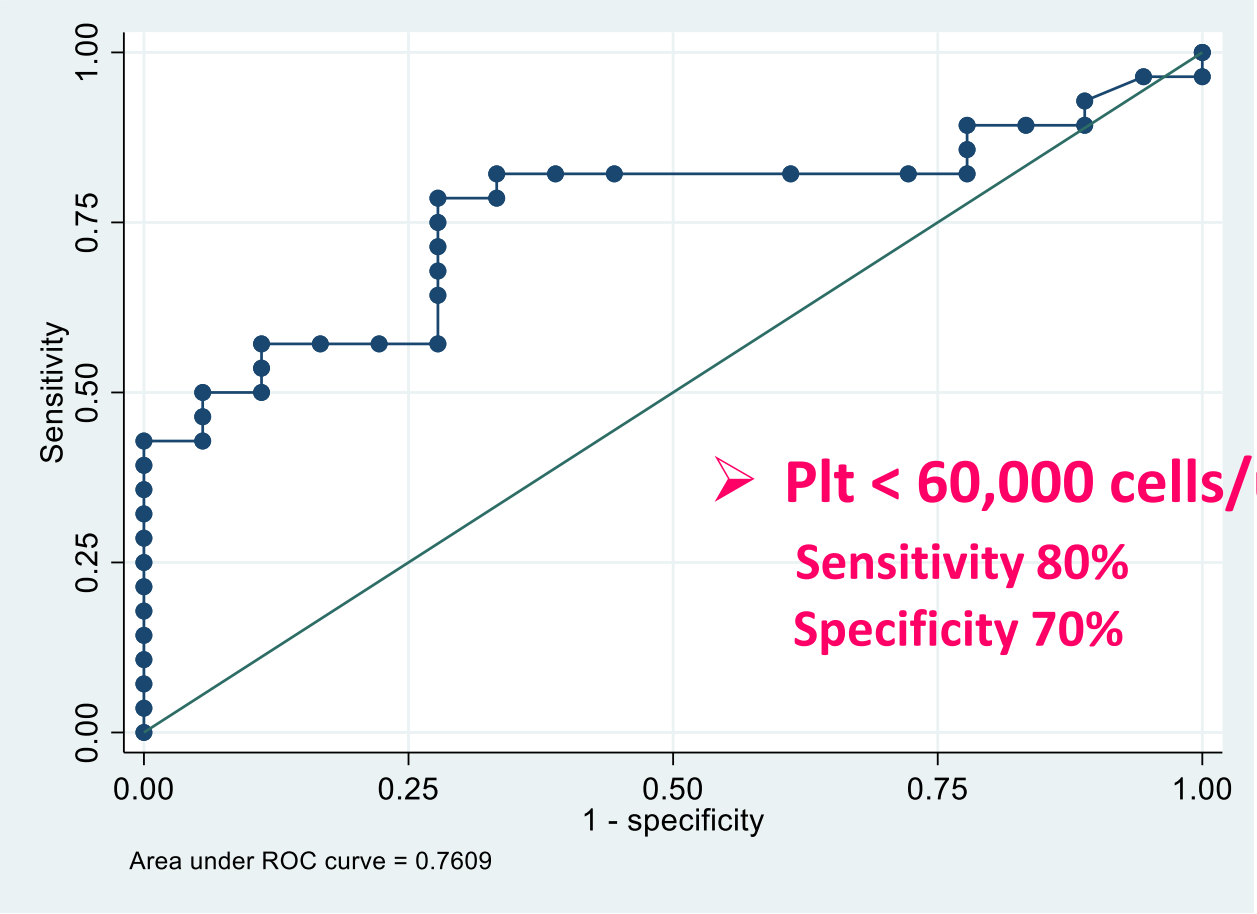
Platelet Count



%IPF

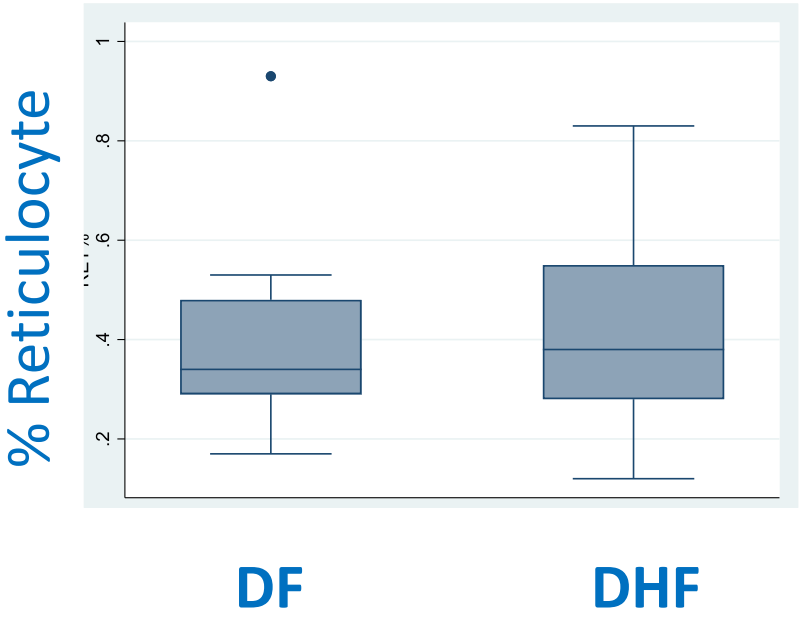


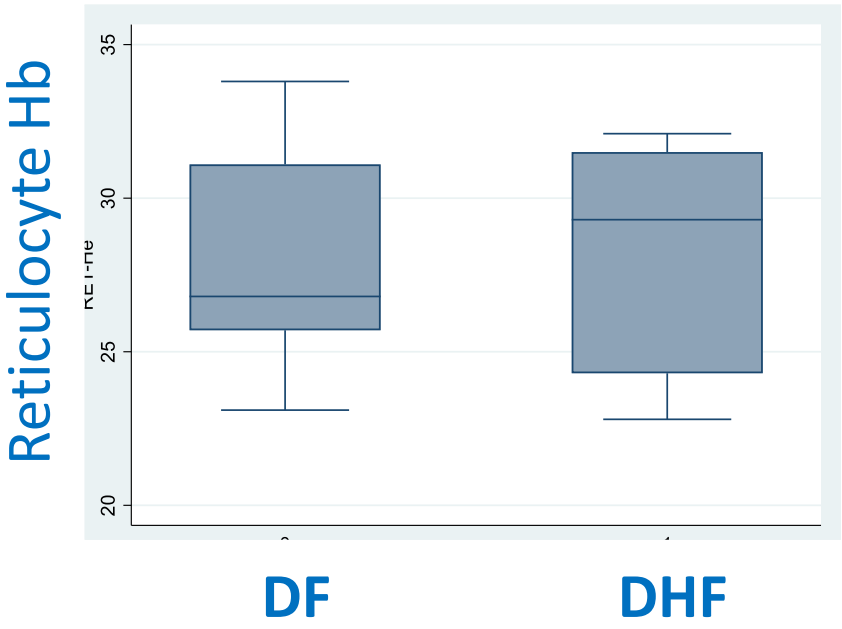
Hematological Parameters at Day 0

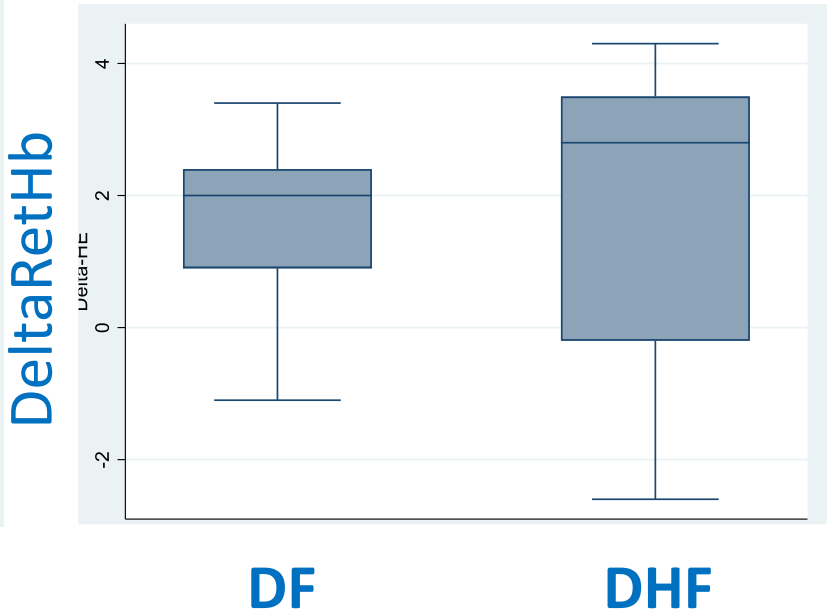


- **Plt < 60,000 cells/uL**
  - **Sensitivity 80%**
  - **Specificity 70%**

Hematological Parameters at Day 0







Cellular Response During Critical Phase

- More lymphocyte reactivity

- More Immature granulocyte count
  - ➢ Contributing to more pathogenicity?

- Lower platelet count but increased Immature platelet
  - ➢ Reflecting increased consumption with increased bone marrow activity

Cellular Response During Critical Phase

In Press, Corrected Proof What's this?

Review

Neutrophils – an understudied bystander in dengue?

Caroline Lin Lin Chua [1], Raika Francesca Morales [2], Po Ying Chia [2 3 4], Tsin Wen Yeo [2 3 4], Andrew Teo [2 3 5]

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Neutrophils existing in various phenotypes and functional statuses playing a role either in viral clearance or immunopathology

Immature Platelet Fraction in Dengue

International Journal of Infectious Diseases 110 (2021) 187–194

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Evaluation of immature platelet fraction as a marker of dengue fever progression

Kah Wai Looi[a], Yukari Matsui[b], Mari Kono[b], Chandramathi Samudi[a], Nozomi Kojima[b], Jin Xu Ong[a], Chin Aun Tan[a], Chong Siang Ang[a], Peter Hao Yuan Tan[a], Hemalatha Shamnugam[a], Shamala Devi Sekaran[c], Sharifah Faridah Syed Omar[a], Lucy Chai See Lum[a,*]

[a] *Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia*
[b] *Scientific Affairs, Sysmex Corporation, Kobe, Japan*
[c] *Faculty of Medicine & Health Sciences, UCSI University, Kuala Lumpur, Malaysia*

Severe Dengue

Significant increase in IPF at day 3-5 of fever

Severe Dengue associated with more platelet consumption

EIPs to Differentiate Common Febrile Illness

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

A novel diagnostic algorithm equipped on an automated hematology analyzer to differentiate between common causes of febrile illness in Southeast Asia

Susantina Prodjosoewojo[1,2], Silvita F. Riswari[1], Hofiya Djauhari[1], Herman Kosasih[3], L. Joost van Pelt[4], Bachti Alisjahbana[1,2], Andre J. van der Ven[5☯], Quirijn de Mast[5☯*]

1 Health Research Unit, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia, **2** Department of Internal Medicine, Hasan Sadikin General Hospital, Bandung, Indonesia, **3** Indonesia Research Partnership of Infectious Disease (INA-RESPOND), Jakarta, Indonesia, **4** Department of Laboratory Medicine, University Medical Centre Groningen, Groningen, The Netherlands, **5** Department of Internal Medicine, Radboud Center for Infectious Diseases, Radboud university medical center, Nijmegen, The Netherlands

☯ These authors contributed equally to this work.
* Quirijn.demast@radboudumc.nl

EIPs to Differentiate Common Febrile Illness

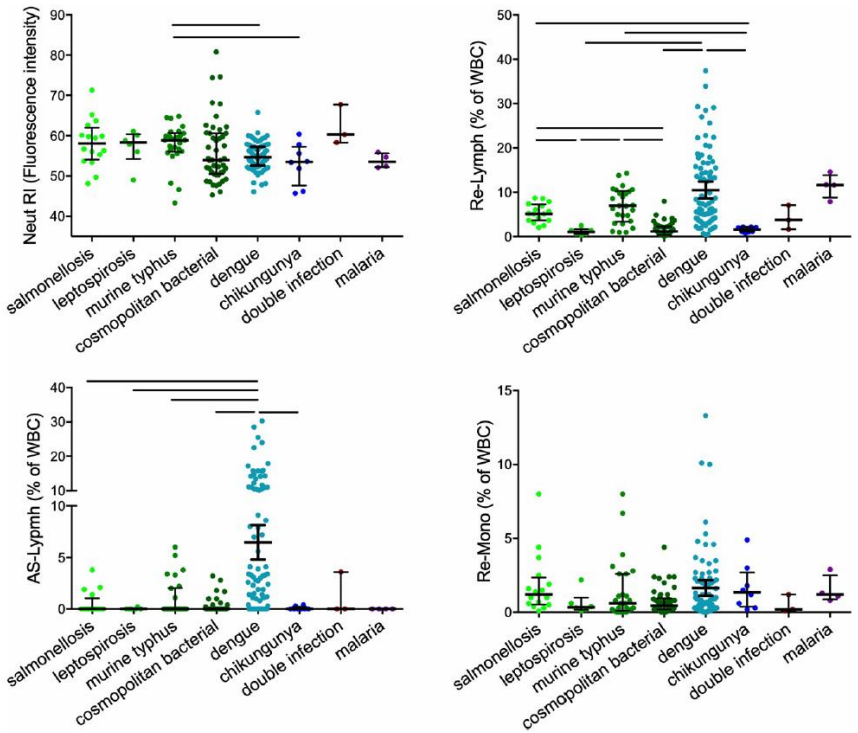

Fig 2. The number or percentage of activated neutrophils (Neut-RI), lymphocytes (Re-Lymph and AS-Lymph), and monocytes (Re-Mono) in patients with a proven infection. The lines indicate median with interquartile ranges. Differences were analyzed using Kruskal Wallis test with post-hoc tests. The lines indicate a statistically significant difference ($P<0.05$) considering correction of the P value for multiple testing (Benjamini-Hochberg). WBC, white blood cells.

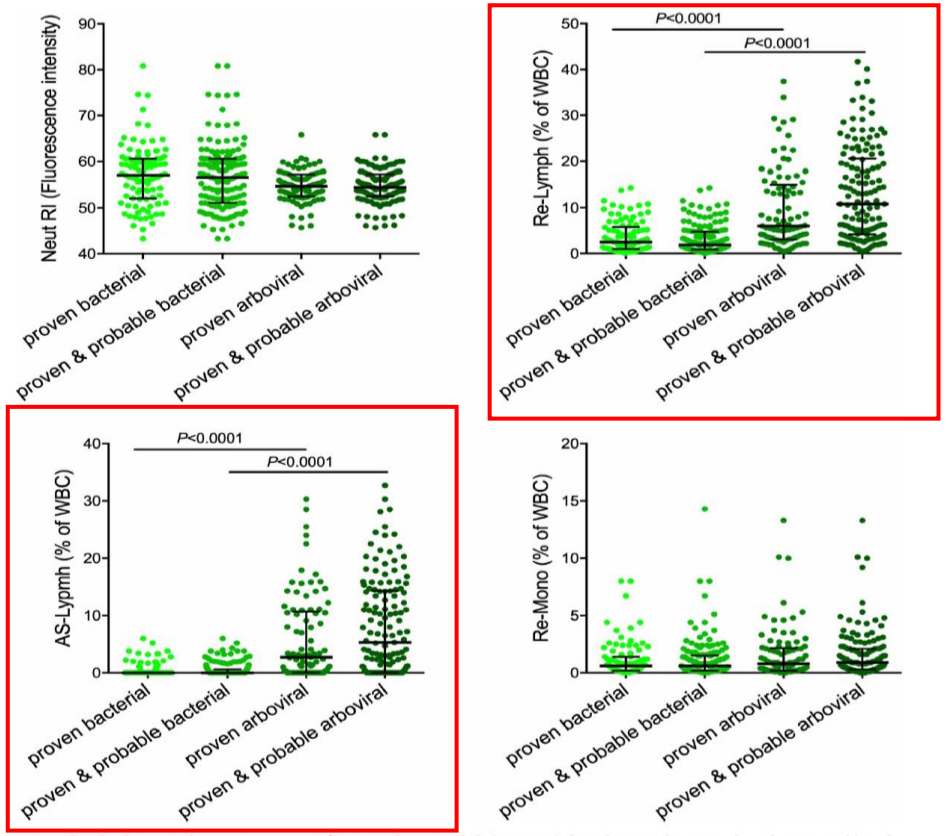

Fig 3. The absolute number or percentage of activated neutrophils (Neut-RI), lymphocytes (Re-Lymph and AS-Lymph) and monocytes (Re-Mono) in patients with proven or proven/probable infections, aggregated in bacterial or arboviral infections. The lines indicate median with interquartile ranges. Differences were analyzed using Kruskal Wallis test with post-hoc tests. WBC, white blood cells.

Prodjosoewojo S, et al. PLoS Negl Trop Dis. 2014;13:e0007183

Strongly associates to Septic cases

Preliminary Data from Chulalongkorn University

Clinical Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2015, Vol. 43(3) 435–451

DOI: 10.1177/0300060514557711
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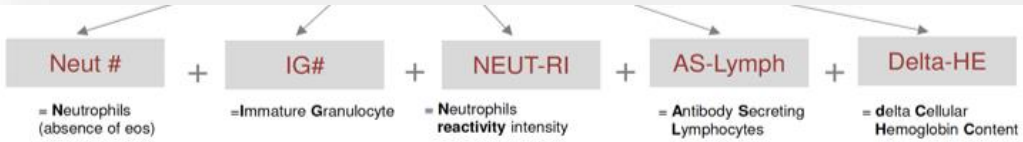
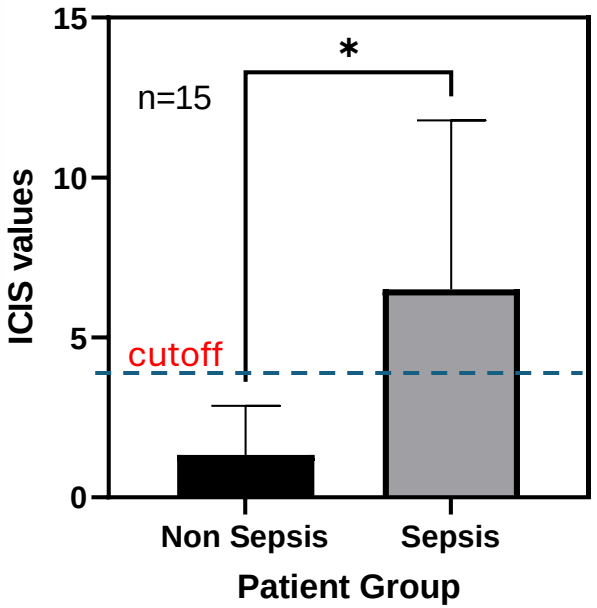
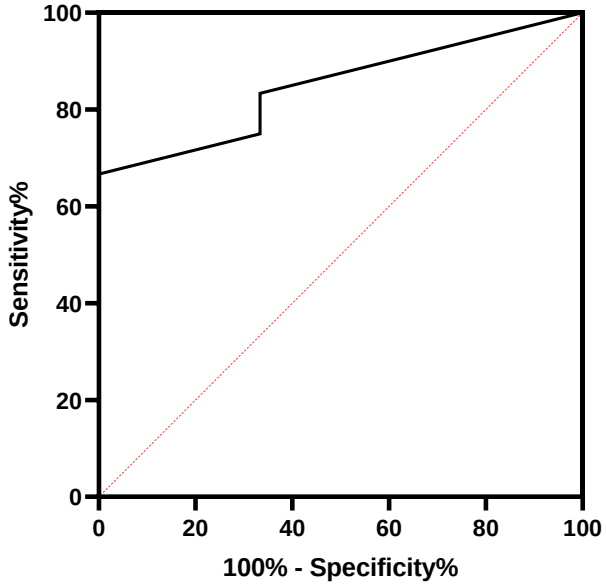
Intensive Care Infection Score – A new approach to distinguish between infectious and noninfectious processes in intensive care and medicosurgical patients

Karin Weimann[1], Mathias Zimmermann[2,3], Claudia D Spies[1], Klaus-Dieter Wernecke[4], Oldrich Vicherek[1], Irit Nachtigall[1], Sascha Tafelski[1] and Andreas Weimann[2,3]



Sysmex Reportable Parameters





Area under the ROC curve	
Area	0.847
Std. Error	0.105
95% confidence interval	0.642 to 1.00
P value	0.0712

Table 3. Receiver operating characteristic curve analysis to determine the best cut-off value for each of four variables, to discriminate between an intensive care unit (ICU) patient group ($n = 175$) with or without infection within the first 5 days of ICU stay. Sensitivity and specificity from calculated best cut-off values are also shown for each variable.

	Variable			
Parameter	CRP	PCT	WBC	ICIS
AUC	0.715	0.689	0.613	0.785
Best cut-off value	>4.8 mg/l	>0.17 µg/l	>13.2 cells/nl	>3
Sensitivity	79.31 (73.56, 85.06)	71.02 (60.63, 81.42)	26.12 (16.61, 35.64)	70.20 (61.03, 79.38)
Statistical significance	NS	NS	$P < 0.001$	–
Specificity	49.78 (42.57, 56.99)	59.65 (51.50, 67.80)	96.25 (93.77, 98.73)	78.68 (73.71, 83.65)
Statistical significance	NS	$P < 0.001$	$P < 0.001$	–

Sensitivity and specificity data presented as % (95% confidence interval).
Statistically significant differences in sensitivity or specificity between ICIS and other variables ($P < 0.05$; ratio estimator).
AUC, area under the curve; CRP, C-reactive protein; ICIS, Intensive Care Infection Score; PCT, procalcitonin; WBC, white blood cell count.

ICIS: Intensive Care Infection Score

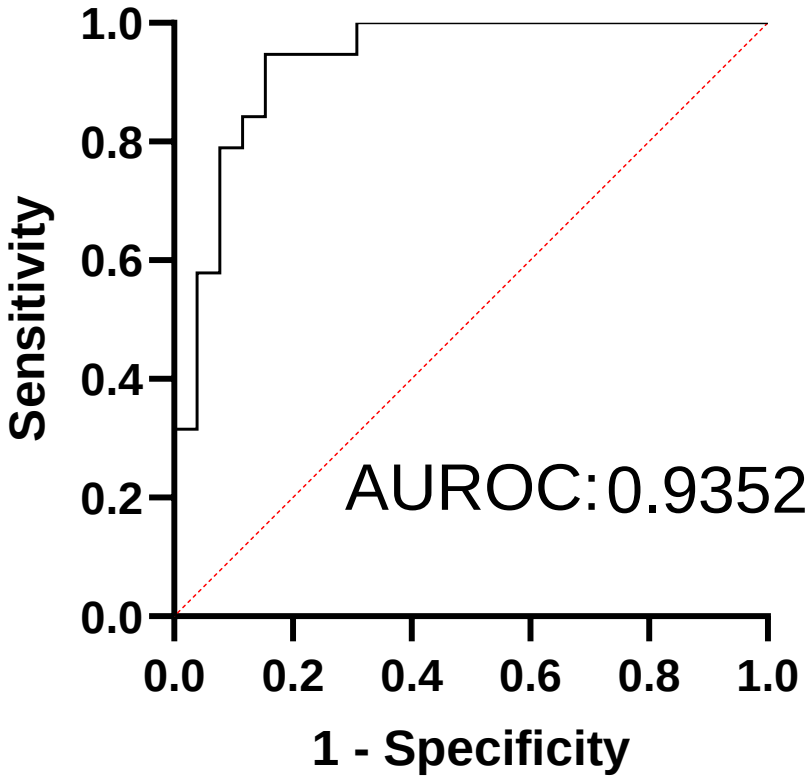
Weimann K et al. Jou Int Med Res 2015

Possible Modeling for DHF Prediction

- At the day of defervescent
  - Immature granulocyte count
  - Reactive lymphocyte count
  - Antibody secreting lymphocyte count
  - Platelet count/IPF

Dengue Fever (DF) vs. Dengue Hemorrhagic Fever (DHF)

A Cross-Sectional Approach Using CBC Parameters



- **CBC Parameters**: PLT, AS-Lymph, RE-Lymph, IG, IPF
- **Cross-sectional view of patient severity** from dengue fever to hemorrhagic fever
- A **multi-parameter approach** enhances **clinical decision-making**

Accuracy of Model

Classification table	Predicted 0	Predicted 1	Total	% Correctly classified
Observed DF	22	4	26	84.62
Observed DHF	2	17	19	89.47
Total	24	21	45	86.67

Negative predictive power (%)	91.67
Positive predictive power (%)	80.95

Acknowledgement

- Department of Pediatrics
- Department of Pathology
  - Faculty of Medicine Ramathibodi Hospital, Mahidol University

- Sysmex, Co Ltd.