



MEDICAL JOURNAL IMJ HEALTH

**VOLUME-11, ISSUE-7,
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Preface

We would like to present, with great pleasure, the inaugural volume-11, Issue-7, July 2025, of a scholarly journal, *International Multispeciality Journal of Health*. This journal is part of the AD Publications series *in the field of Medical, Health and Pharmaceutical Research Development*, and is devoted to the gamut of Medical, Health and Pharmaceutical issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Medical, Health and Pharmaceutical as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical statistics investigations. Its mission is to become a voice of the Medical, Health and Pharmaceutical community, addressing researchers and practitioners in below areas

Clinical Specialty and Super-specialty Medical Science:

It includes articles related to General Medicine, General Surgery, Gynecology & Obstetrics, Pediatrics, Anesthesia, Ophthalmology, Orthopedics, Otorhinolaryngology (ENT), Physical Medicine & Rehabilitation, Dermatology & Venereology, Psychiatry, Radio Diagnosis, Cardiology Medicine, Cardiothoracic Surgery, Neurology Medicine, Neurosurgery, Pediatric Surgery, Plastic Surgery, Gastroenterology, Gastrointestinal Surgery, Pulmonary Medicine, Immunology & Immunogenetics, Transfusion Medicine (Blood Bank), Hematology, Biomedical Engineering, Biophysics, Biostatistics, Biotechnology, Health Administration, Health Planning and Management, Hospital Management, Nephrology, Urology, Endocrinology, Reproductive Biology, Radiotherapy, Oncology and Geriatric Medicine.

Para-clinical Medical Science:

It includes articles related to Pathology, Microbiology, Forensic Medicine and Toxicology, Community Medicine and Pharmacology.

Basic Medical Science:

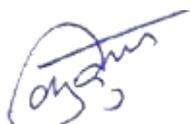
It includes articles related to Anatomy, Physiology and Biochemistry.

Spiritual Health Science:

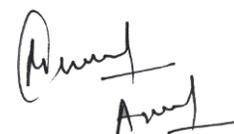
It includes articles related to Yoga, Meditation, Pranayam and Chakra-healing.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within

that community who supported the idea of creating a new Research with *IMJ Health*. We are certain that this issue will be followed by many others, reporting new developments in the Medical, Health and Pharmaceutical Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IMJ Health* readers and will stimulate further research into the vibrant area of Medical, Health and Pharmaceutical Research.



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Research Area: Pediatric Surgery & Laparoscopy.

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INK-4a Downregulated in P53 and P21 Mutation Induce BAT and VSMCs Proliferation: Obesity and Hypertension

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Abstract—

Background: Epidemiology of obesity and hypertension are in high prevalence on low- and middle-neighborhood socioeconomic status (nSES) area. Later, the subjects become Diabetes and Chronic Kidney Diseases (CKDs) 1-5, and cancer due to p53, p21 and p16 mutation in AFB1 exposure population. Neglected p16/INK4a upregulated in early years which induce stunting, later induce proliferation of BAT (Uncoupling Protein UCPs132) or central Obesity, and vascular smooth muscle cells (VSMCs)/Hypertension.

Aims: p16 first upregulated in p21 and p53 mutation due to AFB1 exposure cause stunting, then when p16 downregulated in the older age induce cancer cells proliferation. This study recorded more the proliferation, reveal proliferation of BAT and VSMCs in healthy young age, in the downregulated p16 stage.

Method: Review article of Systematic Review and Meta-Analysis references of p16/INK-4a decreasing induced proliferation, also p16 Knock Out (KO) in p21/ p53 mutation population (AFB1 exposure).

Result: Mutation of p21/p53 in AFB1 exposure, induce p16/INK-4a downregulated in central Obese & VSMCs patients. Silencing/ downregulated p16/INK-4a induced proliferation, but still controversial with the upregulated in p53/p21 mutation (before).

Discussion: Proliferation of UCPs132 BAT cells, proliferation of SMCs, SGLT-2/GLP-1 therapy.

Conclusion: proliferation of BAT UCPs132 (central obesity) or VSMCs (hypertension) is due to downregulated p16 under p21/p53 mutation due to AFB1 exposure in low- and middle-nSES area population.

Keywords— AFB1 exposure population, Hypertension, INK-4a, Obesity, p21/p53.

I. INTRODUCTION

There are so many controversies in Obesity and Diabetes, especially in nature or nurture,¹ geographical or eating habit,² also in p53-p21-p16 upregulated or downregulated in this high prevalence area. p16 is a protein that slows cell division, by slowing the progression of the cell cycle from the G1 phase to the S phase, thereby acting as a tumor suppressor. It is encoded by the P16 gene, the name of p16 is derived from its molecular weight 16 kD, and the similar name INK4 refers to its role in inhibiting CKD4 (cyclin-dependent kinase) involved in regulation of the cell cycle, act as tumor suppressor cells and stunting. Down regulated of p16/INK-4a (by p53 mutation) in UCPs 132,³ VSMCs,⁴ and later proliferation of cancer cells in AFB1 exposure population.⁵ Uncoupling proteins are abundant in mitochondria of Brown Adipose Tissue (BAT) cells, function as thermogenesis metabolism, low of ATP synthesis, like the metabolism of hibernate bears.

The down regulation of p16 is controversial with upregulated p16Ink4, is as a suppressor protein that considered a tumor suppressor protein because both are high in p53 and p21 mutation in cancer patients and senescence.^{5,6} The Paradoxical downregulated⁷ of p16 mRNA with advancing age in BRAF-mutated polyps/adenomas indicates a senescence barrier in the serrated route to colon cancer,⁸ unlike healthy cells. P16 upregulated as a compensate of the failure of p53 and p21 tumor suppressor cells.^{6,7} The p16 pathway is a key regulator of the cell cycle, which controls the passage of cells from G, to S phase. P16 targets CDK4 and prevents Rb phosphorylation. Similar to p16 protein overexpression, is cause by viral E7 protein

(indirect marker of HPV-induced squamous cell carcinoma in head and neck (SCCHN)).⁹ The p16 gene is often mutated or epigenetically-silenced in SCCHN.⁹ This made the survival rate low, and also the prognosis. P16 inhibits cancer cell growth by downregulating eEF1A2 through a direct interaction.¹⁰ So, in p53 and p21 mutation in AFB1 exposure, p16 become the last defense, p16 +/+ or p16 -/- which induced senescence, also depends on Estrogen (year of age), to become Breast cancer after menopause years.^{11,12} Guo et al, 2017, demonstrate that P16 may be associated with the Cigarette smoke extract (CSE)-induced proliferation of vascular smooth muscle cells (VSMCs),¹³ suggesting that P16 serves a role in the development of CS-associated vascular diseases. Cell proliferation and cell cycle distribution were evaluate by flow cytometry, Western blotting for examine protein expression, and bisulfite genomic sequencing polymerase chain reaction was used to determine the hypermethylation of P16 promotor CpG island (repeat CGG).¹⁴ Concentration- and time-dependent exposure induced a downregulation in P16 (all $P < 0.05$).¹³ Significant decrease in gene assay transcriptional activity reduced P16 protein expression in human aortic smooth muscle cells (HAOSMCs) (both $P < 0.01$). Hypermethylation (silencing), mutation, or deletion leads to downregulation of the P16 gene.¹⁴

II. METHOD

Review article of p16 downregulated that induced proliferation, which supported UCPs132/ Brown Adipose Tissue (BAT) and VSMCs proliferation in stunting, obesity, and Diabetes Mellitus high prevalence area/ populations.⁵ Using my Library, and academic search engine mainly ScienceDirect and EBSCOhost. Keywords of Bayesian network of p16 and downregulated were used, Systematic Review and Meta-Analysis references are preferable. P53-p21-p16 downregulated in AFB1 exposure food depends on keywords urine AFM1 in the population area of obesity and hypertension high prevalence. This epidemiology record is long before p16 downregulation is associated with AFB1 exposure in food, but it will be interesting that p16INK-4a downregulated induced UCPs132 cells and VSMCs proliferation in low- and middle-nSES population area.

III. RESULT

The UCPs132 BAT and VSMCs proliferation in AFB1 exposure population, should be supported by down-regulation of p16 gen or epigenetically. P53-p21 mutation due to AFB1 exposure, induced up-regulation of p16.⁶ Paradoxical down-regulation of p16 mRNA with advancing age in AML⁷ and GISTs¹⁵ is similar to aging.⁶ Table 1. describes p16 downregulation that induce proliferation, and the argumentation are supported as follows:

3.1 Epidemiology of p16 downregulation depends on estrogen/senescence:

The upregulation p16 induce stunting¹⁶ but become downregulation after estro ER-/- senescence.¹² Childhood stunting is an important and intractable public health problem that cause about 20% of deaths among children age below 5 y in low- and middle-nSES population.¹⁶ The evaluate interventions to limit exposure and reduce childhood stunting should be promoted. Senescence's after menopause happens because Estrogen promotes estrogen receptor negative BRCA1-deficient tumor initiation and progression in breast cancer.¹² Possible Down Regulation of the p16 gene promoter in individuals with carcinoma,¹⁷ and give poor prognosis.¹⁸ Hepato cellular carcinoma (HCC)¹⁷ and breast cancer (BC) caused by AFB1 exposure has been broadly known⁵

3.2 Epidemiology meet Knock Out p16, induce proliferation:

Down-Regulated of the p16 Gene Promoter in Individuals with negative regulator of the cell cycle. In act of p16, especially promotor,¹⁷ down-reg p16 expression predicts poor prognosis in patients with extrahepatic biliary tract carcinomas patients.¹⁸ The immunohistochemically evaluated down-regulation of p16 in tumor specimen surgical has reported.¹⁸ Over expressed p16, which normally inhibits cell proliferation, induces G1 cell cycle arrest in cervical cancer cells and precancerous lesion.¹⁹ The downregulation of p16 in SiHa and HeLa cells inhibited their proliferation, migration and invasion, also in cervical cancer.¹⁹ p16 maybe a useful strategy in the diagnostic and treatment of cervical cancer.¹⁹ P16 deficiency promotes tumor formation in various tissues.²⁰ Also induced leanness especially in old age, lower body weight as a protection against cerebellar senescence.²⁰

3.3 p16 +/+ vs. +/- vs -/- and Estrogen Reseptor +/+ vs -/-:

p16 deficiency does not alter homeostasis in WAT, so induces leanness especially in old age.²⁰ Expression in estrogen receptor beta (ERB) also increase in deep cerebellar nuclei, implying cross talk between p16 and ERB. Protection against cerebellar senescence by promoting neuronal proliferation and homeostasis via ERB (in response to estrogen).²⁰

p16 mRNA expression in T cells, a marker of cellular senescence, with BC are significant with the increase risk.¹¹ It is differed by age, race, family history of cancer, marital status, annual income, and smoking status,¹¹ which is represented to low- and middle-nSES.⁵

Wang¹² describe the distinctive features of senescent cancer cells and how these changes in proliferation and senolysis.^{11,12} Also the deletion of cell cycle inhibitors p16 is required for development of Brca1-deficient basal-like mammary tumors in ER-positive vs. ER-negative, which estrogen stimulate proliferation and inisating in both ER positive and ER-negative mammary tumor initiation and metastasis (independent of ER).¹²

3.4 Viral E7 protein to p16 down-regulation:

Viral E7 protein Loss of 9p, leads to p16 down-regulation and enables O-6-methylguanine-DNA methyltransferase (MGMT) promotes the anti-proliferative and pro-apoptotic when cervical cancer cells stimulated with 5-Aza-dC.²¹ Methylation of p16 and MGMT was reversed. 5-Aza-dC inhibited E6 and E7 expression and up-regulated p53, p21, and Rb expression.

HPV E6/E7 mRNA tests determine the oncogenic activity of the virus and represent a good clinical biomarker for predicting the risk of developing cervical cancer.²² Also in Epstein Bar Virus (EBV) have shown increase cell proliferation.²³ Which is also paralleled liver Hepatitis B virus (HBV) DNA values.²⁴

TABLE 1
DOWN-REGULATION P16 INDUCES PROLIFERATION

Reference/year	Type of p16 down-regulation	Cases	Effect	pathway
⁷ de Jonge, 2009	P16 mRNA	AML	Aging: p16-p53→ proliferation	P16 up and then downregulation
⁸ Kriegl, 2011	P16 expression	Polyp adenoma (senescence barrier)	Colon Cancer	
¹⁵ Haller, 2008	P16INK4A and P16 mRNA	GISTS	Inhibits the CDK4 from phosphorylating RB	P16 located at 9p21
⁶ Mijit, 2020	P53→p16	Aging	Upregulation p16	p53 mutation- AFB1 exposure
¹⁶ Smith, 2012	P16	Stunting	Upregulation-Estro-/- →downregulation	Food chain AFB1 exposure
¹⁷ Shiraz, 2011	KO p16 (gene promoter)	HCC	Poor prognosis	P16 downregulation
¹⁸ Ichikawa, 2002	P16 down-regulated	Tumor specimen surgical	Extra biliary tract cancer Prognosis	P16 downregulation
⁵ Samsuria, 2018	P53 mutation	Ob-DM-HCC/BC	High prevalence	AFB1: P53-p21
¹⁹ Zhang, 2014	P16 gene downregulation	Cervical Ca	Dx/Rx cervical ca	P16 downregulation
²⁶ Zhang 2015	P16 expression	Breast cancer	Effect of hypoxia	Fibroblast
²⁷ Zhang, 2021	LATS1: Large tumor suppressor kinase 1	VSCMs	Effect of CSE: Cigarette Some Extract	P16-G1 arrest
²⁰ Kim, 2019	P16 deficiency P16-Estrogen Receptor Beta	Various tissue Non WAT	Tumor formation Leanness in old age	P16 downregulated- Estrogen
¹¹ Shen, 2020	P16 mRNA	T cell senescence	Increase BC risk	Age, black, fam history of ca, nSES, smoking status
¹² Wang, 2022	P16 deletion	BC-after menopause	Senescence: change to proliferation	Estrogen Receptor independent
²¹ Chen G-d, 2017	Methylation p16	Cervical cancer	Rx/ 5-Aza-dC inhibit E6/E7 exp	P53, p21, p16, Rb
²² Sharma, 2022	HPV DNA and mRNA	Cervical cancer	proliferation	E6/E7
²³ Uehara, 2021	EBV and HPV double infection	Oral ca tissue samples	proliferation	Reduce P53 induction
²⁴ Pan, 2004	HBV	HPV mRNA and HBV	proliferation	Therapy
²⁵ Kumari, 2021	Upregulation p16	Through ROS, DNA damage, senescence	Aging cells process	P53/p21/WAF1/CIP1 and p16INK4A/pRB play a central role in regulating senescence.
¹³ Guo, 2017	P16	CSE	VSMCs proliferation	
¹⁴ Breuer, 2005	P16 promoter Bisulfite Genomic PCR	Hypermethylation	Concentration and time associated	CpG island (repeat CGG)

Activation of p16 (upregulated p16) through ROS, DNA damage, or senescence leads to the p16 in tissues, and is implicated in aging of cells,²⁵ BAT,³ SMCs,⁴ and Cancer cells.^{18,19,20,21,30} This is about earlier upregulation of P16 gene-stunting then through senescence barrier, change to downregulation which induce proliferation. Conversely, p16 hypermethylation (silencing/KO), mutation, or deletion leads to downregulation of the gene and can lead to cancer through the dysregulation of cell cycle progression.

IV. DISCUSSION

CDKN (inhibitor)2A gene as the main tumor suppressor gene with p53 and p21, has another name P16/INK-4a and act as inhibiting CKD4 (cyclin-dependent kinase), with the help of hormone, antioxidant, antiviral. P16 induced by hypoxia are downregulated has been recorded. Table 2. Urine AFM1 (AFB1 exposure) in low- and middle-nSES home area describe the location of p16 downregulated high prevalence area. The mechanism are as followed:

4.1 Estrogen factors anti inflammation reaction:

Waist Circumference and Breast Cancer are associated with menstrual status⁵ and Estrogen factors act as anti-inflammation protect to P16 down-regulation, inhibit proliferation.

4.2 HBV, EBV, HPV:

HBV. EBV, HPV infection without p16 down-regulation, suppressed tumor cells to proliferated.

Kras activation and p16 inactivation are required to develop pancreatic ductal adenocarcinoma (PDAC). Mutant Kras- and p16-regulated NOX4 activation overcomes metabolic checkpoints in development of PDAC.²⁸ Similar expression profile of KRAS and p16 reported in periampullary cancer.³⁰ In precancerous lesion,²³ reveal the co-expression of low-risk HPV E6/E7 and EBV LMP-1 does not induce malignant transformation, but it allows accumulation of somatic mutations secondary to increase DNA damage and suppression of DNA DDR genes (DNA damage response and repair).²³

4.3 Hypoxia induced proliferation:

Cigarette smoke extract (CSE) exposure, similar to hypoxis, down-regulated p16¹³ and induce Vascular Smooth Muscle Cells (VSMCs) proliferation. The role of P16 in CSE-induced VSMCs proliferation and the underlying mechanism. also has been reported in the CKD1 in low- and middle-nSES AFB1 exposure population/ p53 mutation.^{3,4,5}

4.4 Time-dependent exposure:

Time-dependent exposure induced p16 downregulation-proliferation of precancer cells, BAT cells mitochondrial rich UCPs132 and SMCs proliferation supported.¹³ Not only time-exposure, but the level concentration of exposure are also recorded.¹³

4.5 Reduce p16 expression in HAOSMCs:

Activation of p16 (upregulated p16) through ROS, DNA damage, or senescence leads to the buildup of p16 in tissues and is implicated in aging of cells.²⁵ P53/p21WAF1CIP1 and p16INK4A/pRB play a central role in regulating senescence.²⁵ Boys and Girls in Sierra Leone, West Africa, frequently and constantly exposed by AFB1.³² Birth weight of infant is discussed, and the cord blood aflatoxin found in 58% pregnant woman.³² After the senescence reveal by stunting due to upregulated p16, reduce genetic or epigenic p16 expression induced proliferation in specific tissue e.g. human aortic smooth muscle cells (HAOSMCs),¹³ UCP132 (BAT),³ and fibroblast.²⁶ The loss of p16 expression due to p16 promotor hypermethylation occurs late in carcinogenic process at the level of severe dysplasia.¹⁴

4.6 Fibroblast migration and invasion:

Fibroblast migration and invasion in 83% breast CAFs compared primary cells and also in breast cancer tissues in hypoxia is reported.²⁶

Up and downregulation of p16 expression in BRAF-mutated polyps/ adenomas indicates a senescence barrier in the Colorectal carcinoma (CRC) process.⁸ P16 hypermethylation, mutation, or deletion leads to downregulation of the gene and can lead to cancer through the dysregulation of cell cycle progression. The liberates E2F1 (Transcription Factor) from its bound state in the cytoplasm and allows it to enter the nucleus. Once in the nucleus, E2F1 promotes the transcription of target genes that are essential for transition from G1 to S phase.¹⁵ This pathway connects the processes of tumor oncogenesis and senescence, fixing them on opposite ends of a spectrum. All Obesity and Hypertension high prevalence populations, are in the high prevalence of

stunting with AFB1 exposure-p53 mutation.^{5 and 16} Urine AFM1 describe AFB1 exposure in low- and middle-nSES home area had high Obesity (BAT proliferation) and Hypertension (SMCs proliferation) prevalence.

TABLE 2

IDENTIFIED LITERATURES ON URINE AFM1 (AFB1 EXPOSURE) IN LOW- AND MIDDLE-nSES HOME AREA

Study, year	Area of interest	Adjustment of interest	Comparative urine AFM1	Comorbidities influence	Influence of LC-MS/MS
³³ Ali, 2016	Hot and Humid climate	Bangladesh	Rural vs. Urban	Adult, children	Urine AFM1 as biomarker of AFB1 exposure
³⁴ Gerding, 2015	Germany	23 mycotoxins	Bangladesh Germany Haiti	Prevention of harmful health	Bangladesh and Haiti only, not Germany
³⁵ Jager 2016	UPLC-MS/MS	Brazil	HPLC with fluorescence detection	Prevention of harmful health	Confirmed urine AFM1 very sensitive for AFB1 exp
³⁶ Ezekiel, 2014	Rural North Nigeria	LC-MS/MS multi biomarker	Children Adolescents Adults	Major Public Health Challenge	Call for urgent intervention
³⁷ Warth, 2014	Bangkok	Max AFB1	LC-MS/MS urine AFM1	4 urine biomarkers	First in SEA
³⁸ Mitchell, 2013	AFB1 is a persistent public health issue in Ghana	Urine AFM1	UPSN vs. placebo	intervention	UPSN reduced AFM1 biomarker
³⁹ Solfrizo, 2011	1 st time reported	High pressure LC-MS/MS	Urinary biomarkers	Aflatoxin exposure could be measured	Urine AFM1 can be used
⁴⁰ Solfrizo, 2014	AFB1 exp	Urine AFM1	UPLC MS/MS in pg/mL	Southern Italy ZEA 100%, AFM1 6%	Urine AFM1 detected
⁴¹ Ahn, 2010	Quantitative	Urine AFM1 in pg/ml	LC-MS/MS	Using immunoaffinity column	Korean population 1/12 detected
⁴² De Cassia, 2009	Brazilian population	Urine AFM1	UPLC low pressure with fluorescence detection	Food contamination	AFB1 exposure
³¹ Jonsyn, 2001	Seasonal observational	Urine AFM1 from children in Sierra Leone	Dry and Rainy season	Boys and girls	Frequently and constantly exposed
⁴³ Shephard, 2013	1 st void morning urine	Urinary multi-mycotoxin	LC-MS/MS	Esophageal cancer region	Maize-based evening meal
⁴⁴ Warth, 2012	Quantitative in sub-ppb	AFM1 in Cameroon	LC/ESI-MS/MS	In human urine	Key metabolite
⁴⁵ Chen, 2017	Aptamer AFB1	Fluorescence enhancement	LOD 1.6 ng/ml	DNA strand containing a quencher moiety	+
⁴⁶ Jonsyn, 1999	Aflatoxin	HPLC specimen of infants	High contamination rate	AFB1 G2, OTA, OTB	Urine sample were 100% contaminated
⁴⁷ Jonsyn, 2007	Aflatoxin	Urine sample of school children	High concentration level	57% serum samples + aflatoxin	Low compared to urine
³² Jonsyn, 1995	Cord blood sample from pregnant	OTA	OTA in 25% Aflatoxin in 58%	No urine AFM1	Birth weight of infant

V. LIMITATION:

Many study didn't specify proliferation after stunting (aging) but report proliferation due to p16^{INK4a} downregulation due loss of 9p chromosome of p21.¹⁵ p16(INK4a) upregulated up to p16 downregulation by epimutation/ hypermethylation should be known by the researchers.¹⁴

This study also didn't specify age-dependent p16 epimutation, but proposes as the therapeutic target for colorectal cancer.⁴⁸ And KRAS, p53 are on mutation sequence before DCC gene,⁴⁹ similar to what KRAS activation and p16 downregulation in other precancer.^{28,30} Mutant Kras- and p16-regulated NOX4 activation overcomes metabolic checkpoints in development of Pancreatic ductal adenocarcinoma.

This study also didn't specify that p16 downregulation depends on senescence, while Wang reported exploiting senescence for the treatment of cancer,⁵⁰ where 5-Aza-dC is reveal for the treatment of cancer based on hypermethylation of p16 promotor.²¹ Stunting/ senescence due to p53 mutation.¹⁶ 5-Aza-dC is also reported to other broad range detrimental health cause by hypermethylation other than cancer.⁵¹

This study also didn't characterize Sodium-Glucose Cotransporter 2 (SGLT2) Inhibitors as therapy of afferent contraction due to VSMCs proliferation on afferent glomerulus.^{52, 53, 54} The area of AFB1 exposure could be found in low- and middle nSES (income) in Table 2,⁵⁶ especially in children and infant and moreover since pregnant women.^{57,58,59,60,61} which have associated to growth impairment.^{60,62,63,64} In children, adolescence and adults, is associated with major public health challenge, incl. HIV.³⁶ It is emphasize that AFB1 especially in utero, associated to DNA methylation in white blood cells of infants in the Gambia.⁶⁴

Mild, moderate, severe stunting & underweight on Systematic Analysis in 141 developing countries,^{61,65,66,67} is completed by Sousa reported stunting and overweight/obese high prevalence in Brazilian children (Systematic Review and Meta-Analysis),⁶⁸ and Romero report Urine AFM1 in Brazilian population associated with food consumptions parallel with the highest Diabetes Melitus (IDF), CVD (WHO).⁴² Stern 2001 reported HBV and AFB1 exposure due to p53-codon 249 mutation in China with Meta-Analysis.⁶⁹ It is supported by chronic hepatomegali,⁷⁰ aflatoxin cause stunting in Benin,⁷¹ Aflatoxin exposure in young children Benin and Togo, West Africa,⁷¹ complete this finding of AFB1 exposure induce the proliferation by downregulation of p16INK4a after stunting.^{5,16} p53-p21-p16 axis,⁷³ p16 deletion,⁵⁰ p16 methylation,²¹ which VSMCs function in atherosclerosis-DNA methylation.⁵⁵

VI. CONCLUSION

Proliferation of BAT UCP132 (obesity) or SMCs (hypertension) is due to downregulated p16 under p21/p53 mutation due to AFB1 exposure in low- and middle nSES area population with high childhood stunting (cell senescence) prevalence.

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CONFLICT OF INTEREST

Nothing

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Severe Thrombocytopenia in DLMF/Lymphoma Malignant/ITP: Review and Case report:

A New WHO Dengue Fever classification beyond DHF

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Abstract—

Introduction: Nowadays, incidence of Lymphoma Malignant with mortality is high. Peoples and former health ministry of Indonesia, FS, suspect *Wolbachia-Aedes aegypti* (DHF), but pediatrician thought to DENV-3/-4 secondary heterogeneity infection in endemic DENV -1/-2 in tropical rainforest area.

Method: Review the pathogenesis of the two Lymphoma Malignant dead cases report, in association with DENV-3 or -4 as secondary heterogeneity infection in tropical rainforest area, Indonesia.

Result: Severity Grade of DHF associated with secondary heterogenous DENV-3 or -4 infections. Conclusion: DENV 1-4 is ss RNA in association with emergency pandemic booster of SARS-CoV-2 which is also ss-RNA virus coated, secondary heterogeneity infection, is in one group with virus which could associated as the caused to Leukemia revealed Immune Thrombocytopenia and other tumor incl. Lymphoma Malignant-non-Hodgkin.

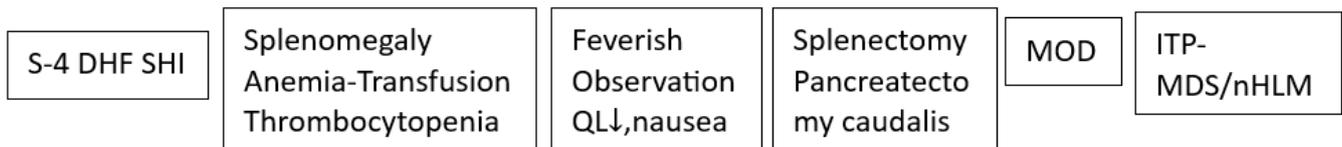
Conclusions: Ss RNA incl. DENV-4 infection as secondary heterogenous DENV-1/-2 or vice versa, which induce anemia and thrombocytopenia ITP with splenomegaly/Lymphoma Malignant, should be classified to DLMF (grade-4) of DHF classification.

Keywords—ITP-MDS, Large Granular Chronic Leukemia, Neutrophil Lymphocyte Ratio (NLR), Secondary heterogeneity infection DENV-4, Sepsis, Splenectomy.

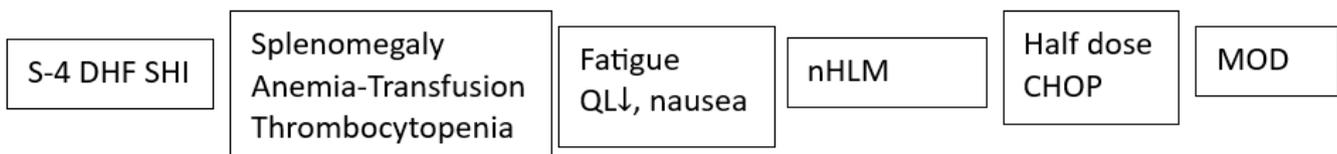
I. INTRODUCTION

Two cases of Acute Lymphoma Malignant mortality in the beginning, and the end of the rainy season in tropical rainforest area (DHF) endemic has shocked peoples in 2024. In Indonesia *Aedes aegypti-Wolbachia* is spreading in big cities incl. West Jakarta, Surabaya, Semarang and Bali. These two cases are diagnosed in one family. Besides, in the other family, there are also such cases. Number of cases on DENV-3/-4 secondary heterogeneity infection (SHI) 2023 and 2024, and the need of tetravalent DHF vaccine (and no booster COVID-19 AZ due to the recollection from circulation) has been reported in this period, is emphasized not because thrombosis and thrombocytopenia syndrome). AZ pull back the ss RNA vaccine circulation of

COVID-19 all over the world on May 7, 2024 due the asking on March 5, 2024, and loose in court on April 2024 caused of Thrombosis with Thrombocytopenia Syndrome (TTS) as a side effect. SS RNA AZ COVID-19 vaccine, and Serotype-3 or -4 DHF secondary heterogeneity infection, both caused TTS. Most COVID-19 vaccine development efforts aim to activate the immune system against the spike protein (glycoprotein spike on a viral capsid or viral envelope). Pfizer and Moderna have already stopped their mRNA vaccine production since the pandemic COVID-19 is over, which is no longer a pandemic-level threat declared by the WHO May 17, 2023 (while global pandemic was revealed on March 11, 2020). Exactly, the permission to use is only on emergency pandemic conditions. So, there are no causes of Wolbachia and booster COVID-19 vaccine in association with thrombocytopenia and these two Lymphoma Malignant non-Hodgkin cases. The aims of this study are no surgery, and no chemotherapy on severe thrombocytopenia. Many nodules in splenomegaly at the beginning, but the spleen is clear post-op (Fig.1). The liver has supple contour, no hepatomegaly and cirrhosis.



a) Case 1: Healthy Man, 73 y with BPJS (government health insurance) limited therapy



b) Case 2: Healthy Man, 70 y with no limit therapy

FIGURE 1: Dx/ Case 1: Splenectomy & cauda pancreatectomy, 3 days after post-op with never conscious/ wake-up. Macroscopy: Clean of nodules, Microcopy/PA: Lymphoma Malignant; Dx/ Case 2: nHLM and Thyroid Cancer & MOD oedema, dead after 1 week chemotherapy.

1.1 Hypothesis: DLMF caused by secondary heterogeneity infection of DENV ssRNA:

The COVID-19 and DHF, both are caused by viral infection which is well known in Indonesia, SEA. SARS and COVID-19 which attack tractus respiratory, and could be fatal, emerge in China, SARS in 2002, while COVID-19 in 2019. In the early 2020 up to 2023, Indonesia has vaccinated due the COVID-19 pandemic. SARS-CoV-1 is SARS virus, which causes severe acute respiratory syndrome (SARS). SARS-CoV-2 is corona virus, which causes respiratory tract infection of COVID-19. This is the name of the virus, and the COVID-19 is the name of the disease. It is like HIV the virus, and AIDS (Acquired Immune Deficiency Syndrome) as the disease, has been well known. MERS-CoV is a different virus than SARS-CoV2 because of the difference vector, it's by camel, not bird or directly from human to human. DENV 1-4 causes Dengue Fever Classic (DFC), DHF, DHF Shock Syndrome, and DF Lymphoma Malignant (DFLM). DSS due to the endothelial cell's dysfunction (Shock Syndrome) AND Development coagulation disorders induced by thrombocyte/platelet-lymph nodes suppression, where monocytes and macrophages are recruited to replicated.¹ These pathogenesis has not yet associated to Lymphoma Malignant (LM) non-Hodgkin as the diagnosis of 2 cases report.^{1,2} Meanwhile CHOP and R-CHOP are reported successful for the therapy of non-Hodgkin LM in the most endemic area top referral hospital in East Java, Dr. Soetomo General Hospital Surabaya.² This inductive phenomena is supported by/ could be associated by Dengue Virus Serotype 4, which is responsible for the outbreak of Dengue in East Java City of Jember, Indonesia.³ Lymphoma Malignant has been associated with DHF infection.^{4,5,6,7.}

1.2 DHF, DSS, thrombocytopenia and epigastric pain, and Wolbachia review:

1) Dengue Fever (DF) classic, usually in children have symptoms: fever, headache, nausea vomitus, epigastric pain, muscle joint, rash after the 4th day. On the 4th day is the most dangerous. Dengue symptoms appear 4 to 7 days after the bite of Aedes sp. mosquito.

DD: typhoid or Upper Respiratory Tract Infection (common cold or staphylococcus).

2) Dengue Hemorrhagic Fever (DHF), DF classic + bruised violet color (arterial damage). Nose, gingiva, or under the skin (petechiae with or without Rumpel Leed test)

3) Dengue Shock Syndrome (DSS), a child and adult with plasma is already extravascular due endothelial apoptosis/leakage, blood pressure: 0/0.

4) Lymphoma Malignant Non-Hodgkin (does not contain Reed-Steinberg cells) B in adult: Splenomegaly. Rx/ Cyclophosphamide (Cytosan) (Endoxan, doxorubicin) are a group chemotherapy which decrease the growth of cancer cells, well known as CHOP regiment with the P for Prednisone.²

Thrombocytopenia cut-off: mild 101,000-140,000 per μ L of blood, severe $<$ 25,000 very severe $<$ 20,000 per μ L, moderate 50-100,000/ μ L. Thrombocytopenia mild, moderate, severe, critical, and cause mortality is the grade to describe severity score (Table 1). Thrombocytopenia during chemotherapy (Lymphoma Malignant) is often accompanied by a reduction of other blood cell counts.

**TABLE 1
CUT-OFF**

		Grade I	75-99			Normal	2.58
Grade I	>500	Grade II	50-74	Normal	40-54 M 36-48 F	Grade I	>3.68
Grade II	2470	Grade III	25-49	Grade I	>54 M, >48 F, >44 C	Grade II	5.5
		Grade IV	<25			Grade III	7.83
						Grade IV	10.84

- a) D- dimer cut-off (ng/mL) N <500
Old age: 90x10
- b) Thrombocytopenia cut-off 100,000/ μ L Successful 16.000 therapy with only NaCl 0,9% is usual in Indonesia
- c) Hematocrit cut-off (%) Automated cell counter: Red cell number x MCV (Millions/mm³ x femto L)
- d) NLR cut-off (%)

**TABLE 2
NLR CUT-OFF WAS 3.0. WHO CATEGORIES FOR COVID-19 SEVERITY (ASYMPTOMATIC 1.92, MILD 2.08, MODERATE 4.79 AND SEVERE 9.9 WERE USED**

Normal	2.58	COVID-19
Grade I asymptomatic	>3.68	1.92
Grade II mild	5.5	2.08
Grade III moderate	7.83	4.79
Grade IV severe	10.84	9.9

Neutrophil Lymphocyte Ratio in COVID-19 is used for describing prognosis, in DHF for severity score diagnosis.⁴

II. METHOD

Review the pathogenesis of the 2 case reports Lymphoma Malignant, in association with DENV 1-4 secondary heterogeneity infection. First, using Chat-GPT, then Science Direct and other search engines preferred PubMed connection.

III. RESULT

Grade of DHF associated to secondary heterogenous DENV-3/-4 infection

3.1 Two Cases Report mortality of DLMF:

These two dead case reports came from fact and report of the case's subject, family and close friends, doctors, and the author meeting with the patients.

3.1.1 Cases 1 DLMF (HUS):

Man > 73 years, reported DHF secondary infection Aedes Aegypti-Wolbachia area DENV-3/-4, eggs outside the female mosquito doesn't yield (to decrease the population of Aedes Aegypti in his home area). SS DEN-3/-4 secondary heterogeneity induces the development of coagulation disorder¹ (marker: D-dimer). Abdominal pain or tenderness, persistent nausea and vomiting, clinical fluid accumulation: ascites and pleural effusion, mucosal bleed and rash on skin are dubious. Lethargy/faint/tired/apathetic/passive/become slow are dominant. Restlessness is not recorded. Liver enlargement > 2 cm. Laboratory finding of increasing Leukocyte, Monocyte, Lymphocyte and Granulocyte with rapid decrease in platelet count. In 1 month, the fever observed in and out of the hospital due to BPJS patients could not be more than 3 days of hospitalization. At 92 years old, at grandma's birthday celebration, the friendly, polite man steps aside and sleeps again and again (End of July 2023). 2 Sept 2023 HUS came to family lunch but didn't want to eat because of nausea (loss appetite). October still with fever observation with hepatosplenomegaly and cauda pancreas full of nodules by USG, and hospitalized again. His brother is also hospitalized for chronic fever observation but survives by taking Ivermectin. HUS died 6 days post splenectomy and cauda pancreatectomy, on Nov 19, 2023, after 3 months feverish observation with low RBC, thrombocytopenia, and high Leukocyte, hypoglycemia and Hb decreasing and drink plenty of fluids. Epigastric pain, postprandial fullness, upper abdominal bloating, early satiation, nausea, vomiting, lethargic. On Nov 11, 2024, 3 days before splenectomy, he asked for high protein milk low carbohydrate high fat which would make him fit. HUS was Dx/ Lymphoma Malignant by PA Post-op splenectomy and cauda pancreatectomy. Oct 2023, HUS decreased albumin levels were strongly associated with DHF (by meta-analysis of multiple studies, $p < 0.05$, while elevated leukocyte Oct 14, 2023. IgG > 2.85 IV: Positive IgG antibody to DFV type- 2/4 detected which may indicate a current or past infection. IgG < 1.64 IV: Negative-No significant level of detectable DFV.

3.1.2 Case 2 DLMF (EeS):

Man 69 years, came to the hospital for BHP medical check-up, go alone with the chauffeur and small luggage, then after 2 weeks Dx/ Lymphoma Malignant with thyroid Ca (T Lymphocyte high production): thymus Dx/ by USG, then were given half dose chemotherapy on 5 May 2024. April 29, 2024 a little bit of moon face. May 3, 2024, couldn't know it was his face. Dead on May 10, 2024 with MOD oedema anasarca.

These 2 cases, endothelial cells dysfunction caused by Imbalance profile of cytokine and other mediators is not reported. Replication phase in hepatocyte and macrophage in spleen, then apoptosis of both supports the diagnosis of Lymphoma Malignant.^{1,2,3,5} Differential diagnosis cirrhosis hepatis due hepatomegaly but no varices esophagus, and the liver is supple and soft in case report 1. HUS. Both DLMF cases, never took a bone marrow biopsy because no indication of blast cells in the periphery blood. Spleen and Liver are working extra hard, but not the bone marrow. Suppressed of hemopoiesis especially erythrocyte and thrombocyte, are clinically shown.¹

IV. DISCUSSION

The author and the whole family are rethinking DHFV-4 because HUS (case 1) loves to go to the garden, his house is at the edge of a rice field, where clean water flood is best habitual for larval mosquitoes of Aedes aegypti. There are so many mosquitos in his house, he got hemolysis, hepatomegaly and splenomegaly. Several times hospitalized, each and every hospitalized person got blood transfusion then the Hb drop again (more frequent transfusion).

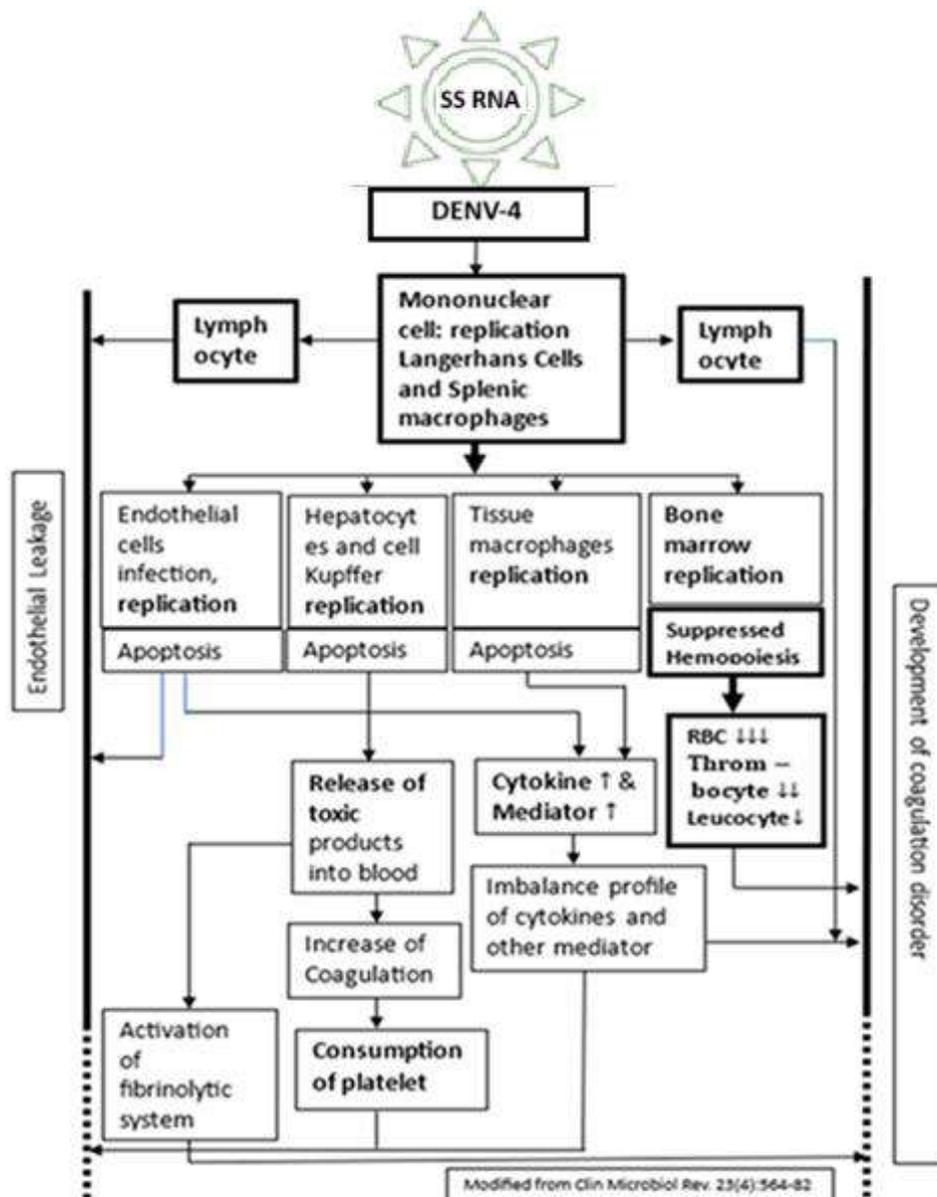


FIGURE 2: Pathogenesis of Lymphoma Malignant non-Hodgkin with drop of Hb and Thrombocyte

Pathogenesis of Development of coagulation disorder (marker: D-dimer) and the endothelial leakage caused by Imbalance profile of cytokine and other mediators in DF, DHF, DSS.¹

Lymphoma malignant is the Pathologic Anatomy Diagnosis of the splenectomy and cauda pancreatotomy surgery in case 1. LM non-Hodgkin is the chemotherapy diagnosis of case 2. There are also Antibody cross react with platelets and plasmin due to mononuclear cells replications.¹

4.1 Review cases:

4.1.1 Review Case report DHF, DSS and DEN 1-4:

Pathophysiology all types are the same, DEN Virus (DENV) has 4-5 serotypes. All types are ssRNA virus. So, if someone got DHF, it could be 3 times DHF again. Each person in at risk of dengue fever endemic area, could be 4 times in their lifetime.

Secondary heterogeneity infection is diagnosed by IgG, IgM, whereas **DENV-3** was the dominant serotype (75.9%).^{5,6,7,8} Secondary infection with heterologous serotypes is more severe than primary DHF infection, could be explained by IgG for old infection usually DENV-1 or -2, IgM for new infection, usually DEN-3 or -4 in endemic area, tropical rainforest climate.

SS-DENV-3 or -4 secondary infection after -1 or -2, induced endothelial cells apoptosis, suppression hemopoiesis to development of coagulation disorder (increase D-dimer). Liver hepatocytes cells and Kupffer cells replication, necrosis and apoptosis in liver. Splenic and Tissue macrophage replication then apoptosis induced cytokine storm – imbalance profile of cytokine and other mediators. Stimulation B cells and T cells Lymphocytes production (Fig.2). Activation of fibrinolytic system (A) and Consumption of platelets (C). Both A & C induce the development of coagulation disorder (marker: D-dimer).¹ Endothelial cells dysfunction caused by Imbalance profile of cytokine and other mediators.¹

Review Case report Grade 1: no evidence of bleeding, RL Tourniquet test Positive. Known as Dengue without warning signs.

Review Case report Grade 2: DHF (and Wolbachia). Adult female mosquitos are estimated to be 10 days old, and the lifespan is 14 days. Only the female mosquito could be a vector of DENV. The egg of a female mosquito with Wolbachia couldn't hatch. Known as Dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing hematocrit with decreasing platelets/thrombocytopenia). Evidence of bleeding episodes.

Review Case Report Grade 3: Dengue Shock Syndrome (DSS): presence of weak and rapid pulse rate, low blood pressure or narrow pulse pressure, severe dengue, dengue with severe plasma leakage, where Rx/Albumin is needed. DSS due to secondary heterogeneity DENV-3 infection. whereas DENV-4 occurs at a low prevalence World Wide⁶ and spreads the least rapidly.⁶ Den-2 has been the most common serotype over the last 50 years. Secondary infection and DENV-3 serotype is most common among dengue patients.⁷

DENV consists of 5 distinct genotypes (I-V). Genotype III is the most widespread and was associated with large outbreaks in Asia, Africa, and America:⁸

DENV-1 and DENV-3 are more pathogenic without immune priming from other serotypes.

Development of Dengue Infection Severity Score is due to thrombocytopenia and leakage of the endothelial cells, and blood pressure/ narrow pulse pressure/ rapid pulse rate, but not in Lymphoma.⁴ The DENV also infect and replicates inside a specialized immune cell located in the skin, a type of dendritic cell called a Langerhans cells.¹

Case report Grade 4 Deadly DLMF: DIC/sepsis (MOD) is when bacteria go inside the blood after post splenectomy or post chemotherapy. And the garden and forest lover only got it although back with antibiotics all the time while needed. Aims: To revised WHO dengue case classification: the system needs to be revised. DENV-4 severity during secondary infection to an antecedent primary DENV-1/-2 infection endemic area. Case DLMF 1 (HUS) only Dx/ by hematology abnormality pre-op, but Dx/ Lymphoma Malignant 3 weeks after Splenectomy and Cauda Pancreatectomy due the extreme drop of Hb and Thrombocyte. Case DLMF 2 (EeS, 70y) only Dx/ non-Hodgkin Lymphoma Malignant (nHLM) & Ca Thyroid by USG (Thymus) before half dose chemotherapy 1 week before mortality. DENV-4 was reported in non-Hodgkin type B with good differentiated (bad prognosis, high grade) vs. poor differentiated (good prognosis, low grade),² summons CHOP/ R-CHOP regiment.²

CD4+ CD8+ double positive (DP) T cells represent a heterogeneous population. One or more groups of clonal T-cells may be present in a person's lymphocyte population without being considered a lymphoma. Clonal selection of T cells taken place in the thymus and is in charge for producing a useful and functional collection of T cells. The DP thymocytes survive for approximately 3 days previous to undergoing apoptosis (programmed cell death). Splenomegaly and full of nodules, then the fact it is clear and supple post operative describe the process of the apoptosis was seen in case DLMF 1 (HUS, 73y).

In Hodgkin Lymphoma, DNA mutation at B Lymphocytes, and Non-Hodgkin Lymphoma, DNA mutation at B and T Lymphocytes. Case report 2 (EeS) was Dx/ Non-Hodgkin Lymphoma Malignant. He died on 10 Mei 2024.

B Lymphocytes are developed in bone marrow, detect intruder/ attacker and form antibodies to fight infection. After having been stimulated by antigen, B lymphocytes develop into cells producing and secreting antibodies (plasma cells, represent the end-stage of the differentiation of B lymphocytes after their stimulation by antigen). They are thus responsible for the antibody type in the immune response. During development, they are influenced by cytokines and growth factors. And can be characterized according to CD markers (CD19, CD20, CD24, CD72), and presence of immunoglobulins molecules on their outside. T Lymphocytes are produced in thymus. T lymphocytes damage cancer cells and cells which is infected. Th / Treg & effector functions (CD4+), Tkiller Tk and T cytotoxic Tc (CD8+) T suppressor: resulting in osmotic swelling (endothelial cells apoptosis) and following killing and lysis.⁴ Th is one of lymphocyte types which help to harmonize immune response so as to approach infection and diseases. In AIDS patients, CD4/CD8 ratio > 1 with CD4 500-1500/mm³ and CD8 Lymphocyte 150-1000/mm³, means the immune system is strong and the patients may not have HIV. Case report DLMF 1 (HUS) has

Suppression hemopoiesis due to replication of stromal cells.¹ The author had enough time to think what it should be like, but no behavior representation give assistance to it. Both DLMF cases, the CD4 never deliver below 200 cells/mm³.

The communication of CD4 with MHC class II greatly become less the number of antigenic peptides required for T cell activation and substantially increases cytokine production by helper T cells.⁹ The interaction of CD8 with MHC I can climb up on to a response against pathogens by produce and discharge cytokines and defend from harm of tumors by directly killing transformed cells. Most Tc cells express T-cell receptors (TCRs) that can identify a specific antigen. CD4 cells lead the fight as protection to infections which help Tc/Tk to lyse infected cells. Tc recognizes any infected cell expressing MHC I. Th also helps B Lymphocyte cells to produce highly matured antibodies. Th also encouraging other immune cells, such as macrophages. Both, CD4 (Th) cells and CD8 (Tc/Tk) cells are growth or development different from the common lymphoid progenitor cells in the bone marrow and go on to full grown in the thymus. Low CD4/CD8 ratio reflects increased immune activation and is associated with an increased risk of severe non-AIDS events or cancers. High CD8 count means that the body is effectively controlling the infections. Development always generates more CD4 than CD8 T cells. During a CD8 T-cell reaction to virus infection, there are 3 characteristic phases: a period at the beginning activation and the action of becoming more extensive, a process of becoming smaller of the death phase, and the establishing and maintaining of memory. Memory CD8 T-cell differentiation throughout viral Infection.^{1,10} It all needs energy, that's why hypoglycemia and lethargic happens.

Neutrophil increase, and Leukocyte decrease. Neutrophil normal 1,450-7,500/ μ L. In viral infection neutrophil can fall quite low and may stay low for many months. Neutrophil to Lymphocyte Ratio: Normal: 1-2. NLR >3 grade I; < 0.7 Low, mirroring a preserved immune balance. 2.3-3.0 early warning cancer, atherosclerosis, infection, inflammation, psychiatric disorders and stress. High grade NLR narrate bacteria that are already inside the blood vessels. Lymphocyte grow describes viral infection. In both DLMF cases high Lymphocytes have been reported. The NLR cut-off was 3.0. WHO categories for COVID-19 severity (asymptomatic 1.92, mild 2.08, moderate 4.79 and severe 9.9 were used.¹¹

Hematocrit cut-off >54 Male, 48 Woman, and Rumpel Leed and bleeding, none reported in both DLMF cases. Secondary infection and Den-3 serotype most common among dengue patients.^{7,8}

4.1.2 SsRNA vs. DsRNA vs. DsDNA, and mRNA:

SsRNA DHF Virus, and also AZ vaccine which is March 2024 recollected from the circulation used for booster. ds RNA or RNAi synthesis to silencing DNA for plant, insect, agriculture and aquaculture. Ds-DNA Wolbachia outside the human body, Adenovirus is one of the DNA viruses.

Messenger RNA (mRNA) is an intermediate which brings genetic information from a gene in the nucleus to a ribosome in the cytoplasmic cell. Transcription is the synthesis of RNA from DNA template where the code in the DNA is converts to a complementary RNA code. Translation is the synthesis of a protein from an mRNA template, where the code in the mRNA is converted into an amino acid sequence in a protein, e.g. spike protein. So ssRNA could be from DHF virus,¹ but not from Wolbachia.

TABLE 3
SSRNAV, DSRNAV AND DNAV
1. VIRUS & DISEASE

Virus Name	Disease Name	Contains SS/DS/DNA
SARS-CoV-1 (SARS virus)	Severe Acute Respiratory Syndrome (SARS)	SS RNA
SARS-CoV-2 (corona virus)	breath canal infection of COVID-19	SS RNA
HIV	AIDS (Acquired Immune Deficiency syndrome)	Retrovirus that contains SS RNA
MERS-CoV	Difference than corona virus, Vector: Camel	SS RNA
DENV 1-4	Dengue Fever Classic, DHF, DHF Shock Syndrome, and DF Lymphoma Malignant	SS RNA

MERS (Middle East Respiratory Syndrome)

DENV-3/4 causes: Endothelial cells dysfunction/ leakage (Shock Syndrome) AND Development coagulation disorders (thrombocyte/platelet- LM non-Hodgkin)

2. VACCINE & MANUFACTURE:

Vaccine	Manufacture	Contains SS/DS/mRNA
SARS-CoV-2 (COVID-19)	AZ-Oxford, and J&J	SS RNA spike protein -> B cell used vector adenovirus chimpanzee
SARS-CoV-2 (COVID-19)	Pfizer-BioNTech	mRNA-BNT162b
SARS-CoV-2 (COVID-19)	Moderna	mRNA -1273
SARS-CoV-2 (COVID-19)	Sinovac	Deadly V
DHF Tetravalent (DENV1-4)	Takeda	SS RNA 1-4

4.2 MOD, Endothelial cells dysfunction AND Development coagulation disorders beyond DLMF:

Development coagulation disorders only after post-op (case 1, HUS) and after chemotherapy (case 2, EeS) due to the drop of Leukocytes and T Lymphocyte, Erythrocyte, Thrombocytes, but no report of endothelial cells and development coagulation disorders: Fig 2. Edema anasarca due to multi organ damages (MOD) has been reported in case 2 due to sepsis. Endothelial cells dysfunction AND development coagulation disorders is a good condition to the infection of bacteria into the blood (sepsis) and developed to the end stage known as MOD.

Although live in Wolbachia spreading area Semarang, and Jakarta Barat-Bali, both cases also haven't got ssRNA booster, but high-risk exposure to ssRNA DENV-4 with no IgG and IgM reported. EeS died from MOD post CHOP chemotherapy, also with no endothelial cell's dysfunction and no clinical coagulation disorders. While a long process of fever observation frequently hospitalized since before Sept 2023 and deadly operation of Splenectomy and Cauda Pancreatectomy with 3 days never to wake-up due the knock-down to pain anesthesiology till 19 November 2023. Case DLMF 2 (EeS, 70y) – ssRNA type-4 serologies IgG and IgM were also disregarded, although in Wolbachia spreading area West Jakarta, but no risk of DENV-4. Due to his BHP, fever, and lacking energy, faint and tiredness, EeS has done his demonstration of friendship, and getting in touch with the inheritance, reveals the chronicity of the illness.

Both patients got hepatomegaly and splenomegaly. Case 1 with full nodule in the cauda pancreas, could be due to extreme extra glucose per infusion and per oral. Hypoglycemic is due to replication phase and storm and imbalance cytokine and mediators.¹

D-dimer cut-off before post op and chemotherapy, increases extremely after post-operation and chemotherapy due to sepsis and the end stage mainly MOD. Thrombocytopenia in the beginning, before operation/chemotherapy, drops at the last days. Decrease Hb in the beginning, before op/chemotherapy, drop at the last days. Hypoglycemia, infused dextrose 5%, extreme glucose per oral, and cauda pancreatomy in DLMF case 1 (HUS, 73y). Ca thyroid diagnosis in DLMF case 2 (EeS,70y), before chemotherapy, is due to the development in thymus,¹² which is normally atrophy in adolescence, which is located in the thyroid area.

Case 1 DLMF (HUS 73 y) splenectomy, and cauda pancreatectomy, 6 days post op Knock-Down due anesthesia pain killer, never wake-up again. When the author asked his sister (Clinical Pathologist) about the argument of hemolysis or for hematopoiesis as the cause of splenomegaly, she didn't give the description of the Blood Smear Morphology-Peripheral. BMP had never been done. And she commented in May 24, 2024 that she didn't know if hemolysis or strong hematopoiesis is the cause of the splenomegaly. He eats dirty street food randomly without any reason, maybe he got chronically poisoned. Sudden splenectomy was indicated by full nodules and quickly dropped the thrombocyte, and cauda pancreatomy by hypoglycemia for 3 months with full nodule on it (Dx/Ca pancreas). He swims 1x/ week, September still swimming, out of first hospitalization also still swimming. Mid November 2023 the last hospitalized, the condition is sleeping all the time, could not open the eyes,

sleepy continuously, difficult to open handphone, then could not open handphone, moreover could not grab the hp, could not self-drinking, could not walk. So, everything drops, Hb, glucose, thrombocyte, while splenomegaly and cauda pancreas increase the size and nodules. It is hemopoiesis due the splenomegaly and also destruction of blood cells, not hemolysis because no shivering was reported. September 29, 2023, he asked to go home, because September 30 he wants to celebrate his birthday. It is all to describe that no one becomes aware of the disease, except it is easy to sleep. Hospitalized not because of fever, or hypoglycemia, or low of Hb, but faint and don't want to eat. Abdominal Pain is a commonly reported symptom in DF.^{4,13}

Severe hepatic damage is not common in DENV infections, but elevated liver enzymes suggest that the liver is affected.^{1,4} The first diagnosis as cirrhosis has not ever been heard again post operation and post chemotherapy.

Case 2 DLMF (EeS 70 y) got chemotherapy for LGC disorder, 3 days post chemotherapy, lethargy, never talk again. Before the chemotherapy he can contact his cousin indistinctly. On a death case report to the family, a doctor said the thrombocyte become only 200, and could not be saved.

Case 1 (HUS, 73y) 3 months in and out the hospitalized for blood transfusion and hypoglycemic. After 3 months he said Leukemia and severe thrombocytopenia, but then hematology malignancy. PA 3 weeks after Splenectomy and cauda pancreatomy: Lymphoma Malignant.

Case 2 (EeS, 70y) never being hospitalized, come to the hospital himself with fever observation for BHP medical check-up, go out with Dx/ Lymphoma malignant, Ca thyroid with thrombocytopenia and edema anasarca caused by Multiple Organ Damages (MOD).

The thrombocytopenia in dengue is caused by the suppression of bone marrow, platelet destruction by antibodies to the dengue virus, excessive consumption of platelets, viral-replication-mediated destruction of platelets by complement-mediated lysis, and apoptosis.^{1,14}

4.3 Comparison Serotype DEN 1-4:

The flavivirus genome consists of \pm 11,000bps, which translates into three structural and 7 nonstructural proteins (See "Chimeric virus vaccines"), later. A successful extra valent dengue vaccine should concurrently protect against all 4 serotypes because each person could 4 times be infected by DENV. DENV1-4 have different 3 phases (fever, critical, and recovery), DENV-2 Hemorrhagic critical, DENV-3 Shock Syndrome critical, and DENV-4 have a long recovery phase which could be DLMF. Mutation by DsDNA could be ignoring the stop codon (i.e. UAA, UAG and UGA), which is a generated readthrough mechanism, continuing on that makes a longer string of amino acids. Sometimes this function is combined with the ribosomal frame-shifting to produce an even greater variety of viral proteins.¹⁵ which induced Lymphoma Malignant,¹⁶ which could be by STAT3 mutation,^{17,18,19} The global trends in research on endothelial cells and sepsis 2002-2022 has been reported,²⁰ and the mechanism supported the mechanism in these 2 cases. Wolbachia 16S rRNA haplotypes detected in wild *Anopheles stephensi* has been reported in eastern Ethiopia,²¹ whereas detection of Wolbachia genes in patient with non-Hodgkin's lymphoma already has been set up and reported before.²² Clinical assessment of dengue and identification of risk factors for severe disease protocol for a multicenter study in 8 countries, incl. hematocrit, white blood cell count, lymphocyte count, thrombocyte count, etc. as well as SGOT/SGPT, plasma albumin etc.²³ Identification of early serological correlates of serious dengue are included, but not yet implicit Lymphoma malignant,²³ although splenomegaly 67% has reported caused by STAT3 -T-Cell Large Granular Lymphocytic Leukemia.¹⁹ STAT3 mutations show the presence of subclinical T-cell clones in a subset of aplastic anemia (AA) and myelodysplastic syndromes (MDS) patients,²⁴ supported by Lymphotropic virus: chronic inflammation bringing rise of cancer.²⁵ It seems Adult T-Cell Leukemia/Lymphoma as new biologic insight, and new direction in treatment since 2017 by Mehta-shah, should not be neglected. Prevalence in Indonesia -1 (33.6%), -3 (28.4%); -2(20.5%), and -4(14.9%): 22Oct 2020.² Low carbohydrate, high fat, high protein should be given in this early biomarker for prediction of serious thrombocytopenia in DLMF new classification in tropical rainforest area (low- to middle- income countries during epidemics,²⁷ whereas decreased albumin level was strongly associated with DHF.²⁷ ITP associated SarCov2 has been revealed.²⁸

LIMITATION

This study did not elaborate specifically severe score DENV-1 and DENV-3, which are more pathogenic without immune priming from other serotypes.⁴

It is also recorded unexplained cytopenia in MDS associated with excellence prognosis of Low-Risk MDS without detectable Myeloid-Related mutations.²⁹ The early detection of DLMF from DF to ITP/MDS/AML³⁰ and high protein therapy³¹ support this study, early mass diagnosis and therapy, but not covered.

V. CONCLUSION

DENV 1-4 is Ss RNA incl. DENV-4 infection as secondary heterogenous DENV-1/-2 or visa versa, which induce anemia and thrombocytopenia ITP with splenomegaly/Lymphoma Malignant, should be classified to DLMF (grade-4) of DHF classification.

Ss RNA incl. DENV-4 infection as secondary heterogenous DENV-1/-2 or vice versa, which induce anemia and thrombocytopenia ITP, then splenomegaly/Lymphoma Malignant, should be classified to DLMF (grade-4) of Dengue Fever classification.

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AstraZeneca and detik.com, who exposed the withdrawal of SS-RNA all over the world in May 7, 2024.

CONFLICT OF INTEREST

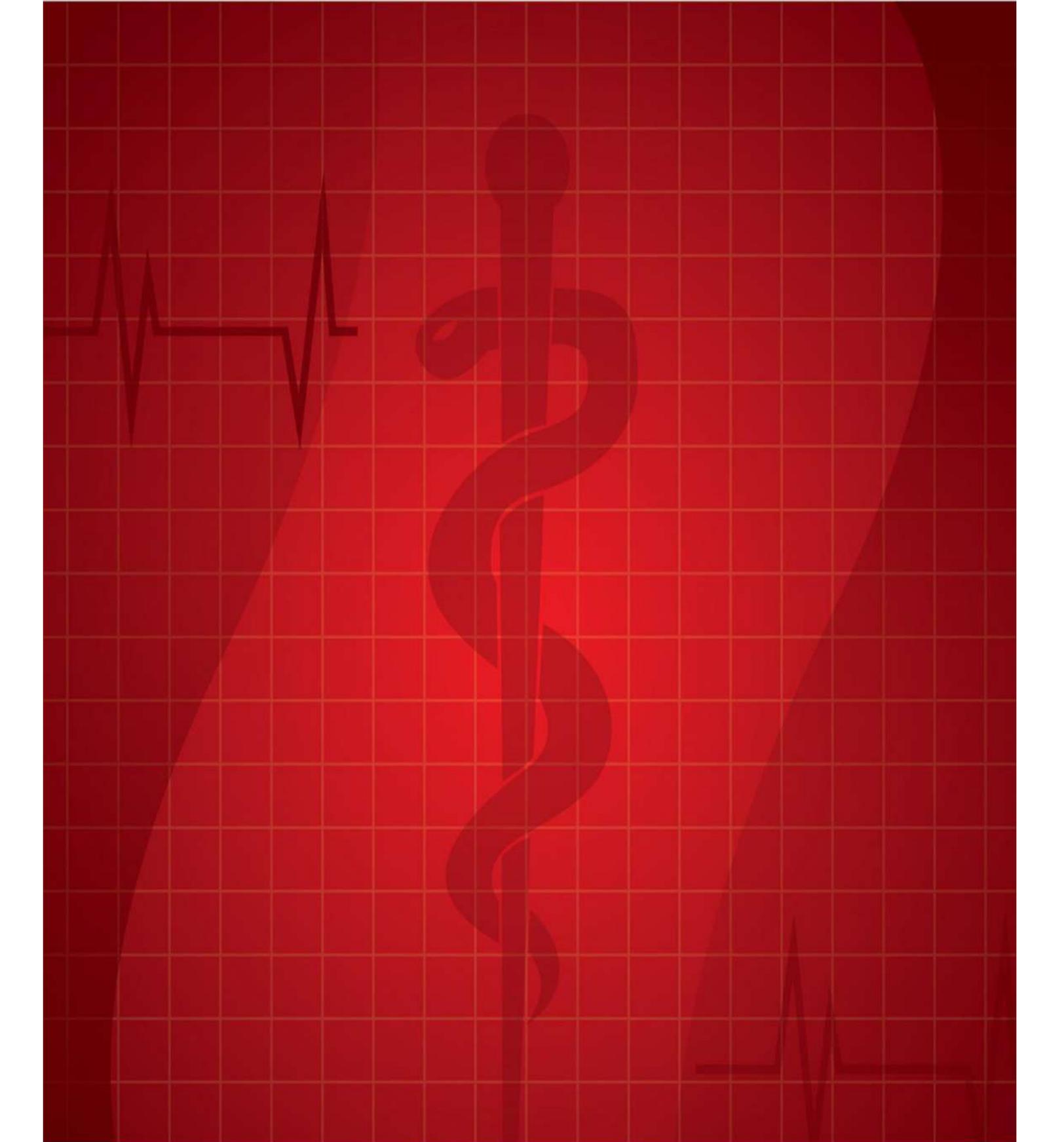
The author declares No competing interests.

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