UPDATE ON DENGUE DIAGNOSTICS and TREATMENT GUIDELINES

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Introduction

- the most prevalent mosquito-borne viral hemorrhagic fever.
- Flaviviridae family, four antigenically distinct serotypes of this virus
- Endemic virus transmission, coupled with frequent cyclical epidemics →half of the world's population at risk of infection each year
- encroaching into nonendemic areas due to increasing international travel and rapid urbanization with poor public health infrastructure
- global warming could further exacerbate the spread of dengue vectors both northward and southward
- the cost of acute illness to society is considerably resulting from loss of productivity to costs arising from medical care

Overview of dengue pathology

infect Langerhans cells at the site of mosquito inoculation

↓

migrates through the lymphatics

↓

spreads to other cells of hematopoietic lineages

↓

systemic dissemination

↓

Viremia - 1–2 days prior to the onset of symptoms, peaks during days 1–2 of fever and declines over next 3–5 days until resolution of fever

Overview of dengue pathology

- stimulates both innate and acquired immunity
- Innate immune response - complement and type I interferon (IFN)
- Acquired immune response - involves serotype specific CD4+ and CD8+ T cells result in lysis and production of cytokines
- Antibody response
  - targeted at prM, E and NS1 viral proteins
  - Antibodies may neutralize DENV by blocking virus attachment to cells or by blocking viral fusion with cellular membranes
  - if antibody titers are **below the threshold necessary for DENV neutralization**
    - opsonize DENV and facilitate viral entry into cells
    - increase in virus replication and higher risk of severe dengue

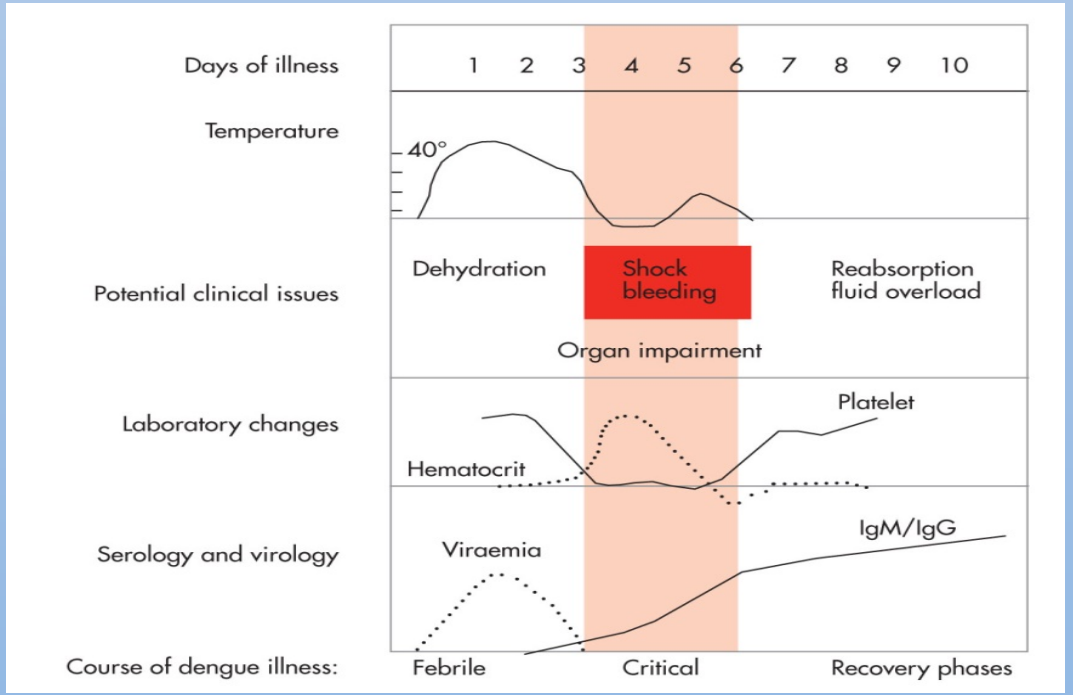
"antibody-dependent enhancement (ADE) of infection"

Clinical presentation

- Approximately a quarter of dengue infections are symptomatic
- a wide spectrum of presentation and often with unpredictable clinical evolution and outcomes

  - three phases
    - the febrile phase
    - critical phase
    - recovery phase

(Dengue guideline for Diagnosis, Treatment, Prevention and Control – WHO 2009)



Clinical presentation

- incubation period - 4–10 days,
- febrile phase
  - lasts for 2–7 days
  - acute fever and constitutional symptoms; chills, malaise, headache, arthralgia, myalgia, retro-orbital pain, anorexia, nausea, vomiting, lethargy and rash
  - Thrombocytopenia, leukopenia and variable rise in hematocrit
  - Dehydration is common
- critical phase
  - Lasts for 24-48 h
  - fever defervescence and viremia resolution
  - capillary leakage, plasma volume loss and, if untreated or improperly managed, shock
  - It is hypothesized that clinical complications are virus-driven immunological responses

Clinical presentation

- recovery phase begins after 48–72 h
  - symptoms improve, hemodynamic status stabilizes with reabsorption of extravasated fluid
  - rapid improvement in white cell and platelet counts
  - ascites and respiratory distress from massive pleural effusion, pulmonary edema or congestive heart failure

Diagnosis of dengue

1. Clinical diagnosis
   - Starts with clinical suspicion
   - 3 phases
   - Symptoms are not unique to dengue

2. Laboratory diagnosis
   - Definitive diagnosis requires laboratory confirmation

Clinical diagnosis

- starts with a clinical suspicion
- prompted by the recognition of a collection of presenting symptoms and signs
- often present with a history of sudden onset fever, which is often accompanied by nausea, aches and pains in the early acute febrile phase of illness
- symptoms are not unique to dengue
- onset of a maculopapular rash, retro-orbital pain, petechiae or bleeding nose or gums are more pathognomonic of dengue
- usefulness for early diagnosis would be more limited

Symptoms differentiating dengue infection from other febrile illnesses

Symptoms	Den|OFIs	p-value	Children (<15 years)|adults (>15 years)	p-value
Nausea	50.0|28.9% 68.0|49.0%† 51.3|30.5%	<0.00001 <0.05 <0.001	50.2|76.4%	<0.001
Vomitting	16.4|8.4% 16.2|8.5% 57.0–64.0|31.0–46.0%† 70.0|52.0%†	<0.00001 0.03 <0.01 <0.05	50.2|76.4%	<0.001
Retro-orbital pain	26.0|15.9% 26.6|13.5% 10.01§	<0.00001 0.003 0.001	8.7|29.1%	<0.001
Aches/pains	1.4§	<0.0001	20.3|36.4%	0.012
Rash	11.2–41.2|3.0–6.4%	<0.003/0.007	NA	NA
Tourniquet test positive	34.0|19.0% 42.0|5.0%† 43.0–65.0|21.0–39.0% 1.86§	0.02 <0.01 <0.1 <0.001	NA	NA
Leukopenia	3.8×10^3|7.3×10^3/μl 4.5×10^3|8.1×10^3/μl <4.5×10^3/μl: 72.1|11.5%	<0.0001 <0.1 <0.001	NA	NA
Thrombocytopenia (platelet/mm³)	16|4% (≤100,000)† 16|82%‡ (≤100,000) 66|95%‡ (≤100,000) 14.9|1.5% (≤100,000) 32,000|96,500 163,500|239,000 70,000|104,000¶	NA NA <0.01 <0.001 <0.001 <0.0001 NA	NA	NA

†Studies perfomed in children younger than 15 years.
‡Dengue and severe dengue comparison, performed in children younger than 15 years.
§Risk ratio.
¶Average.
Den: Confirmed dengue cases; NA: Not applicable; OFI: Other febrile illness.

Kin Fai Tang & Eng Eong Ooi (2012) Diagnosis of dengue: an update, expert review of Anti-infective Therapy

The Early Clinical Features of Dengue in Adults: Challenges for Early Clinical Diagnosis

Jenny G. H. Low[1], Adrian Ong[1], Li Kiang Tan[2], Shera Chaterji[3], Angelia Chow[3], Wen Yan Lim[3], Koon Wui Lee[4], Robert Chua[3], Choon Rong Chua[2], Sharon W. S. Tan[2], Yin Bun Cheung[3,5], Martin L. Hibberd[6], Subhash G. Vasudevan[3], Lee-Ching Ng[2], Yee Sin Leo[1], Eng Eong Ooi[3,4]*

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Abstract

Background: The emergence of dengue throughout the tropical world is affecting an increasing proportion of adult cases. The clinical features of dengue in different age groups have not been well examined, especially in the context of early clinical diagnosis.

Methodology/Principal Findings: We structured a prospective study of adults (≥18 years of age) presenting with acute febrile illness within 72 hours from illness onset upon informed consent. Patients were followed up over a 3–4 week period to determine the clinical outcome. A total of 2,129 adults were enrolled in the study, of which 250 (11.7%) had dengue. Differences in the rates of dengue-associated symptoms resulted in high sensitivities when the WHO 1997 or 2009 classification schemes for probable dengue fever were applied to the cohort. However, when the cases were stratified into age groups, fewer older adults reported symptoms such as myalgia, arthralgia, retro-orbital pain and mucosal bleeding, resulting in reduced sensitivity of the WHO classification schemes. On the other hand, the risks of severe dengue and hospitalization were not diminshed in older adults, indicating that this group of patients can benefit from early diagnosis, especially when an antiviral drug becomes available. Our data also suggests that older adults who present with fever and leukopenia should be tested for dengue, even in the absence of other symptoms.

Conclusion: Early clinical diagnosis based on previously defined symptoms that are associated with dengue, even when used in the schematics of both the WHO 1997 and 2009 classifications, is difficult in older adults.

Low JGH, Ong A, Tan LK, Chaterji S, Chow A, et al (2011). The Early Clinical Features of Dengue in Adults: Challenges for Early Clinical Diagnosis. PLoS Negl Trop Dis 5(5): e1191.

Decision Tree Algorithms Predict the Diagnosis and Outcome of Dengue Fever in the Early Phase of Illness

Lukas Tanner[1,9], Mark Schreiber[1,9], Jenny G. H. Low[2], Adrian Ong[2], Thomas Tolfvenstam[3], Yee Ling Lai[4], Lee Ching Ng[4], Yee Sin Leo[2], Le Thi Puong[5], Subhash G. Vasudevan[1], Cameron P. Simmons[6], Martin L. Hibberd[3], Eng Eong Ooi[7]*

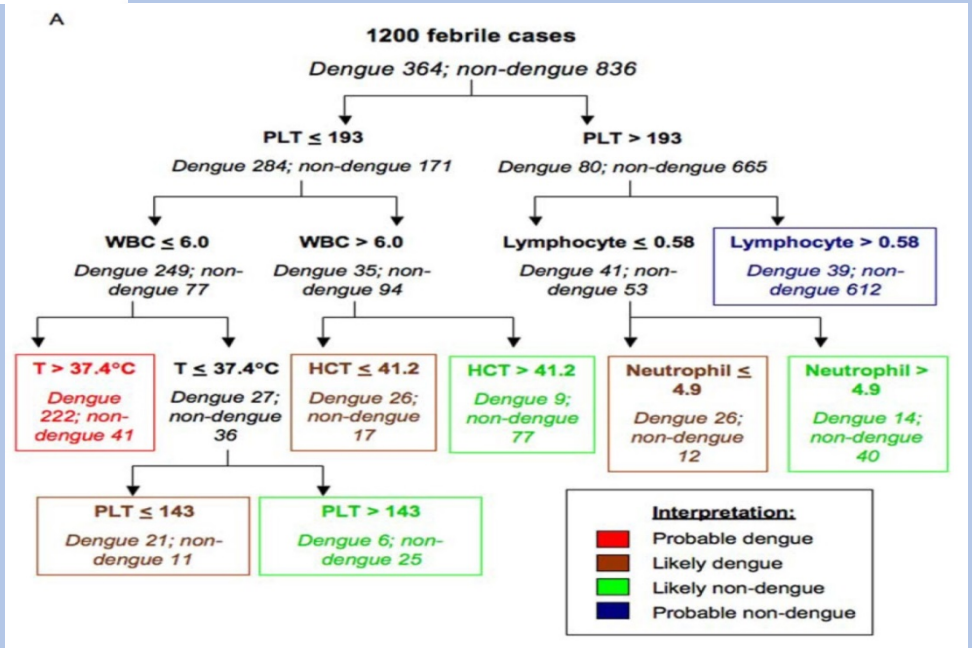
Methods and Findings: A total of 1,200 patients presenting in the first 72 hours of acute febrile illness were recruited and followed up for up to a 4-week period prospectively; 1,012 of these were recruited from Singapore and 188 from Vietnam. Of these, 364 were dengue RT-PCR positive; 173 had dengue fever, 171 had dengue hemorrhagic fever, and 20 had dengue shock syndrome as final diagnosis. Using a C4.5 decision tree classifier for analysis of all clinical, haematological, and virological data, we obtained a diagnostic algorithm that differentiates dengue from non-dengue febrile illness with an accuracy of 84.7%. The algorithm can be used differently in different disease prevalence to yield clinically useful positive and negative predictive values. Furthermore, an algorithm using platelet count, crossover threshold value of a real-time RT-PCR for dengue viral RNA, and presence of pre-existing anti-dengue IgG antibodies in sequential order identified cases with sensitivity and specificity of 78.2% and 80.2%, respectively, that eventually developed thrombocytopenia of 50,000 platelet/mm^3 or less, a level previously shown to be associated with haemorrhage and shock in adults with dengue fever.

Conclusion: This study shows a proof-of-concept that decision algorithms using simple clinical and haematological parameters can predict diagnosis and prognosis of dengue disease, a finding that could prove useful in disease management and surveillance.

Tanner L, Schreiber M, Low JGH, Ong A, Tolfvenstam T, et al (2008). Decision Tree Algorithms Predict the Diagnosis and Outcome of Dengue Fever in the Early Phase of Illness. PLoS Negl Trop Dis 2(3): e196.

Decision Node Feature	OR	95% CI (OR)	*p* value
Platelet count ≤ 193 X 1000/mm^3	13.8	13.6, 14.1	<0.0001
White cell count ≤ 6.0 x 1000 cells/mm^3	8.7	8.3, 9.1	< 0.0001
Body temperature > 37.4°C	7.2	6.6, 7.8	< 0.001
Platelet < 143 x 1000/mm^3	8.0	5.7, 11.3	< 0.01
Hematocrit ≤ 41.2	13.1	11.3, 15.2	< 0.001
Lymphocyte count ≤ 0.58 x 1000 cells/mm^3	12.1	11.6, 12.6	<0.001
Neutrophil count < 4.9 x 1000 cells/mm^3	5.9	4.6, 7.5	<0.01

(Tanner et al, 2008)



Laboratory diagnosis

1. Virus isolation
2. Viral RNA detection
3. Antigen detection
4. Antibody detection
5. Dengue neutralizing antibody detection
6. Combined antigen/antibody detection

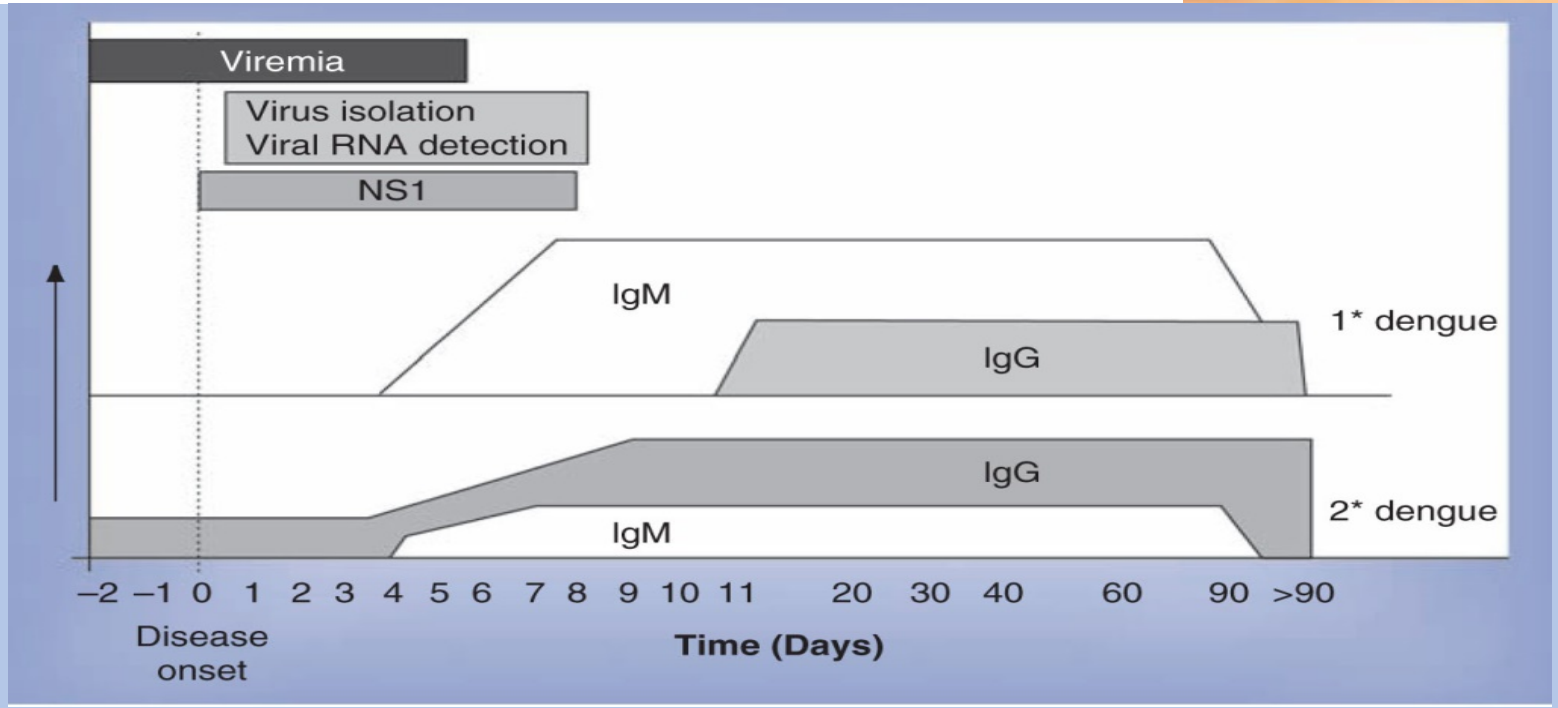
Laboratory diagnostics for dengue: sensivity and specificity

Category	Technique	Parameters		
		Sensitivity (%)	*Specificity (%)*	*Detection limit*
Viral detection	Virus isolation (mosquitoes)	71.5–84.2	100	NA
	Virus isolation (mouse intrecerebral inoculation)	NA	NA	NA
	Virus isolation (cell culture)	40.5	100	≥1 viable virus
	Viral RNA RT-PCR (conventional)	48.4–100	100	1–50 PFUs
	Viral RNA RT-PCR (real-time detection)	58.9–100	100	0.1–3.0 PFUs
	Viral RNA RT-PCR (NASBA)	98.5	100	<25 PFUs/ml
	Viral antigen detection (NS1 detection)	54.2–93.4	92.5–100	0.2 ng/ml[†]
Antibody detection	IgM detection	61.5–100	52.0–100	NA
	IgG detection	46.3–99.0	80.0–100	NA
	Rapid IgM detection (strips)	20.5–97.7	76.6–90.6	NA
Antigen/antibody combined detection	NS1 and IgM	89.9–92.9	75.0–100	NA
	NS1 and IgM/IgG	93.0	100	NA

NA: Not applicable; NASBA: Nucleic acid sequence-based amplification; PFU: Plaque forming unit; RT-PCR: Reverse transcriptase PCR.
[†]Data taken from [110].

Kin Fai Tang & Eng Eong Ooi (2012). Diagnosis of dengue: an update, Expert Review of Anti-infective Therapy, 10:8, 895-907

Approximate window of detection for dengue diagnostics



Viral isolation

- viremia - from 2 to 3 days prior to the onset of fever to up to 5.1 and 4.4 days after the onset of the disease for 1· and 2· infection respectively
- blood, serum or plasma samples can be used for virus isolation
- Mosquito, mouse brain or cell lines innoculation
- Mosquito inoculation - the most sensitive, Cell line innoculation – more widely used
- the isolation rate in patients with 1·dengue (91.0%) was higher than those with 2· dengue (77.6%)
- could be due to the interference of cross reactive antibodies with virus isolation
- Advantage - genome sequencing, virus neutralization and infection studies
- Disadvantage -highly specific but sensitivity only 40% in cell line based virus isolation, requires highly trained operators, a dependence on sample integrity and a short viremia period ---> a narrow window of opportunity from illness onset
- not widely used in routine diagnostic laboratories

Am. J. Trop. Med. Hyg., 84(2), 2011, pp. 218–223
doi:10.4269/ajtmh.2011.09-0798

Factors Influencing Dengue Virus Isolation by C6/36 Cell Culture and Mosquito Inoculation of Nested PCR-Positive Clinical Samples

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Abstract. Dengue viral isolation is necessary for definitive diagnosis, pathogenesis and evolutionary research, vaccine candidates, and diagnostic materials. Using standardized techniques, we analyzed isolation rates of 1,544 randomly selected polymerase chain reaction (PCR)-positive samples, representing all four dengue serotypes, from patients with serologically confirmed dengue infections and evaluated whether clinical and laboratory results could be predictive of isolation using standard and mosquito isolation techniques. Viruses were isolated from 62.5% of the samples by direct application to C6/36 cells and increased to 79.4% when amplifying C6/36 negative samples by intrathorasic inoculation in *Toxyrhynchites splendens* mosquitoes. High viremia, measured by reverse transcriptase (RT)-PCR, was a strong predictor for viral isolation by either method. Isolation was most successful in samples collected early in the disease, had low antibody levels, temperatures greater than 38°C, and had a final clinical diagnosis of dengue fever. Dengue serotypes also played a role in the success of viral isolation.

Viral RNA detection

- By reverse transcriptase PCR (RT-PCR) detection of dengue viral RNA extracted from blood, serum or plasma
- a rapid, sensitive and specific method for dengue infection confirmation
- conventional RT-PCR and real-time RT-PCR
- sensitivity of conventional RT-PCR ranges from 48.4 to 98.2%
- RT-PCR is rarely positive in a case of dengue after 6 days from illness onset
- Fluorescence-based real-time RT-PCR has a better reported sensitivity (58.9–100%)
- Multiplex RT-PCRs can differentiate DENV serotypes in a single assay
- Dengue viral RNA can be detected in whole blood, serum, plasma, formalin-fixed tissue specimens, urine and saliva samples using real-time RT-PCR
- Drawback - sensitivity of RT-PCR is also highly dependent on the short window of viremic period

Antigen detection (Dengue NS1)

- highly conserved glycoprotein essential for DENV viability and is secreted from infected cells
- Serum or plasma level has been found to correlate with viremia titer and disease severity
- can be found in the peripheral blood circulation for up to 9 days from illness onset but can persist for up to 18 days from illness onset in some cases
- a larger window of opportunity for diagnosis of dengue compared with virus isolation, RT-PCR

Antigen detection (Dengue NS1)

- Commercially available NS1 capture-based **detection kits** with **sensitivities** that **ranged from 54.2 to 93.4%**
- able to confirm dengue infection in serum specimens that were RT-PCR negative and secondary dengue infection
- less sensitive in secondary dengue infection (67.1–77.3%) compared with primary dengue cases (94.7–98.3%) probably owing to the presence of cross-reactive anti-NS1 antibodies that impedes the detection of free NS1 proteins in the serum or plasma
- can also detect infection in other sample sources; tissues, liver, lung and kidney through immunohistochemistry
- could be useful in postmortem studies
- Serotype specific NS1 monoclonal antibodies have been raised and applied for NS1-based dengue serotype identification assays

Vascular Leakage in Severe Dengue Virus Infections: A Potential Role for the Nonstructural Viral Protein NS1 and Complement

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Background. Vascular leakage and shock are the major causes of death in patients with dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Thirty years ago, complement activation was proposed to be a key underlying event, but the cause of complement activation has remained unknown.

Methods. The major nonstructural dengue virus (DV) protein NS1 was tested for its capacity to activate human complement in its membrane-associated and soluble forms. Plasma samples from 163 patients with DV infection and from 19 patients with other febrile illnesses were prospectively analyzed for viral load and for levels of NS1 and complement-activation products. Blood and pleural fluids from 9 patients with DSS were also analyzed.

Results. Soluble NS1 activated complement to completion, and activation was enhanced by polyclonal and monoclonal antibodies against NS1. Complement was also activated by cell-associated NS1 in the presence of specific antibodies. Plasma levels of NS1 and terminal SC5b-9 complexes correlated with disease severity. Large amounts of NS1, complement anaphylatoxin C5a, and the terminal complement complex SC5b-9 were present in pleural fluids from patients with DSS.

Conclusions. Complement activation mediated by NS1 leads to local and systemic generation of anaphylatoxins and SC5b-9, which may contribute to the pathogenesis of the vascular leakage that occurs in patients with DHF/DSS.

Avirutnan P, Punyadee N, Noisakran S et al(2006). Vascular leakage in severe dengue virus infections: a potential role for the nonstructural viral protein NS1 and complement. J. Infect. Dis. 193(8), 1078–1088

Diagnostic Accuracy of NS1 ELISA and Lateral Flow Rapid Tests for Dengue Sensitivity, Specificity and Relationship to Viraemia and Antibody Responses

Vu Ty Hang[1], Nguyen Minh Nguyet[1,2], Dinh The Trung[1,2], Vianney Tricou[1], Sutee Yoksan[3], Nguyen Minh Dung[2], Tran Van Ngoc[2], Tran Tinh Hien[2], Jeremy Farrar[1,4], Bridget Wills[1,4], Cameron P. Simmons[1,4]*

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Abstract

Background: Dengue is a public health problem in many countries. Rapid diagnosis of dengue can assist patient triage and management. Detection of the dengue viral protein, NS1, represents a new approach to dengue diagnosis.

Methodology/Principal Findings: The sensitivity and specificity of the Platelia NS1 ELISA assay and an NS1 lateral flow rapid test (LFRT) were compared against a gold standard reference diagnostic algorithm in 138 Vietnamese children and adults. Overall, the Platelia NS1 ELISA was modestly more sensitive (82%) than the NS1 LFRT (72%) in confirmed dengue cases. Both ELISA and LFRT assays were more sensitive for primary than secondary dengue, and for specimens collected within 3 days of illness onset relative to later time points. The presence of measurable DENV-reactive IgG and to a lesser extent IgM in the test sample was associated with a significantly lower rate of NS1 detection in both assays. NS1 positivity was associated with the underlying viraemia, as NS1-positive samples had a significantly higher viraemia than NS1-negative samples matched for duration of illness. The Platelia and NS1 LFRT were 100% specific, being negative in all febrile patients without evidence of recent dengue, as well as in patients with enteric fever, malaria, Japanese encephalitis and leptospirosis.

Conclusions/Significance: Collectively, these data suggest NS1 assays deserve inclusion in the diagnostic evaluation of dengue patients, but with due consideration for the limitations in patients who present late in their illness or have a concomitant humoral immune response.

Citation: Ty Hang V, Minh Nguyet N, The Trung D, Tricou V, Yoksan S, et al. (2009) Diagnostic Accuracy of NS1 ELISA and Lateral Flow Rapid Tests for Dengue Sensitivity, Specificity and Relationship to Viraemia and Antibody Responses. PLoS Negl Trop Dis 3(1): e360. doi:10.1371/journal.pntd.0000360

J Clin Virol. 2011 Apr;50(4):314-9. doi: 10.1016/j.jcv.2011.01.001. Epub 2011 Feb 1.

The development of a novel serotyping-NS1-ELISA to identify serotypes of dengue virus.

Puttikhunt C[1], Prommool T, U-thainual N, Ong-ajchaowlerd P, Yoosook K, Tawilert C, Duangchinda T, Jairangsri A, Tangthawornchaikul N, Malasit P, Kasinrerk W.

STUDY DESIGN: The monoclonal antibodies (Mabs) against NS1 of each DENV serotype were produced and characterized for their serotype-specificity. To develop serotyping-NS1-ELISA, the selected serotype-specific anti-NS1 Mabs were applied to detect the NS1 antigen, which was previously captured by a flavivirus cross-reactive anti-NS1 Mab. Serotyping accuracy of the developed assay was validated with NS1 from DENV-infected cell culture supernatants and from well-characterized clinical specimens.

RESULTS: Of 30 anti-NS1 Mabs, 1 serotype-specific anti-NS1 Mab to each DENV serotype was selected based on NS1 capture ELISA results for developing the serotyping-NS1-ELISA. Using DENV-infected cell culture supernatants for validation, the selected antibodies were shown to be capable of differentiating four DENV serotypes. When acute phase plasma from DENV-infected patients was used for validation, 65 out of 85 specimens (76.5% overall sensitivity) were positive to one of the four serotypes developed in our assay. Interestingly, identification of DENV serotypes by our serotyping-NS1-ELISA was 100% accurate for DENV1, 3 and 4 and 82.4% for DENV2 as compared with standard RT-PCR. Assay specificity was 100% (90/90).

CONCLUSIONS: The developed serotyping-NS1-ELISA provides an alternative for simultaneous detection of DENV NS1 and identification of its serotype in acute patients' specimens. The assay would be applicable for dengue diagnosis and epidemiological studies.

Antibody detection (IgM & IgG)

- Detection of antidengue antibodies (IgM and IgG) is the most widely used test in diagnosis of dengue
- kits are either in the form of Ig capture or direct Ig detection and are configured to detect IgM, IgG or both simultaneously
- two versions of these tests: ELISA or strip format (rapid test)
- ELISA provides greater sensitivity, the strip format is amenable for bedside use
- IgM can be detected as early as 3–5 days after illness onset
- levels of IgM continue to increase for approximately 2 weeks thereafter and may persist for approximately 179 and 139 days following primary and secondary infection, respectively

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Estimation of Dengue Virus IgM Persistence Using Regression Analysis▽

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Dengue virus IgM persistence was estimated using follow-up sera from 98 patients (60 with primary infections and 38 with secondary infections) whose first-specimen IgM index was strongly positive, suggesting recent disease onset. Regression analysis of the follow-up IgM index versus days between samples yielded a trend line that reached the cut-point index (1.10) at 179 days for the primary infection group and 139 days for the secondary infection group. This difference reflected significantly higher first-sample IgM indices in primary infections than in secondary infections rather than differences in IgM decay rates.

- single IgM raises the likelihood that a febrile patient has dengue
- a definitive diagnosis may require the use of paired sera to demonstrate rising IgM titers

Trans R Soc Trop Med Hyg. 2006 Aug;100(8):775-84. Epub 2006 Mar 23.

A systematic review and meta-analysis of the diagnostic accuracy of rapid immunochromatographic assays for the detection of dengue virus IgM antibodies during acute infection.

Blacksell SD[1], Doust JA, Newton PN, Peacock SJ, Day NP, Dondorp AM.

Abstract

A meta-analysis of rapid (</=60 min) dengue diagnostic assays was conducted to determine accuracy and identify causes of between-study heterogeneity. A systematic review identified 302 potentially suitable studies, of which 11 were selected for meta-analysis. All selected studies evaluated the immunochromatographic test (ICT) manufactured by Panbio Pty Ltd. Individual study results for sensitivity ranged from 0.45 to 1.0, specificity 0.57-1.0, diagnostic odds ratio 4.5-1287, and positive:negative likelihood ratios 2.3-59 and 0.01-0.56, respectively. Results indicated that the ICT evaluated in the selected studies can both rule in and rule out disease but is more accurate when samples are collected later in the acute phase of infection. Limitations of this meta-analysis were significant between-study heterogeneity caused by inconsistencies in evaluation methodologies, and the evaluation of only the Panbio ICT. It is recommended that additional, standardized evaluations are required for other dengue ICTs.

Evaluation of Commercially Available Anti–Dengue Virus Immunoglobulin M Tests

Elizabeth A. Hunsperger, Sutee Yoksan, Philippe Buchy, Vinh Chau Nguyen, Shamala D. Sekaran, Delia A. Enria, Jose L. Pelegrino, Susana Vázquez, Harvey Artsob, Michael Drebot, Duane J. Gubler, Scott B. Halstead, María G. Guzmán, Harold S. Margolis, Carl-Michael Nathanson, Nidia R. Rizzo Lic, Kovi E. Bessoff, Srisakul Kliks, and Rosanna W. Peeling

Anti–dengue virus immunoglobulin M kits were evaluated. Test sensitivities were 21%–99% and specificities were 77%–98% compared with reference ELISAs. False-positive results were found for patients with malaria or past dengue infections. Three ELISAs showing strong agreement with reference ELISAs will be included in the World Health Organization Bulk Procurement Scheme.

Hunsperger EA, Yoksan S, Buchy P et al(2009). Evaluation of commercially available anti-dengue virus immunoglobulin M tests. Emerging Infect. Dis. 15(3), 436–440.

Characteristics of 9 tests used for detection of IgM against dengue virus

ELISAs

Test name	Dengue IgM capture	Pathozyme dengue M	Pathozyme dengue M capture	Dengue fever virus IgM capture DxSelect	Dengue IgM capture
Company, location	Panbio Diagnostics, Windsor, Queensland, Australia	Omega Diagnostics, Alva, UK	Omega Diagnostics	Focus Diagnostics, Cypress, CA, USA	Standard Diagnostics, Kyonggi-do, South Korea
Detection method	IgM capture	Indirect IgM detection	IgM capture	IgM capture	IgM capture
Format	12 strips of 8 wells	12 strips of 8 wells	12 strips of 8 wells	12 strips of 8 wells	12 strips of 8 wells
No. tests/package	96	96	96	96	96
Antigen	Recombinant DENV 1–4	Purified DENV 2	DENV 1–4	DENV 1–4	DENV 1–4
Sample volume, μL	10	10	20	10	10
Total incubation time	130 min at 37°C	120 min at 37°C	110 min at 37°C	240 min at room temperature	130 min at 37°C
Storage conditions, °C	2–30	2–8	2–8	2–8	2–8

Rapid diagnostic tests

Test name	Dengue duo cassette	Hapalyse dengue-M PA kit	Dengucheck WB	SD dengue IgG/IgM
Company, location	Panbio Diagnostics	Pentax, Tokyo, Japan	Zephyr Biomedicals, Panaji, India	Standard Diagnostics
Assay principle	Lateral flow	Particle agglutination	Lateral flow	Lateral flow
Target antibody	IgM and IgG	IgM	IgM and IgG	IgM and IgG
Format	Cassette	12 strips of 8 wells	Cassette	Cassette
No. tests/package	25	96	25	25
Antigen	Recombinant DENV 1–4	DENV 1–4	Recombinant DENV (serotype not specified)	Recombinant DENV 1–4 envelope protein
Specimen type	Serum, plasma, or whole blood	Serum or plasma	Serum, plasma, or whole blood	Serum or plasma
Volume of sample required, μL	10	1	5	5
Duration of test, min	15	90	15	15–20
Storage conditions, °C	2–30	2–8	4–30	1–30
Additional equipment required	No	Yes (e.g., micropipette)	No	No

*Ig, immunoglobulin; DENV, dengue virus.

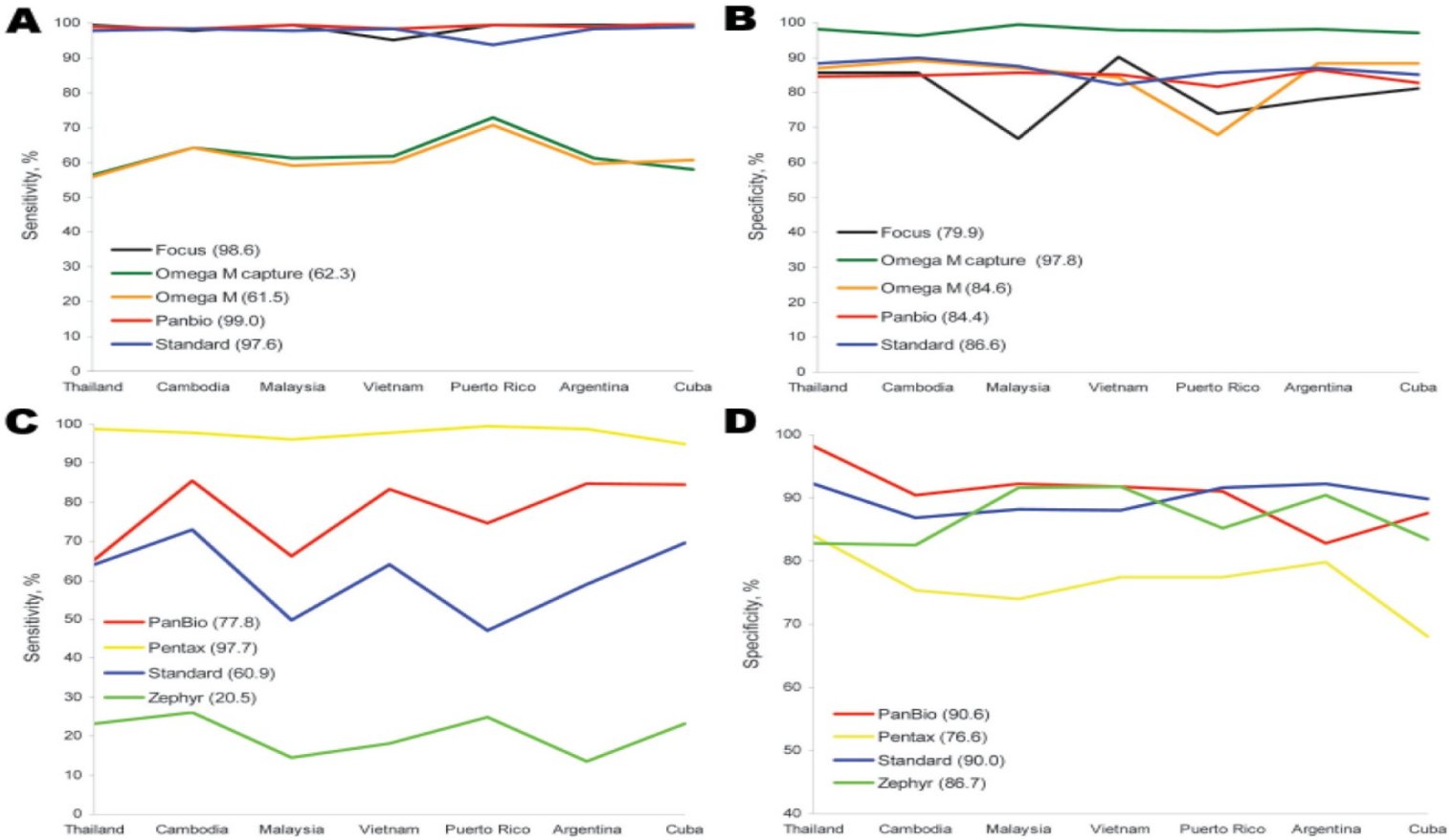

Figure A) Sensitivity and B) specificity of 5 microplate ELISAs used at laboratories in 7 countries for detecting immunoglobulin (Ig) M against dengue virus compared with reference solid-phase IgM antibody-capture ELISAs used by the Centers for Disease Control and Prevention (Atlanta, GA, USA) and the Armed Forces Research Institute of Medical Science (Bangkok, Thailand). Mean sensitivities and specificities for the 5 tests are shown in parentheses. C) Sensitivity and D) specificity of 4 rapid diagnostic tests used at laboratories in 7 countries for detecting IgM against dengue virus compared with solid-phase IgM antibody-capture ELISAs. Mean sensitivities and specificities for the 4 tests are shown in parentheses.

Antibody detection (IgM & Ig G)

- Early antidengue **IgM** response (<2 months) has been found to be cross-reactive to all four DENV serotypes and other flaviviruses
- False positives have also been observed in patients with previous dengue or malaria infection
- During primary infection, IgG can only be detected after 10 days from illness onset, making it less useful for early diagnosis
- the rapid increase of **IgG** levels during secondary infection (as early as day 4 from illness onset) can be suggestive of dengue when the ratio of IgM and IgG is used

Dengue neutralizing antibody detection

- Neutralizing antibodies provide greater specificity in distinguishing antibodies to DENV from other cross reactive flavivirus antibodies
- can be measured by using plaque reduction neutralizing tests (PRNTs)
- PRNT remains the most widely used assay for immunity studies
- labor intensive, time consuming and has low throughput and is therefore not routinely used in dengue diagnostics
- New tests - ELISA-based microneutralization test (ELISA-MN), the fluorescent antibody cell sorter-based Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin expressor DC assay and the enzyme-linked immuno sorbent spot microneutralization assay have been developed to overcome the limitations
- ?? good agreement or poor agreement in association with vaccination or secondary infection

Combined antigen/antibody detection

- Combination of the NS1 antigen, antidengue IgM and IgG antibody into a single reaction for ease of use

- diagnostic **sensitivity (89–93%)** and **specificity (75.0–100%)**

- rapid diagnostic tools – single or duo, sensitivities and specificities are uniformly lower than the equivalent laboratory-based assays

Tricou *et al. BMC Infectious Diseases* 2010, **10**:142
http://www.biomedcentral.com/1471-2334/10/142

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Comparison of two dengue NS1 rapid tests for sensitivity, specificity and relationship to viraemia and antibody responses

Vianney Tricou*[1], Hang TT Vu[1], Nhu VN Quynh[1], Chau VV Nguyen[2], Hien T Tran[2], Jeremy Farrar[1,3], Bridget Wills[1,3] and Cameron P Simmons[1,3]

Methods: The sensitivity and specificity of the Bio-Rad NS1 Ag Strip and SD Dengue Duo (NS1/IgM/IgG) lateral flow rapid tests were evaluated in a panel of plasma samples from 245 Vietnamese patients with RT-PCR confirmed dengue and 47 with other febrile illnesses.

Results: The NS1 rapid tests had similar diagnostic sensitivities (respectively 61.6% and 62.4%) in confirmed dengue cases but were 100% specific. When IgM/IgG results from the SD Dengue Duo were included in the test interpretation, the sensitivity improved significantly from 62.4% with NS1 alone to 75.5% when NS1 and/or IgM was positive and 83.7% when NS1 and/or IgM and/or IgG was positive. Both NS1 assays were significantly more sensitive for primary than secondary dengue. NS1 positivity was associated with the underlying viraemia as NS1-positive samples had a significantly higher viraemia than NS1-negative samples.

Conclusions: These data suggest that the NS1 test component of these assays are highly specific and have similar levels of sensitivity. The IgM parameter in the SD Duo test improved overall test sensitivity without compromising specificity. The SD Dengue Duo lateral flow rapid test deserves further prospective evaluation in dengue endemic settings.

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1556-6811/11/$12.00 doi:10.1128/CVI.05285-11

Evaluation of Six Commercial Point-of-Care Tests for Diagnosis of Acute Dengue Infections: the Need for Combining NS1 Antigen and IgM/IgG Antibody Detection To Achieve Acceptable Levels of Accuracy▽†

Stuart D. Blacksell,[1,2]* Richard G. Jarman,[3] Mark S. Bailey,[4] Ampai Tanganuchitcharnchai,[1] Kemajittra Jenjaroen,[1] Robert V. Gibbons,[3] Daniel H. Paris,[1,2] Ranjan Premaratna,[5] H. Janaka de Silva,[5] David G. Lalloo,[6] and Nicholas P. J. Day[1,2]

Six assays were evaluated in this study to determine their suitability for the diagnosis of acute dengue infection using samples from 259 Sri Lankan patients with acute fevers (99 confirmed dengue cases and 160 patients with other confirmed acute febrile illnesses): (i) the Merlin dengue fever IgG & IgM combo device (Merlin), (ii) the Standard Diagnostics Dengue Duo nonstructural 1 (NS1) antigen and IgG/IgM combo device (Standard Diagnostics, South Korea), (iii) the Biosynex Immunoquick dengue fever IgG and IgM (Biosynex, France) assay, (iv) the Bio-Rad NS1 antigen strip (Bio-Rad, France), (v) the Panbio Dengue Duo IgG/IgM Cassette (Inverness, Australia), and (vi) the Panbio dengue NS1 antigen strip (Inverness, Australia). The median number of days of fever prior to admission sample collection was 5 days (interquartile range, 3 to 7 days). Sensitivity and specificity of the NS1 antigen tests ranged from 49 to 59% and from 93 to 99%, respectively, and sensitivity and sensitivity of the IgM antibody test ranged from 71 to 80% and from 46 to 90%, respectively. Combining the NS1 antigen and IgM antibody results from the Standard Diagnostics Dengue Duo test gave the best compromise of sensitivity and specificity (93% and 89%, respectively) and provided the best sensitivity in patients presenting at different times after fever onset. The Merlin IgM/IgG antibody tests correctly classified 64% and 86% of the primary and secondary dengue infection cases, respectively, and the Standard Diagnostics IgM/IgG antibody tests correctly classified 71% and 83% of the primary and secondary dengue infection cases, respectively. This study provides strong evidence of the value of combining dengue antigen- and antibody-based test results in the rapid diagnostic test (RDT) format for the acute diagnosis of dengue.

Key issues in diagnosis in DENV

- Clinical diagnosis using the 1997–2009 WHO dengue classification schemes has high sensitivity but lacks specificity
- Decision algorithms for diagnosis have been proposed
- Choice of diagnostic assays should be guided by the time from illness onset
- The presence of pre-existing antibodies from a previous heterologous dengue virus infection or a previous flavivirus infection can affect the sensitivity or specificity of many diagnostic assays

Rationale for dengue therapy

- treatment for dengue is largely supportive
- no licensed therapeutic drug
- Several clinical trials of therapeutic interventions against acute dengue
- target the virus, host immune responses or host factors required by DENV to complete replication cycle
- studies reported higher viremia levels (>tenfold) in patients with severe compared with nonsevere during early infection

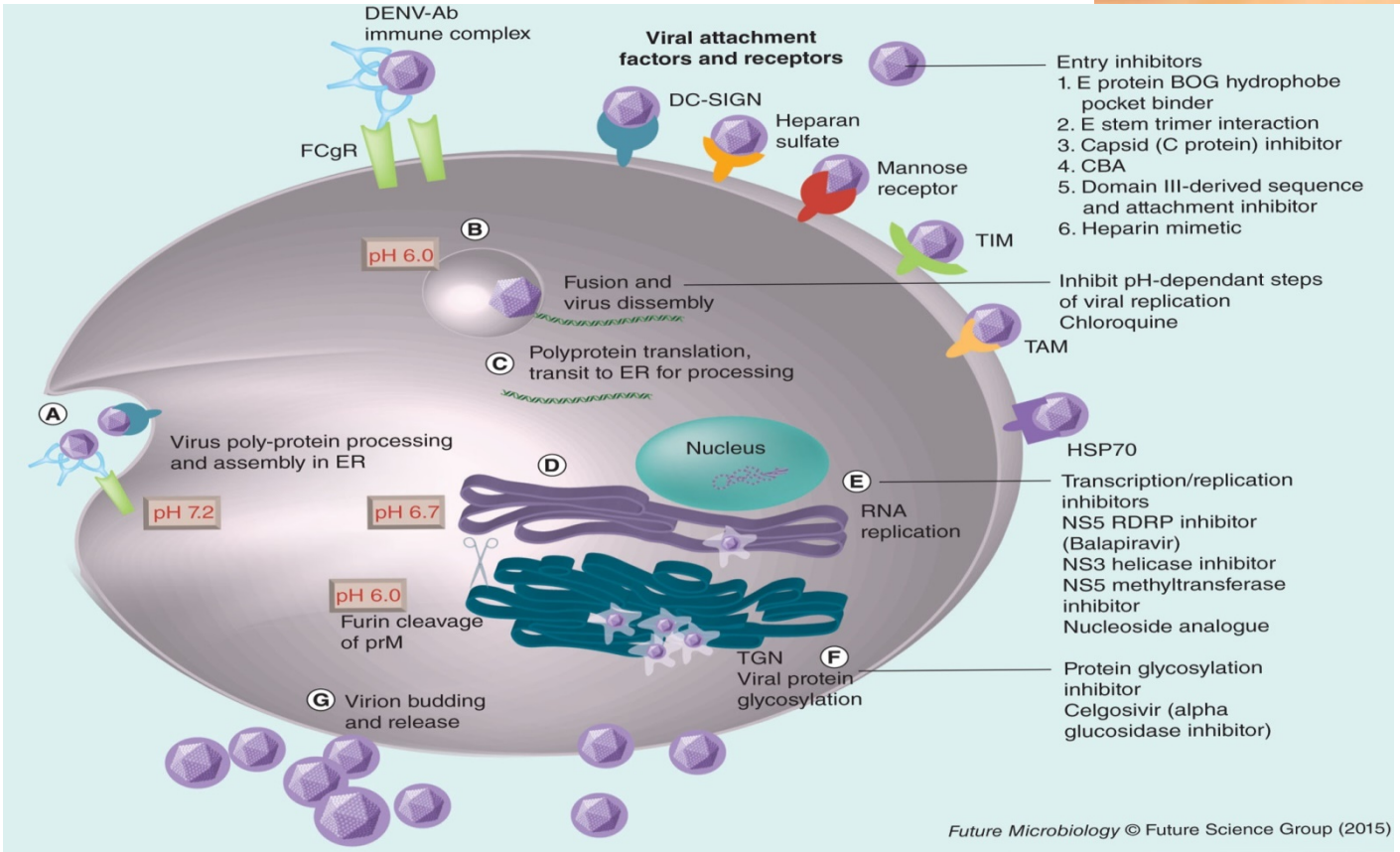
- hypothesis → reducing viremia by antiviral therapy in the early phase of dengue infection may reduce severity of clinically apparent disease

Target Profile of a dengue therapeutic

Ideal properties of a dengue therapeutic

Drug parameters	Ideal properties
Spectrum	Effective against all four DENV serotypes
Clinical outcome	Rapid resolution of symptoms, reduced severity
Safety	Well tolerated, minimal toxicity and does not require lab monitoring
Bioavailability	Fast acting, high volume of distribution
Pharmacokinetic	Sufficient half-life to enable infrequent (e.g., once daily) dosing to promote compliance
Route of administration	Oral rather than parenteral
Interactions	Minimal with common drugs
Target population	Adults, children, infants, pregnant women, patients with co-morbidities (renal, hepatic)
Others	Long shelf-life, stable without refrigeration to facilitate rural distribution
Cost	Affordable in dengue-endemic countries

Life cycle of DENV, drug targets and candidate drugs



I. Drugs targeting dengue proteins

Direct-acting antiviral agents

1. Entry/fusion inhibitors
2. Replication & transcription inhibitors
3. MTase inhibitors
4. Nucleoside analog
5. Helicase inhibitors
6. Protease inhibitors
7. NS4B inhibitor

? Antiviral therapy in early phase

Differing Influences of Virus Burden and Immune Activation on Disease Severity in Secondary Dengue-3 Virus Infections

Daniel H. Libraty,[1,3] Timothy P. Endy,[2,3,a] Huo-Shu H. Houng,[2] Sharone Green,[1] Siripen Kalayanarooj,[4] Saroj Suntayakorn,[5] Wanya Chansiriwongs,[4] David W. Vaughn,[2] Ananda Nisalak,[3] Francis A. Ennis,[1] and Alan L. Rothman[1]

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Dengue hemorrhagic fever (DHF), the most severe form of illness following infection with a dengue virus, is characterized by plasma leakage, thrombocytopenia, and hepatic inflammation. The interrelationships among virus burden, immune activation, and development of DHF were examined in 54 children with secondary dengue-3 virus infections participating in a prospective, hospital-based study. DHF was associated with higher mean plasma viremia early in illness and earlier peak plasma interferon-γ levels. Maximum plasma viremia levels correlated with the degree of plasma leakage and thrombocytopenia. Maximum plasma levels of interleukin (IL)–10 and soluble tumor necrosis factor receptor–II correlated with the degree of thrombocytopenia, independently of viremia levels. Hepatic transaminase elevation correlated with plasma soluble IL-2 receptor levels and not with viremia levels. Quantitative differences in virus burden and host immune responses, and the timing of type 1 cytokine responses, have differing influences on the severity of disease manifestations during secondary dengue-3 virus infections.

Libraty DH, Endy TP, Houng H-SH et al. Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. J. Infect. Dis. 185(9), 1213–1221 (2002).

? Antiviral therapy in early phase

High Levels of Plasma Dengue Viral Load during Defervescence in Patients with Dengue Hemorrhagic Fever: Implications for Pathogenesis

Wei-Kung Wang,*,[1] Day-Yu Chao,† Chuan-Liang Kao,‡ Han-Chung Wu,§ Yung-Ching Liu,[¶] Chien-Ming Li,‖ Shih-Chung Lin,*,* Shih-Ting Ho,†,† Jyh-Hsiung Huang,‡,‡ and Chwan-Chuen King†

Studies of the pathogenesis of dengue hemorrhagic fever (DHF), a potentially life-threatening disease, have revealed the importance of initial high levels of virus replication. However, the possible involvement of virus during the transition from fever to defervescence, a critical stage in determining the severity of disease, has not been appreciated. Using quantitative reverse transcription-polymerase chain reaction, we examined the levels of plasma dengue viral load during both fever and defervescence periods in patients from a DEN-3 outbreak in southern Taiwan in 1998. Higher levels of plasma dengue viral RNA were found in DHF patients than in DF patients. During defervescence, while the level of plasma dengue viral RNA was undetectable in most DF patients, it remains high in all DHF patients. Using a modified immunoprecipitation assay, we demonstrated for the first time that the plasma dengue viruses persisting during defervescence were in the immune complexes for most DHF patients. These findings suggest that continued active viral replication or delay in the clearance of viremia contributes to the pathogenesis of DHF. Moreover, high levels of plasma dengue viral RNA during defervescence may serve as a disease marker for DHF.

Wang W-K, Chao D-Y, Kao C-L et al (2003). High levels of plasma dengue viral load during defervescence in patients with dengue hemorrhagic fever: implications for pathogenesis. Virology 305(2), 330–338

? Antiviral therapy in early phase

Slower Rates of Clearance of Viral Load and Virus-Containing Immune Complexes in Patients with Dengue Hemorrhagic Fever

Wei-Kung Wang,[1,3] Hui-Ling Chen,[1] Chao-Fu Yang,[1] Szu-Chia Hsieh,[1] Chung-Chou Juan,[5] Shu-Mei Chang,[5] Cheng-Ching Yu,[6] Li-Hui Lin,[6] Jyh-Hsiung Huang,[4] and Chwan-Chuen King[2]

Methods. We investigated plasma dengue viral load, virus in immune complexes, antibody response, complements, and cytokines for 54 patients with dengue fever (a relatively mild form of disease) and 49 patients with DHF. The patients had confirmed secondary infection with dengue virus type 2 from a large outbreak in southern Taiwan in 2002.

Results. Patients with DHF had a significantly higher viral load and a slower rate of clearance than patients with dengue fever. For viral loads >5.7 log RNA copies/mL on the day of defervescence, the positive and negative predictive values for DHF are 0.88 and 0.95, respectively. A higher level and slower decline of dengue virus–containing immune complexes (and a subsequently higher elevation of C5a and soluble interleukin 2 receptor) were found in patients with DHF, compared with patients with dengue fever.

Conclusions. These findings indicate that slower rates of clearance of viral load and virus-containing immune complexes are associated with subsequent immune activation and contribute to the progression of DHF at this critical stage. Moreover, viral load on the day of defervescence can predict cases of DHF.

Wang W-K, Chen H-L, Yang C-F et al. Slower rates of clearance of viral load and virus-containing immune complexes in patients with dengue hemorrhagic fever. Clin. Infect. Dis. 43(8), 1023–1030 (2006).

Practical challenges in development of Dengue therapy

- present to healthcare settings may be too late to benefit from antiviral therapy
- clinically difficult in differentiating dengue from other febrile diseases
- lacking of diagnostic tests in resource limited settings
- Challenging in identifying patients at risk of severe disease
- antiviral treatment must be fast acting as viremia declines rapidly after illness onset

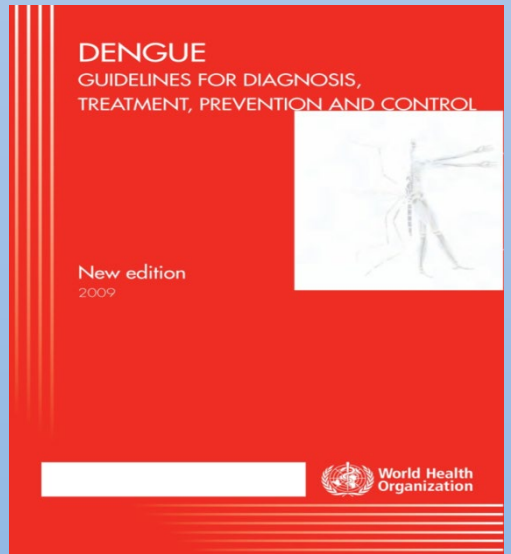
Dengue therapeutics development must be coupled with improved diagnostic and prognostic tools in order to translate research into clinical practice

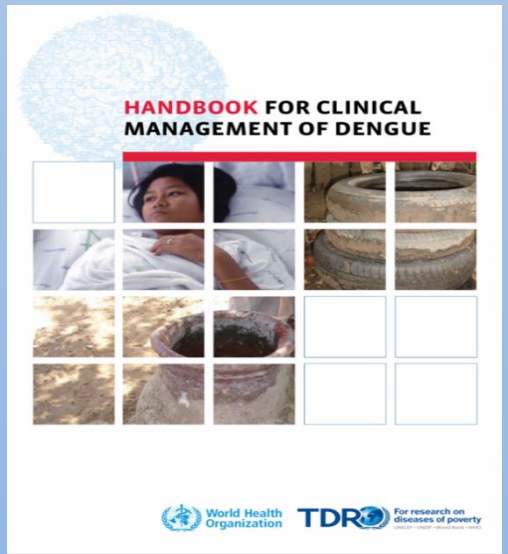
Gold Standard – clinical intervention

(Supportive therapies during acute dengue)

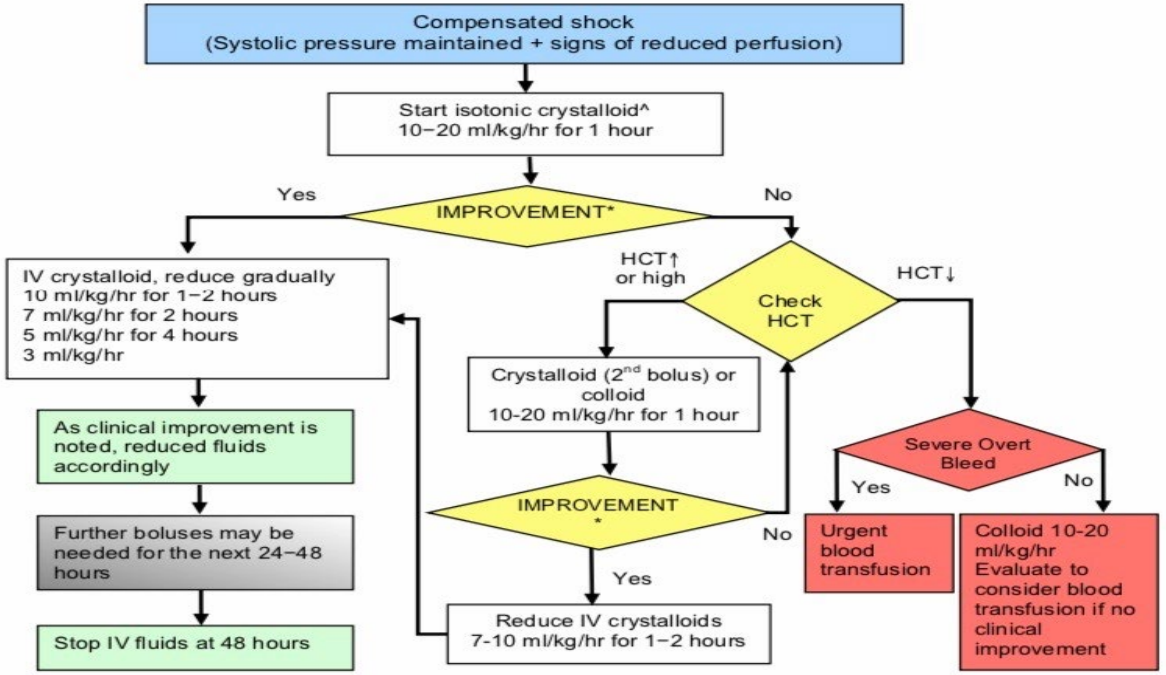
II. Fluid Therapy

- Sensible fluid resuscitation is of paramount importance in the management of patients with severe dengue
- a clinical algorithm in the latest WHO guidelines
- goal is to prevent the complications of vascular leakage and hypovolemic shock



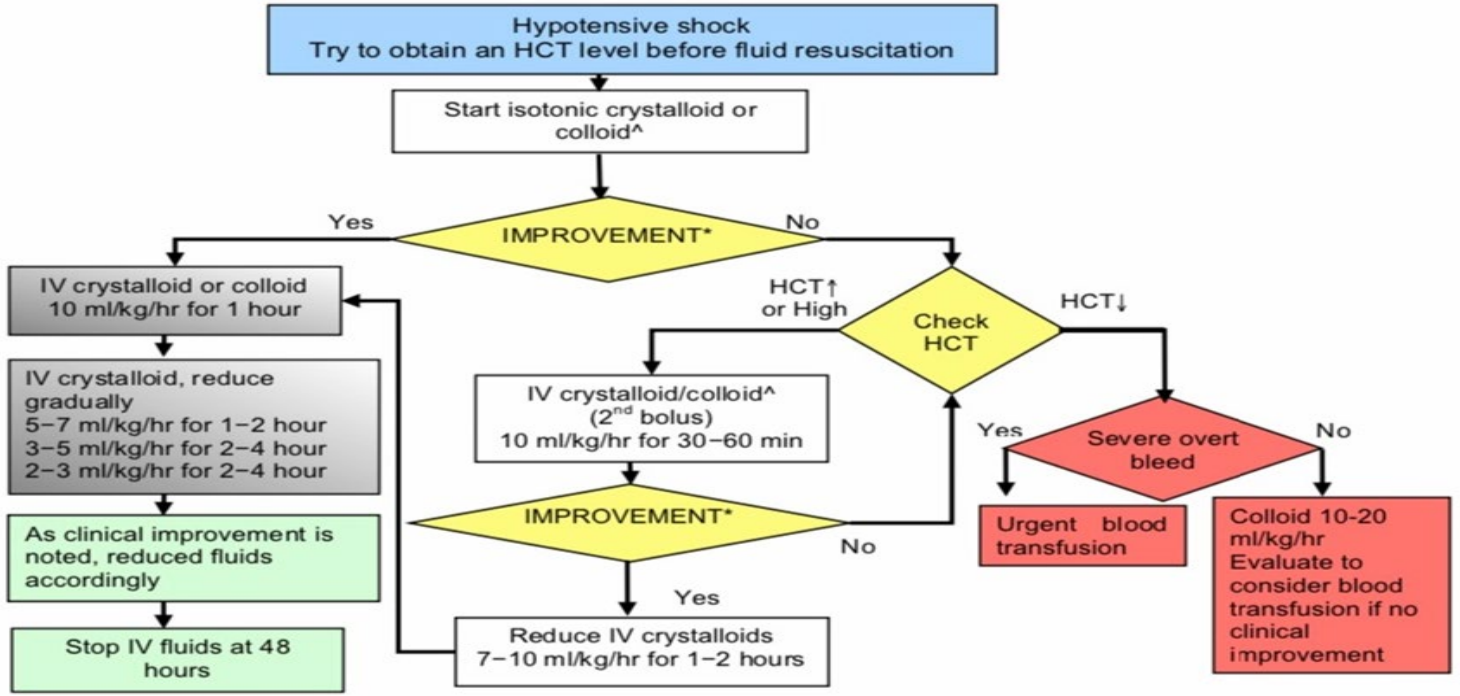


Algorithm for fluid management of compensated shock: in infants and children



^Colloid is preferable if the patient has already received previous boluses of crystalloid
*Reassess the patient's clinical condition, vital signs, pulse volume, capillary refill time and temperature of extremities.IV = intravenous; HCT = haematocrit; ↑ = increased; ↓ = decreased

Algorithm for fluid management in hypotensive shock – infants, children and adults



^Colloid is preferable if the patient has already received previous boluses of crystalloid
*Reassess the patient's clinical condition, vital signs, pulse volume, capillary refill time and temperature of extremities.
IV = intravenous; HCT = haematocrit; ↑ = increased; ↓ = decreased

Fluid therapy

- **Colloids**
  - provide volume expansion over and above the actual fluid volume infused
  - molecules increase plasma oncotic pressure and promote retention of fluid in the intravascular compartment
  - magnitude of this effect is determined by the average molecular weight of the colloid molecule and circulation retention time determines the duration of the effect

- **Crystalloids**
  - Plasma volume-expanding capacity is related to sodium concentration
  - NaCl 0.9% (154 mM) >>> Ringer's lactate (131 mM)(theoretical risks of worsening tissue acidosis and lactate accumulation when large volumes are infused)

Fluid Replacement in Dengue Shock Syndrome: A Randomized, Double-Blind Comparison of Four Intravenous-Fluid Regimens

N. M. Dung, N. P. J. Day, D. T. H. Tam, H. T. Loan, H. T. T. Chau, L. N. Minh, T. V. Diet, D. B. Bethell, R. Kneen, T. T. Hien, N. J. White, and J. J. Farrar

From The Centre for Tropical Diseases and the Wellcome Trust Clinical Research Unit, Ho Chi Minh City, Vietnam, and the Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, United Kingdom

Dengue hemorrhagic fever and dengue shock syndrome (DSS) are major causes of childhood morbidity and mortality in many tropical countries. Increased intravascular permeability leading to shock is the cardinal feature of DSS. Fluid resuscitation to counteract massive plasma leakage is the mainstay of treatment. A double-blind, randomized trial comparing four intravenous-fluid regimens for acute resuscitation of 50 children with DSS was conducted. Colloids (dextran 70 or the protein digest gelafundin 35,000) restored cardiac index and blood pressure and normalized hematocrit more rapidly than crystalloids (Ringer's lactate or 0.9%-weight/volume saline). Dextran 70 provided the most rapid normalization of the hematocrit and restoration of the cardiac index, without adverse effects, and may be the preferred solution for acute resuscitation in DSS. Further large-scale double-blind trials are required to provide an evidence-based approach to the management of DSS.

Dung NM, Day NP, Tam DT et al (1999). Fluid replacement in dengue shock syndrome: a randomized, double-blind comparison of four intravenous-fluid regimens. Clin. Infect. Dis. 29(4), 787–794

Acute Management of Dengue Shock Syndrome: A Randomized Double-Blind Comparison of 4 Intravenous Fluid Regimens in the First Hour

Ngo Thi Nhan,[1] Cao Xuan Thanh Phuong,[1,a] Rachel Kneen,[2,3] Bridget Wills,[2,3] Nguyen Van My,[1] Nguyen Thi Que Phuong,[1] Chu Van Thien,[1] Nguyen Thi Thuy Nga,[1] Julie A. Simpson,[2,3] Tom Solomon,[2,3] Nicholas J. White,[2,3] and Jeremy Farrar[2,3]

[1]Dong Nai Paediatric Hospital, Bien Hoa, Dong Nai Province, [2]Wellcome Trust Clinical Research Unit, Centre for Tropical Diseases, Ho Chi Minh City, Vietnam; and [3]Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

Dengue hemorrhagic fever is an important cause of morbidity among Asian children, and the more severe dengue shock syndrome (DSS) causes a significant number of childhood deaths. DSS is characterized by a massive increase in systemic capillary permeability with consequent hypovolemia. Fluid resuscitation is critical, but as yet there have been no large trials to determine the optimal fluid regimen. We undertook a randomized blinded comparison of 4 fluids (dextran, gelatin, lactated Ringer's, and "normal" saline) for initial resuscitation of 230 Vietnamese children with DSS. All the children survived, and there was no clear advantage to using any of the 4 fluids, but the longest recovery times occurred in the lactated Ringer's group. The most significant factor determining clinical response was the pulse pressure at presentation. A comparison of the colloid and crystalloid groups suggested benefits in children presenting with lower pulse pressures who received one of the colloids. Further large-scale studies, stratified for admission pulse pressure, are indicated.

Ngo NT, Cao XT, Kneen R et al (2001). Acute management of dengue shock syndrome: a randomized double-blind comparison of 4 intravenous fluid regimens in the first hour. Clin. Infect. Dis. 32(2), 204–213

Comparison of Three Fluid Solutions for Resuscitation in Dengue Shock Syndrome

Bridget A. Wills, M.R.C.P., Nguyen M. Dung, M.D., Ha T. Loan, M.D., Dong T.H. Tam, M.D., Tran T.N. Thuy, M.D., Le T.T. Minh, M.D., Tran V. Diet, M.D., Nguyen T. Hao, M.D., Nguyen V. Chau, M.D., Kasia Stepniewska, Ph.D., Nicholas J. White, F.R.C.P., and Jeremy J. Farrar, F.R.C.P.

ABSTRACT

BACKGROUND

Dengue shock syndrome is characterized by severe vascular leakage and disordered hemostasis and progresses to death in 1 to 5 percent of cases. Although volume replacement is recognized as the critical therapeutic intervention, World Health Organization management guidelines remain empirical rather than evidence-based.

METHODS

We performed a double-blind, randomized comparison of three fluids for initial resuscitation of Vietnamese children with dengue shock syndrome. We randomly assigned 383 children with moderately severe shock to receive Ringer's lactate, 6 percent dextran 70 (a colloid), or 6 percent hydroxyethyl starch (a colloid) and 129 children with severe shock to receive one of the colloids. The primary outcome measure was requirement for rescue colloid at any time after administration of the study fluid.

RESULTS

Only one patient died (<0.2 percent mortality). The primary outcome measure — requirement for rescue colloid — was similar for the different fluids in the two severity groups. The relative risk of requirement for rescue colloid was 1.08 (95 percent confidence interval, 0.78 to 1.47; P=0.65) among children with moderate shock who received Ringer's lactate as compared with either of the colloid solutions, 1.13 (95 percent confidence interval, 0.74 to 1.74; P=0.59) among children who received dextran as compared with starch in the group with severe shock, and 0.88 (95 percent confidence interval, 0.66 to 1.17; P=0.38) among children who received dextran as compared with starch in the combined analysis. Although treatment with Ringer's lactate resulted in less rapid improvement in the hematocrit and a marginally longer time to initial recovery than did treatment with either of the colloid solutions, there were no differences in all other measures of treatment response. Only minor differences in efficacy were detected between the two colloids, but significantly more recipients of dextran than of starch had adverse reactions. Bleeding manifestations, coagulation derangements, and severity of fluid overload were similar for all fluid-treatment groups.

From the Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, and the Centre for Clinical Vaccinology and Tropical Medicine, Oxford University, Oxford, United Kingdom (B.A.W., K.S., N.J.W., J.J.F.); and the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam (N.M.D., H.T.L., D.T.H.T., T.T.N.T., L.T.T.M., T.V.D., N.T.H., N.V.C.). Address reprint requests to Dr. Wills at Oxford University Clinical Research Unit, Hospital for Tropical Diseases, 190 Ben Ham Tu, Quan 5, Ho Chi Minh City, Vietnam, or at bridgetw@hcm.vnn.vn.

N Engl J Med 2005;353:877-89.

No clear advantage to use of colloids in term of overall outcome

Study (year)	Des			Participants (n)	Fluid	Intervention	Conclusion
Dung *et al.* (1999)	Double-blin randomized, controlled trial		ts	50	NaCl 0.9%; dextran 7; 3% gelatin; Ringer's lactate	Study fluid was administered for the first 72 h	Colloids may be superior to crystalloids, with more rapid restoration of hematocrit and cardiovascular stability
Ngo *et al.* (2001)	Double-blind, randomized trial	Vietnam	(a DSS hemorr		Dextran 70; 3% gelatin; Ringer's lactate; NaCl 0.9%	Study fluid was administered for the first 1 h, followed by Ringer's lactate (WHO guideline*)	No clear advantage to using any of the fluids in cardiovascular recovery time. Colloids were more likely to benefit patients severe shock
Wills *et al.* (2005)	Double-blind, randomized trial	Vietnam	Pediatric ICU pa (aged 2–15 years) w DSS	severe ived s sever	sta	Study fluid was administered for the first 2 h, followed by Ringer's lactate*. Patients with persistent shock after inistration of study re given rescue	Requirement for rescue colloid was similar for the different regimens. Significantly, more recipients in the dextran group had coagulopathy and fluid overload than starch. Colloids performed similarly in severe DSS. Ringer's lactate should be used in children with moderately severe DSS
Kalayanarooj (2008)	Randomized, single-blinded trial	Thailand	Pediatric DHF patients (aged 8.6 ± 3.9 years) who initially received crystalloid, but failed to improve or developed fluid overload	104	Colloids – hydroxyethyl 10% dextran 40	s	Both colloids were equally effective with no difference in mplications with regard to verload, renal functions coagulation

*By WHO guideline 1997.
#Abstract only.
§Clinical diagnosis according to the WHO 1999 guideline.
DHF: Dengue hemorrhagic fever; DSS: Dengue shock syndrome.

Recommandations for fluid therapy

1. Ringer's lactate – theoretical risks of worsening tissue acidosis and lactate accumulation

2. No significant advantage of colloids compared with lactate infusion

3. Effect of colloids are transient

4. Colloids may be the preferred choice in those with PP< 10 mmHg

5. **Importance of carefully titrated fluid therapy to maintain vital functions during the vascular leakage period without overfilling the intravascular space**

III. Blood products

- Thrombocytopenia (platelet count below 150×10^9/l) is a hallmark of Dengue infection and usually observed between days 3 and 8 following the onset of illness
- Platelet count plummets in parallel with a rising hematocrit, indicative of progression to the critical phase of the disease
- Platelet count reaches nadir during defervescence (days 3–6) followed by gradual spontaneous recovery
- Minor bleeding (mucosal, petechiae) without hemodynamic instability is common and usually resolves spontaneously

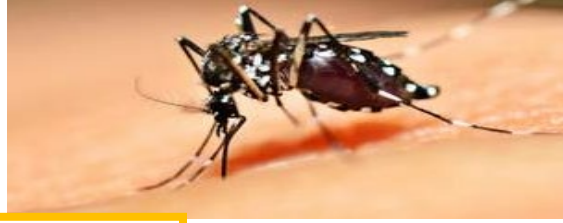
Blood products

- In patients with severe thrombocytopenia (defined as ≤20 × 109/l), strict bed rest and avoidance of NSAIDs and intramuscular injections are usually sufficient to reduce the risk of severe bleeding

- correlation between platelet count and bleeding risk is lacking

- the benefits of treatment are outweighed by significant risks including fluid overload, transfusion-associated lung injury, blood-borne infections and allergic reactions

Platelets transfusion in Dengue



Recommendations: Other Supportive Therapy of Severe Sepsis

K. Blood Product Administration

1. Once tissue hypoperfusion has resolved and in the absence of extenuating circumstances, such as myocardial ischemia, severe hypoxemia, acute hemorrhage, or ischemic heart disease, we recommend that red blood cell transfusion occur only when hemoglobin concentration decreases to <7.0 g/dL to target a hemoglobin concentration of 7.0 –9.0 g/dL in adults (grade 1B).
2. Not using erythropoietin as a specific treatment of anemia associated with severe sepsis (grade 1B).
3. Fresh frozen plasma not be used to correct laboratory clotting abnormalities in the absence of bleeding or planned invasive procedures (grade 2D).
4. Not using antithrombin for the treatment of severe sepsis and septic shock (grade 1B).
5. In patients with severe sepsis, administer platelets prophylactically when counts are <10,000/mm^3 (10 x 10^9/L) in the absence of apparent bleeding. We suggest prophylactic platelet transfusion when counts are < 20,000/mm^3 (20 x 10^9/L) if the patient has a significant risk of bleeding. Higher platelet counts (≥50,000/mm^3 [50 x 10^9/L]) are advised for active bleeding, surgery, or invasive procedures (grade 2D).

Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock: 2012

Prophylactic platelet transfusion in patients without bleeding when platelet falls below 10–20 × 109/l is widely practiced in **sepsis**, but is not supported by evidence in **dengue** management

The JOURNAL of PEDIATRICS

Risk factors for hemorrhage in severe dengue infections*

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Article Info

The purpose of this study was to identify the early indicators of hemorrhage in severe dengue infections in 114 patients; 24 patients had severe hemorrhage and 92 had no hemorrhage. The platelet counts were not predictive of bleeding. The duration of shock (OR, 2.11; 95% CI, 1.13 to 3.92; P =.019) and low-normal hematocrit at the time of shock (OR, 0.72; 95% CI, 0.55 to 0.95; P =.020) were risk factors of severe hemorrhage. (J Pediatr 2002;140:629-31)

Confirmed adult dengue deaths in Singapore: 5-year multi-center retrospective study

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Abstract

Background: Dengue re-emerges in Singapore despite decades of effective vector control; the infection predominantly afflicts adults. Severe dengue not fulfilling dengue hemorrhagic fever (DHF) criteria according to World Health Organization (WHO) 1997 guideline was increasingly reported. A new WHO 2009 guideline emphasized warning signs and a wider range of severe dengue manifestations. We aim to evaluate the utility of these two guidelines in confirmed adult dengue fatalities.

Methods: We conducted a multi-center retrospective chart review of all confirmed adult dengue deaths in Singapore from 1 January 2004 to 31 December 2008.

Results: Of 28 adult dengue deaths, median age was 59 years. Male gender comprised 67.9% and co-morbidities existed in 75%. From illness onset, patients presented for admission at a median of 4 days and death occurred at a median of 12 days. Intensive care admission was required in 71.4%. Probable dengue was diagnosed in 32.1% by WHO 1997 criteria and 78.6% by WHO 2009. The earliest warning sign was persistent vomiting at a median of 1.5 days. Hematocrit change ≥20% concurrent with platelet count <20 × 10^9/L was associated with the shortest interval to death at a median of 3 days. Only 35.7% of death cases fulfilled DHF criteria by WHO 1997 versus severe dengue in 100.0% by WHO 2009 criteria. Deaths were due to shock and organ failure. Acute renal impairment occurred in 71.4%, impaired consciousness 57.1% and severe hepatitis 53.6%.

Conclusions: In our adult fatal dengue cohort, WHO 2009 criteria had higher sensitivity in diagnosing probable dengue and severe dengue compared with WHO 1997. As warning signs, persistent vomiting occurred early and hematocrit change ≥20% concurrent with platelet count <20 × 10^9/L preceded death most closely.

Leo Y-S, Thein TL, Fisher DA et al (2011). Confirmed adult dengue deaths in Singapore: 5 year multi-center retrospective study. BMC Infect. Dis. 11, 123

Ceylon Med J. 2008 Jun;53(2):36-40.

Is fresh frozen plasma effective for thrombocytopenia in adults with dengue fever? A prospective randomised double blind controlled study.

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Abstract

RATIONALE: Thrombocytopenia is a common problem which causes concern and complications in dengue fever. If proven effective, intravenous fresh frozen plasma is a simple and widely available therapeutic option to manage thrombocytopenia.

OBJECTIVE: To test the efficacy of fresh frozen plasma (FFP) on thrombocytopenia in patients with dengue fever.

DESIGN: 109 serologically confirmed dengue patients with platelet counts <40 000/mm3 were randomised into two groups. Group A (treatment) comprised 53 patients and group B (control) 56 patients. Group A received an intravenous infusion of 3 units (600 ml) of FFP over 90 minutes. Group B received an intravenous infusion of an equal volume of isotonic saline over the same period. The primary outcome measure was the difference between pre- and post-interventional platelet counts at 12, 24 and 48 hours.

RESULTS: Following Intervention, the mean platelet count was significantly higher in Group Athan in Group B at 12 hours (p=0.04; t-test). The mean platelet counts continued to be higher in Group A than in Group B at 24 and 48 hours post-intervention, but the differences were not statistically significant.

CONCLUSIONS: In dengue patients with thrombocytopenia, infusion of 600 ml FFP may contribute to a significant increase in platelet count in the first 12 hours, but not thereafter.

Lack of Efficacy of Prophylactic Platelet Transfusion for Severe Thrombocytopenia in Adults with Acute Uncomplicated Dengue Infection

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Thrombocytopenia in dengue infection raises concerns about bleeding risk. Of 256 patients with dengue infection who developed thrombocytopenia (platelet count, $<20 \times 10^3$ platelets/μL) without prior bleeding, 188 were given platelet transfusion. Subsequent bleeding, platelet increment, and platelet recovery were similar between patients given transfusion and patients not given transfusion. Prophylactic platelet transfusion was ineffective in preventing bleeding in adult patients with dengue infection.

Lye DC, Lee VJ, Sun Y, Leo Y-S (2009). Lack of efficacy of prophylactic platelet transfusion for severe thrombocytopenia in adults with acute uncomplicated dengue infection. Clin. Infect. Dis. 48(9), 1262–1265

Effectiveness of Platelet Transfusion in Dengue Fever: A Randomized Controlled Trial

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Summary

Background: Scientific data regarding effects of platelet transfusion on platelet count in dengue-related thrombocytopenia is scanty. **Methods:** A single center, randomized non-blinded trial was conducted on adult patients with dengue fever and platelet counts less than 30,000/µl. Patients were randomized to treatment and control group. Treatment group received single donor platelets. Patients with post-transfusion platelet increment (PPI) ≥10,000/µl and/or corrected count increment (CCI) ≥5,000/µl 1 h post-transfusion were considered responders. Primary outcome was platelet count increments at 24 and 72 h. **Results:** 87 patients were enrolled, and 43 (48.2%) received platelet transfusion. Mean PPI and CCI at 1 h post-transfusion in the treatment group were 18,800/µl and 7,000/µl respectively. 22 (53.6%) patients in the treatment group were non-responders. Mean platelet increments at 24 and 72 h were higher in the treatment group as compared to the control group. Responders showed significantly higher increments when compared to non-responders and the control group at 24 h ($p = 0.004$ and $p < 0.001$, respectively) and 72 h ($p = 0.001$ and $p < 0.001$, respectively). Significant differences were found between non-responders and the control group at 24 h ($p < 0.001$), but not at 72 h ($p = 0.104$). Patients with lower baseline platelet count were more likely to be non-responders. Platelet transfusion neither prevented development of severe bleeding nor shortened time to cessation of bleeding. Three severe transfusion reactions and two deaths occurred in treatment group. **Conclusion:** In this trial, almost half the patients showed no response to a high-dose platelet transfusion. Platelet transfusion did not prevent development of severe bleeding or shorten time to cessation of bleeding and was associated with significant side effects. Therefore, platelet transfusion should not be routinely done in the management of dengue fever.

Assir MZK, Kamran U, Ahmad HI et al (2013). Effectiveness of platelet transfusion in dengue fever: a randomized controlled trial. Transfus. Med. Hemother. 40(5), 362–368

Summary of clinical trials that assessed prophylactic blood product transfusion in dengue patients

Study (year)	Design	Setting	Patients	Participants (n)	Treatment	Conclusion	Favor transfusion?
Sellahewa *et al.* (2008)	Randomized, double-blind-controlled trial	Sri Lanka	Dengue adult patients with platelet count <40,000/mm^3 without bleeding	109	Prophylactic FFP	FFP may contribute to an increased platelet count in the first 12 h, but no difference was observed at 24–48 h	No
Assir *et al.* (2013)	Randomized, nonblinded trial	Pakistan	Adult dengue patients with platelet count <30,000/μl without bleeding or with mild bleeding	87	Prophylactic platelets	No benefit in the prevention or resolution of bleeding. 7% developed severe side effect	No
Lye *et al.* (2009)	Nonrandomized, retrospective cohort study	Singapore	Adult patients with severe thrombocytopenia (<20 × 10^3/μl) without clinical bleeding	266	Prophylactic platelets	No benefit	No

Major bleeding

- usually arises from the gastrointestinal tract and/or vagina in adult females

- Patients at risk of major bleeding
  - 1st commonest cause - prolonged or refractory shock
  - 2nd - renal or liver failure
  - 3rd - persistent metabolic acidosis
  - 4th - nonsteroidal anti-inflammatory drugs or anticoagulants (e.g., heparin, warfarin)
  - 5th - preexisting peptic ulcer disease

In the event of severe bleeding, timely transfusion of packed red cells, platelets and fresh frozen plasma may be lifesaving

IV. Therapies targeting the host immune response

1. Corticosteroids

- Inhibitory effects

Study (year)	Design	Setting	Patients	Participants (n)	Drug	Conclusion	Favor steroids?
Pongpanich *et al.* (1973)	Nonblinded, randomized	Thailand	Children with dengue shock	26	IV hydrocortisone	No difference in duration of shock or need for fluid replacement	No
Min *et al.* (1975)	Randomized, double-blinded	Thailand	Children with dengue shock	98	IV hydrocortisone for 3 days	Favors steroids in children >8 years. Case fatality 44% (22 of 50) in nonsteroid group, and 18.75% (9 of 48) in steroid group	Yes
Sumarmo *et al.* (1982)	Randomized, nonblinded	Indonesia	Children <10 years with dengue shock	97	IV hydrocortisone × 1 dose	No difference in duration of shock or need for fluid requirement. No difference in mortality	No
Tassniyom *et al.* (1993)	Randomized, double-blinded	Thailand	Children <15 years with dengue shock	63	IV methylprednisolone × 1 dose	No difference in mortality	No
Kularatne *et al.* (2009)	Randomized, placebo-controlled, double-blinded	Sri Lanka	Dengue patients aged 12–65 years with platelets <50 × 10^9/l	200	IV dexamethasone for 24 h	Ineffective in increasing platelet count	No
Tam *et al.* (2012)	Randomized, placebo-controlled, double-blind	Vietnam	Dengue patients aged 5–20 years ≤72 h	255	Oral prednisolone for 3 days	Steroids not associated with prolonged viremia	(Underpowered)
Shashidhara *et al.* (2013)	Randomized, controlled, nonblinded	India	Adults with dengue aged >18 years	61	IV dexamethasone for 4 days	Ineffective in increasing platelet count	No

*** Use of steroids cannot be recommended

Cochrane Library
Cochrane Database of Systematic Reviews

Corticosteroids for dengue infection (Review)

Zhang F, Kramer CV

Zhang F, Kramer CV.
Corticosteroids for dengue infection.
Cochrane Database of Systematic Reviews 2014, Issue 7. Art. No.: CD003488.
DOI: 10.1002/14651858.CD003488.pub3.

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Main results

We included eight studies enrolling 948 participants in this review.

Paitents with dengue-related shock

Four studies enrolled children younger than 15 years with dengue-related shock at hospitals in Southeast Asia and evaluated intravenous corticosteroids. The trials did not detect an effect on death (four trials, 284 participants, *very low quality evidence)*, the need for blood transfusion (two trials, 89 participants, *very low quality evidence*), pulmonary haemorrhage (one trial, 63 participants, *very low quality evidence*), convulsions (one trial, 63 participants, *very low quality evidence*), or duration of hospitalization (one trial, 63 participants, *very low quality evidence*). The body of evidence is too small to confidently prove or exclude clinically important effects. Furthermore, the trials are more than 20 years old with several methodological limitations.

Patients with dengue at an early stage

Four studies enrolled 664 children and adults with dengue at an early stage of infection (without shock) in Columbia, India, Sri Lanka and Vietnam. In these participants there were no evidence of effects of oral or intravenous corticosteroids on mortality (four trials, 664 participants, *low quality evidence),* or on the development of complications of severe dengue such as shock (two trials, 286 participants, *very low quality evidence*), severe bleeding (two trials, 425 participants, *very low quality evidence*), severe thrombocytopaenia (one trial, 225 participants, *very low quality evidence*), ascites (one trial, 178 participants, *very low quality evidence*) and intensive care unit (ICU) admissions (two trials, 286 participants, *very low quality evidence*).

Authors' conclusions

The evidence from trials using corticosteroids in dengue is inconclusive and the quality of evidence is low to very low. This applies to both the use of corticosteroids in dengue-related shock and for dengue at an early stage. There is insufficient evidence to evaluate the effects of corticosteroids in the treatment of early stage dengue fever and dengue-related shock outside of the context of a randomized controlled trial.

Therapies targeting the host immune response

2. IVIG

Lack of Efficacy of High-Dose Intravenous Immunoglobulin Treatment of Severe Thrombocytopenia in Patients with Secondary Dengue Virus Infection

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Abstract. Because most cases of secondary dengue virus infection are associated with an increased level of platelet-associated IgG, a high dose of intravenous immunoglobulin (IVIG) may have an effect on the development of severe thrombocytopenia in this disease. A randomized, controlled study was conducted with two treatment groups consisting of a treatment (IVIG) group (n = 15) and a non-treatment (non-IVIG) group (n = 16) to determine whether a high dose of IVIG is effective in hastening the recovery from thrombocytopenia in patients with secondary dengue virus infection. No significant difference was found in the baseline demographic data between the two groups. No adverse effect of IVIG was observed, but no effect in hastening the recovery of platelet counts was found in patients with secondary dengue infections. The lack of efficacy of IVIG suggests that platelet clearance by macrophages through Fc γ receptors is not a primary mechanism in this disease.

***failed to show efficacy of IVIG in promoting platelet recovery

Dimaano EM, Saito M, Honda S et al (2007). Lack of efficacy of high-dose intravenous immunoglobulin treatment of severe thrombocytopenia in patients with secondary dengue virus infection. Am. J. Trop. Med. Hyg. 77(6), 1135–1138 .

Therapies targeting the host immune response

3. Mast cell inhibitors

St John AL, Rathore APS, Raghavan B, Ng M-L, Abraham SN (2013). Contributions of mast cells and vasoactive products, leukotrienes and chymase, to dengue virus-induced vascular leakage. eLife 2, e00481

Contributions of mast cells and vasoactive products, leukotrienes and chymase, to dengue virus-induced vascular leakage

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Abstract Dengue Virus (DENV), a flavivirus spread by mosquito vectors, can cause vascular leakage and hemorrhaging. However, the processes that underlie increased vascular permeability and pathological plasma leakage during viral hemorrhagic fevers are largely unknown. Mast cells (MCs) are activated in vivo during DENV infection, and we show that this elevates systemic levels of their vasoactive products, including chymase, and promotes vascular leakage. Treatment of infected animals with MC-stabilizing drugs or a leukotriene receptor antagonist restores vascular integrity during experimental DENV infection. Validation of these findings using human clinical samples revealed a direct correlation between MC activation and DENV disease severity. In humans, the MC-specific product, chymase, is a predictive biomarker distinguishing dengue fever (DF) and dengue hemorrhagic fever (DHF). Additionally, our findings reveal MCs as potential therapeutic targets to prevent DENV-induced vasculopathy, suggesting MC-stabilizing drugs should be evaluated for their effectiveness in improving disease outcomes during viral hemorrhagic fevers.

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V. Drugs targeting host factors required by DENV to complete its life cycle

1. Chloroquine
   - by interfering with pH-dependent steps of viral replication
   - also has immunomodulatory effects by suppressing release of TNF-α and IL-6
2. Celgosivir
   - an alkaloid castanospermine derived from the Moreton Bay Chestnut tree
   - inhibited all four serotypes at submicromolar concentrations
   - result in misfolding of E, prM and NS1 proteins during virus replication
   - could also reduce cellular apoptosis due to virus-induced ER stress
3. Others (HMG-CoA-reductase inhibitors)
   - Inhibition of cholesterol synthesis may result in faulty viral particle assembly and protein glycosylation
   - Statin also exhibit anti-inflammatory and endothelial-stabilizing effects and possible antiviral effect targeting DENV virion assembly
   - lovastatin increased survival rate when administered either before or after infection

VI. Therapeutic antibodies

1. Therapeutic human serum **polyclonal** antibodies (IgG)
   - prepared from pools of plasma obtained from multiple healthy blood donors
   - Disadvantage - batch-to-batch variations, risk of blood-borne pathogens and allergic reactions

2. Therapeutic **monoclonal** antibodies (mAbs)
   - can be produced in high quantities, specificity and consistency with significantly reduced adverse events associated with polyclonal IgGs
   - Mouse-derived or human mAbs

- Have only been studied in vitro and in animal models

Conclusion

1. Acute systemic viral infection presents with wide clinical spectrum from mild form to severe and even fatal syndrome

2. Death is usually primarily by plasma leakage, leading to shock, organ failure and hemorrhage

3. Clinical assessment and laboratory tests are required to assess disease phase and severity

4. Judicious fluid therapy is currently the corner stone of managing acute dengue to prevent complication

Conclusion

5. No clear advantage to the use of colloids over crystalloids

6. Colloids may be preferred in severe or refractory shock

7. Blood transfusion is indicated in patients with major hemorrhage

8. Prophylactic platelet or FFP transfusion is not beneficial and not recommended

9. Drugs treatment targeting host immune response, host factors required by dengue virus to complete life cycle and denue proteins or direct acting antiviral agents are in cinical trial

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THANK YOU