

Shock Syndrome in Primary Dengue Infections

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OBJECTIVE: To document by intensive laboratory investigation primary dengue infection associated with dengue shock syndrome (DSS).

BACKGROUND: During the year 1974 five patients presented at the Bangkok Children's Hospital (BCH) with shock and preliminary laboratory findings suggestive of primary dengue infections. This report summarizes the clinical histories of these patients and reviews laboratory findings, establishing them as primary dengue infections with shock.

DESCRIPTION: The detection of patients for this study has been previously described, (SEATO Medical Research Laboratory Annual Report 1974-1975). Briefly, patients with a hospital admission diagnosis compatible with dengue infection were studied. Clinical diagnosis of dengue hemorrhagic fever and grading of the severity of disease followed criteria previously reported (SEATO Medical Research Laboratory Annual Report 1974-1975). Virus isolation used a direct and delayed plaque technique on LLC-MK₂ cells. Isolates were identified by plaque reduction neutralization tests. Serum obtained from each individual was tested for antibodies by hemagglutination inhibition (HI) and plaque reduction neutralization (PRNT). Primary dengue infections were tentatively identified as patients with convalescent HI titers of less than 1:640.

Final classification of primary infections was based on classical characteristics, i.e., absent or low titered antibodies in the acute serum and the development of IgM during convalescence. IgM was separated from serum proteins by sucrose gradient ultracentrifugation. Fractions were tested for content of IgM by radial immuno-diffusion and for antibody activity by HI. Antibody activity was proved to be associated with IgM by a significant reduction in HI titer following treatment of the fraction with 2-mercaptoethanol.

Previous investigations of shock associated with secondary dengue infections have indicated a fall in B1c/B1a concentrations in acute serum related to the severity of disease. B1c/B1a levels were determined in primary patients using commercially prepared radial diffusion plates (Hyland Laboratories).

Three patients with shock met the preliminary criteria for primary dengue. Clinical and laboratory findings on these patients are presented.

Patient 77, a four year old Thai female, had a five day history of fever, anorexia and vomiting. On the day of admission, she had a cough, a rash on both arms, and became increasingly lethargic. Physical examination revealed an axillary temperature of 38.5°C, a pulse of 116 and a blood pressure (BP) of 120/80 mm Hg (pulse pressure 40 mm Hg). There were petechiae on both arms. The tourniquet test was positive. Her posterior cervical lymph nodes were enlarged and her liver was palpated at the right costal margin. Her hematocrit was 42%, WBC, 6,900 per mm³; and platelet count, 186,000 per mm³.

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Despite the administration of intravenous fluids, vascular collapse developed (Figure 1 a). Plasma was administered intravenously with satisfactory improvement and the patient was discharged from the hospital on the ninth day of disease.

Patient 91, a 12 year old Thai female, had a five day history of fever, headache and abdominal pain. On the day of admission a rash developed and the child became increasingly obtunded. Physical examination revealed mild dehydration, an axillary temperature of 37°C, and a pulse of 100. A light macular rash was present over her entire body and petechiae were on the face and arms. The tourniquet test was positive. Her liver was palpable below the right costal margin. The patient was in shock with cold, clammy extremities; a weak, thready pulse; and a BP of 110/90 mm Hg (pulse pressure 20 mm Hg). Her hematocrit was 47%; WBC, 6,400 per mm³; and platelet count, 42,000 per mm³.

Intravenous fluids led to improvement (Figure 1 b). During the first 12 hours in hospital, the patient passed a black tarry stool and the petechiae became more widespread. The patient was discharged on the eighth day of disease.

Patient 103, an eight year old Thai female, had a four day history of fever and vomiting. On admission, she had a temperature of 38°C; a pulse of 124 and a BP of 90/80 mm Hg (pulse pressure 10 mm Hg). Her tonsils were injected and her liver was palpable 3 cm below the right costal margin. No petechiae were noted, however, the tourniquet test was positive. Her hematocrit was 47%, WBC, 3,300 cells per mm³; and platelet count, 146,000 per mm³.

Intravenous fluid was administered and the blood pressure stabilized at 100/60 mm Hg (Figure 1 c). The patient was discharged on the seventh day of disease.

The above three primary infections may be compared to the following patient with a secondary infection and shock.

Patient 6, a seven year old Thai female, was admitted with a five day history of fever, drowsiness and abdominal pain. On the day of admission she became increasingly lethargic and manifested clinical signs of shock. Her temperature was 35.5°C, her pulse was 120 and her BP was 80/60 mm Hg (pulse pressure 20 mm Hg). Her liver was palpable 1 cm below the right costal margin. Petechiae were noted on both upper extremities; the tourniquet test was positive. Her hematocrit was 48%; WBC, 5,600 per mm³; and platelet count, 85,000 per mm³.

During the first six hours in the hospital, the pulse pressure fell to 10 mm Hg (BP 80/70 mm Hg), the pulse rate rose to 130, and the hematocrit rose to 52%. Administration of intravenous fluid led to recovery and the patient was discharged on the eighth day of illness (Figure 1 d).

Laboratory Studies :

A strain of D3 virus was isolated from the acute serum of Patient 103. The other two patients with primary dengue infections did not yield virus isolates; however, D1 virus was isolated from two siblings of Patient 77 with undifferentiated fevers.

HI tests of sequential serum specimens showed a broadly reactive antibody response (Table 1). PRNT antibody gave the best indication of the infecting virus type, since they were nearly monospecific; Patients 77 and 91 had D 1 and Patient 103 had D 3.

Low titers of PRNT antibody were found in the convalescent sera of Patient 77 to D 2, D 3, D 4 and chickungunya. Unlike the others, Patient 77 had received plasma infusions. The plasma administered was not tested for antibodies; however, nearly 100% of adults in Bangkok have antibodies to dengue and chickungunya (chik) viruses. The low titers of antibodies found in this patient may have been acquired from the plasma transfused. The HI and PRNT titers of Patients 77, 91 and 103 may be compared with those of Patient 6 in whom high titered cross reacting antibodies were found.

Using sucrose gradient ultracentrifugation, immunoglobulin separations were performed on sera collected early and late in the course of infection (Table 2). IgM was found in fractions two to five while IgG was found in fractions six to twelve. In the early sera, the IgM fractions contained higher HI titers of anti-dengue immunoglobulin than did the IgG fractions. Convalescent sera contained predominantly IgG antibody. Although IgM antibody was often present against more than one type of dengue, the titer to the infecting virus type appeared to be higher and to last longer. Immunoglobulin separations on early convalescent sera from Patient 77 showed only IgG antibodies to chik. This strengthened the impression that antibodies to this agent were passively acquired through transfusion.

Concentrations of B1c/B1a were determined on sequential sera from the presumptive primary dengue patients (Figure 2). The concentrations of these proteins were found to be subnormal in the acute sera from each case. By the time convalescent sera were obtained, the levels had risen to the normal range. These findings were similar to those previously reported in secondary dengue infections and were also seen in Patient 6.

Fatal Cases :

There were five fatal cases studied in 1974. Of the fatal cases, two (Patients 16 and 112) did not have detectable HI antibody in their acute sera. Since convalescent sera were unavailable, sequential studies of antibody titers and complement levels could not be performed. These two patients are presented here as possible primary dengue infections.

Patient 16, a 12 year old Thai female, had a five day history of fever, myalgia and lethargy. Two days prior to admission she developed anorexia and abdominal distention. On admission, a physical examination revealed an obtunded child with an axillary temperature of 38.5°C. Petechiae were present in the axilla and subclavicular areas and the tourniquet test was positive. Her axillary and right submandibular lymph nodes were enlarged, her abdomen was distended and her liver was not palpable. The patient was in shock with pallor, cold extremities, restlessness and tachypnea. Her pulse was 120 and BP was 90/80 mm Hg (pulse pressure 10 mm Hg). Her hematocrit was 54 %, WBC, 6,800 per mm³; and platelet count, 17,000 per mm³.

Rapid administration of plasma led to a fall in the hematocrit to 40 %; however, neither the pulse pressure nor the blood pressure improved. Intravascular coagulation and acidosis developed. Despite treatment with plasma, sodium bicarbonate and heparin, she died eight hours after admission to the hospital.

Patient 112, a seven year old Chinese male, had a four day history of fever, anorexia and abdominal pain. One day prior to admission he developed diaphoresis, cold extremities and lethargy. A rash was noted on his arms and legs. On admission he was lethargic with an axillary temperature of 39.5°C. His pulse rate was 102 and BP was 110/90 mm Hg (pulse pressure 20 mm Hg). His hematocrit was 44%; WBC, 4,150 per mm³; and platelet count, 110,000 per mm³.

Intravenous fluid administration led to a transient improvement in clinical status and a rise in pulse pressure to 30 mm Hg. However, after three hours the child became restless and vomited copious amounts of fresh blood. The pulse pressure fell to 10 mm Hg (BP 110/100 mm Hg) and shock reappeared. Plasma and fresh whole blood were administered, but the hematemesis continued. Despite volume replacement, there was no improvement and the child died 12 hours after admission.

Laboratory Studies :

Dengue type 2 was isolated from the blood of Patient 16 and type D 3 from Patient 112. Although there were no detectable HI antibodies, the PRNT on the acute sera revealed low titers of antibody against the infecting virus in both patients. Immunoglobulin separations on the acute specimens were not informative as the concentrations of dengue antibodies were too low to be

detected. From Patient 112 a second serum was obtained after death. HI antibody determinations on this serum showed low titer IgG antibodies against D 1-4, JE and chik. These antibodies were probably passively introduced by transfusion. The fatal cases also had markedly depressed concentrations of B 1 c / B 1 a.

DISCUSSION : The development of dengue shock syndrome in older children has been associated epidemiologically with secondary dengue infections (1). It was suggested that anamnestic antibody in the presence of dengue antigens triggered an immunologic mechanism (2). Three patients described here, however, had no evidence of prior dengue infections. They demonstrate that primary dengue infections can cause the dengue shock syndrome in older children. Furthermore, in two fatal dengue infections, the absence of HI antibodies and the low titered PRNT antibodies against the isolated virus types in acute sera are also consistent with primary infections. Although not substantiated by studies on convalescent sera, these data suggest that primary dengue may also be associated with fatalities.

DSS presenting in primary dengue infections had similar clinical and laboratory courses to those previously reported for secondary infections (3). The demonstration of a depletion in complement factor three in primary infections supports an immunological mechanism for shock. The development of immune complexes may be related to viral and host factors as yet unknown. Immune complexes may be more likely in secondary dengue infections because of the anamnestic antibody response during the time when viral antigens are circulating. Conceivably immune complexes may occur in primary dengue infections in patients who produce the appropriate amount and type of antibody at the appropriate time.

In Bangkok, primary dengue with shock is infrequently recognized. It occurred in less than 5% of the DHF patients admitted to the BCH in 1974. Dengue is endemic in Bangkok. The true incidence of primary dengue with shock could not be calculated as no information was available on the number of individuals infected or at risk. However, each secondary dengue infection must be preceded by a primary infection. If the severe manifestations of dengue occurred as commonly in primary infections as they do in secondary, the number of patients hospitalized with primary infections should be similar to the number hospitalized for secondary infections. Further studies are required to elucidate both the mechanism and epidemiology of DSS in primary and secondary infections.

SUMMARY : During 1974, 114 patients with dengue hemorrhagic fever were studied at the Bangkok Children's Hospital. Over 40% of the patients had dengue shock syndrome. Five fatal cases were included in the study. Primary dengue infections were identified by absent or low titered antibodies in acute sera and the development of IgM antibodies during convalescence. Three patients age 4, 8 and 12 years had primary dengue infections with shock. Although no convalescent sera could be tested, two other patients with fatal disease, age 7 and 12 years, also appeared to have primary infections. At the time of shock, patients with primary infections had subnormal concentrations of complement factor 3. The data show that in older children dengue shock syndrome associated with complement depression can occur during primary as well as secondary infections.

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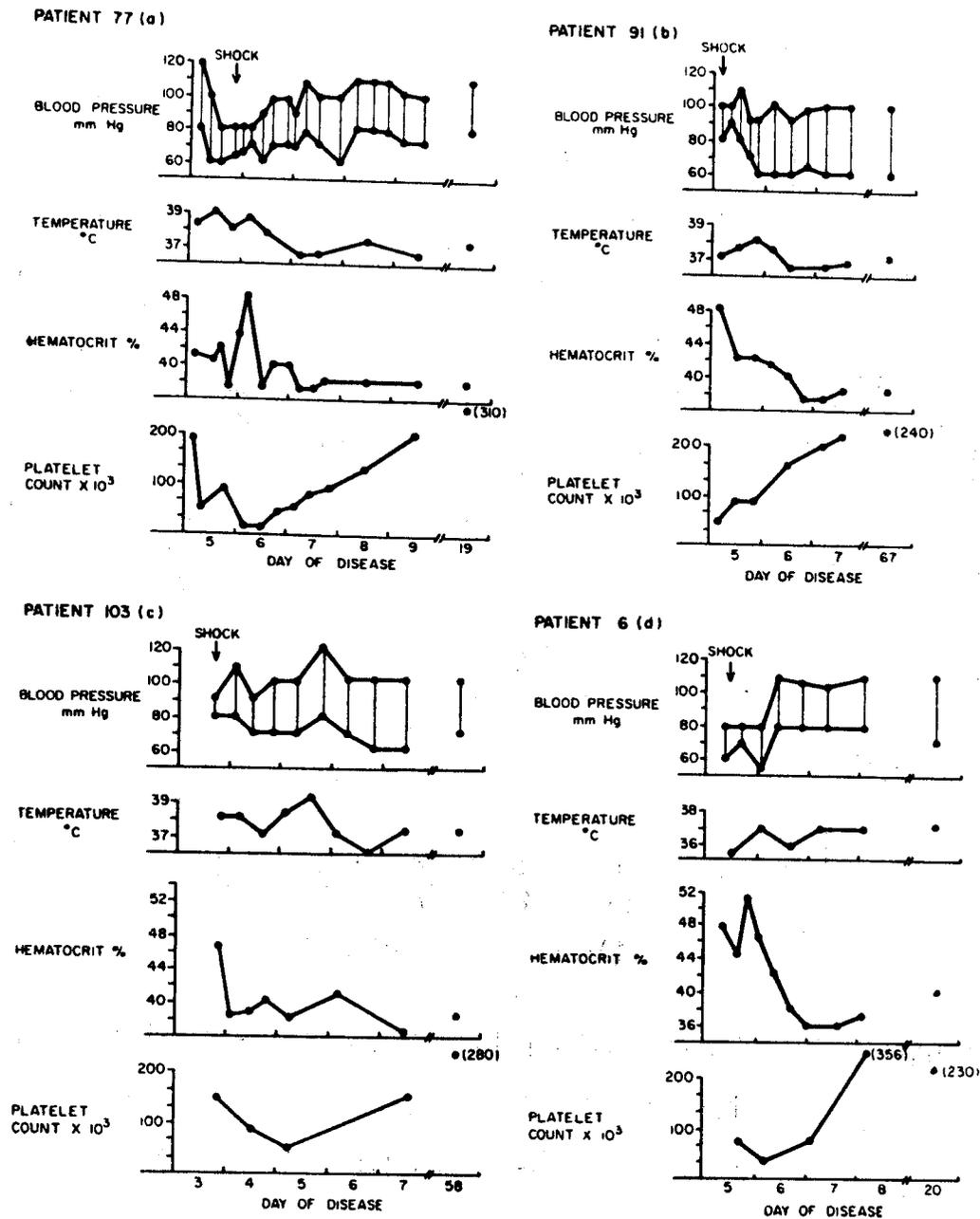


Figure 1 Diagrams of the clinical courses of primary dengue patients (a) 77, (b) 91 and (c) 103, showing the relationship of several clinical and laboratory variables to the onset of shock. A secondary dengue patient, (d) 6 is diagrammed for comparison.

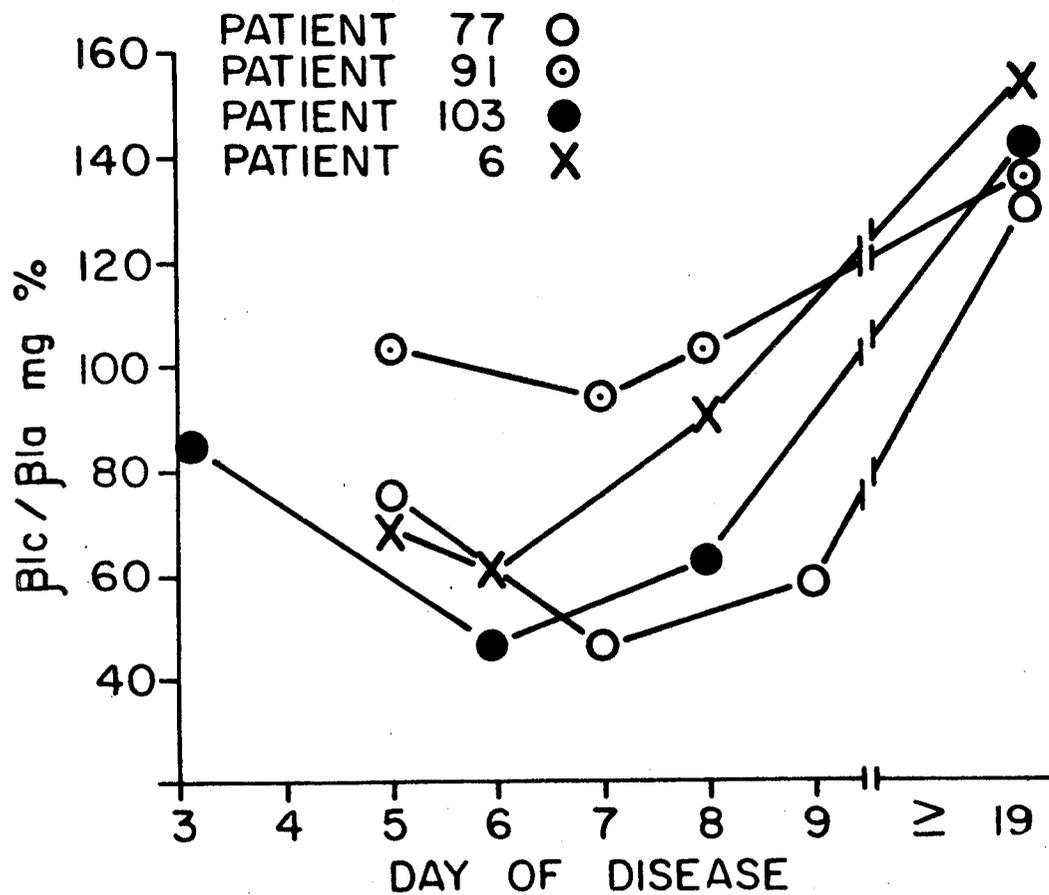


Figure 2. Sequential β_{lc}/β_{la} concentrations from patients with primary and secondary dengue infections.

Table 1. Antibody Titers in Patients with Dengue Infections

Patient Number	Day of Disease	Reciprocal HI Antibody Titer				Reciprocal PRNT Antibody Titer					
		D1	D2	D3	D4	D1	D2	D3	D4	Chik	
77	5	>20	<20	<20	<20	<20	<20	<20	<20	<20	<20
	17	80	40	160	160	≥1280	40	120	40	160	160
	36	40	20	40	40	≥1280	40	230	40	120	120
91	5	40	<20	<20	<20	20	<20	<20	<20	NT	NT
	11	160	40	80	160	NT ^a	NT	NT	NT	NT	NT
	67	160	80	80	160	500	<20	<20	<20	<20	<20
103 ^b	3	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
	7	<20	20	40	<20	<20	<20	40	<20	<20	<20
	58	20	20	160	80	20	<20	70	<20	<20	<20
6	6	10240	10240	10240	≥20480	≥5120	≥5120	≥5120	≥5120	≥5120	<10
	11	≥20480	10240	10240	≥20480	NT	NT	NT	NT	NT	NT
	20	≥20480	10240	≥20480	≥20480	≥5120	≥5120	≥5120	≥5120	≥5120	<10

a NT = Not tested

b Dengue 3 virus isolated from this patient

Table 2. Hemagglutination Inhibition Antibody Titers of Serum IgM and IgG Fractions in Patients with Dengue Infections

Patient Number	Day of Disease	Immuno- ^a globulin	Reciprocal HI Antibody Titer								
			D1		D2		D3		D4		
			Not Treated	2ME Treated	Not Treated	2ME Treated	Not Treated	2ME Treated	Not Treated	2ME Treated	
77	5	IgM	<4	<4	<4	<4	<4	<4	<4	<4	<4
		IgG	<4	<4	<4	<4	<4	<4	<4	<4	<4
	9	IgM	64	<4	32	<4	8	<4	64	<4	<4
		IgG	8	8	8	16	16	16	16	16	16
	17	IgM	16	<4	4	<4	8	<4	8	<4	<4
		IgG	16	32	8	16	16	32	32	32	32
	36	IgM	8	<4	<4	<4	4	<4	<4	<4	<4
		IgG	8	16	4	8	16	16	8	8	8
91	5	IgM	4	<4	<4	<4	<4	<4	<4	<4	<4
		IgG	<4	<4	<4	<4	<4	<4	<4	<4	<4
	8	IgM	64	<4	4	<4	8	<4	8	<4	<4
		IgG	8	4	<4	<4	4	4	8	8	8
	37	IgM	8	<4	<4	<4	<4	<4	<4	<4	<4
		IgG	128	128	32	32	64	128	128	64	64
103	3	IgM	<4	<4	<4	<4	<4	<4	<4	<4	<4
		IgG	<4	<4	<4	<4	<4	<4	<4	<4	<4
	7	IgM	<4	<4	<4	<4	32	<4	<4	<4	<4
		IgG	<4	<4	<4	<4	<4	4	<4	<4	<4
	58	IgM	<4	<4	<4	<4	<4	<4	<4	<4	<4
		IgG	32	32	16	16	128	256	64	64	64
6	6	IgM	4	<4	4	4	4	<4	4	4	4
		IgG	≥4096	≥4096	≥4096	≥4096	≥4096	≥4096	≥4096	≥4096	≥4096
	11	IgM	8	4	8	4	4	<4	8	4	4
		IgG	≥4096	≥4096	≥4096	≥4069	≥4096	≥4096	≥4096	≥4096	≥4096
	20	IgM	<4	4	4	4	4	<4	4	4	4
		IgG	≥4096	≥4096	≥4096	≥4096	≥4096	≥4096	≥4096	≥4096	≥4096

a. The sucrose gradient fraction with the highest concentration of IgM or IgG is shown.