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Kinetics of IgM and IgG seroconversion in Nipah virus infection

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Abstract

This is a study of the kinetics of serum IgM and IgG seroconversion in 176 patients from Seremban Hospital and University Hospital, Kuala Lumpur. One hundred and forty two patients with Nipah encephalitis and 34 patients with non-encephalitic Nipah virus infection were studied. The serology tests were done at day of admission, a week later and subsequently. The daily rate of positive serology showed that serum anti-Nipah IgM antibody was 44%-50% positive at day one, 60%-71% positive at day 4 and 100% positive by day 12 of illness. The daily rate of positivity for serum anti-Nipah IgG antibody was 7%-29% on day 1-10, 100% by day 25-26. The positive serology for IgG was persistent throughout the period of study of about 8 months. The positive serology for Serum IgM persisted for at least three months in most patients. In some patients, it persisted for longer than 7 months. *Conclusion:* Serum IgM anti-Nipah antibody is sensitive in early diagnosis of symptomatic Nipah virus infection.

Key words: Nipah encephalitis, serology

INTRODUCTION

A recent outbreak of Nipah encephalitis was seen from late 1998 to mid 1999 in Malaysia among the pig farm workers that started.[1,2] With the mass culling of pigs from the outbreak areas, the epidemic has since subsided. The outbreak caused severe and fatal illness to man and pigs.[2,3] Nipah virus was a novel virus closely related to the Hendra virus that was reported in Australia.[1,4] The clinical, epidemiological, radiological and EEG features of the illness have been previously described.[2,5-7]

It has been found that the mortality of Nipah encephalitis is associated with positive viral culture in cerebrospinal fluid. However, the presence of anti-Nipah virus antibodies in the cerebrospinal fluid did not influence the virus isolation.[8] The presence of CSF IgM also did not correlate with mortality and morbidity, suggesting that humoral immune response in the CNS probably plays a minor role in the disease process and recovery.[8,9] Nevertheless, serology plays an important role in the diagnosis of the infection. It has been found to be positive in 76% of the patients.[2] This study aims to look at the kinetics of the serum antibody response in patients with symptomatic Nipah virus infection, so as to form the basis for interpretation of the serology investigations.

METHODS

The study subjects consisted of Nipah encephalitis or non-encephalitic Nipah virus infection who were admitted to the Seremban Hospital and University Hospital, Kuala Lumpur from December 1998 to June 1999. The two hospitals were the epicenter treating more than 80% of the patients in the outbreak. The study subjects were followed through till 31st of December, 1999. Two inclusion criteria were used: the "serology" criteria required the presence of serum IgG or IgM anti-Nipah virus antibody and the patients from outbreak area. Those with evidence of cerebral involvement were classified as Nipah encephalitis. Those with febrile illness without cerebral involvement were classified as non-encephalitic Nipah virus infection. Encephalitis may be based on clinical, abnormal cerebrospinal fluid findings or characteristic findings in the MRI. The "epidemiology" criteria was applied to patients with Nipah encephalitis only. It did not require a positive serology, but patients should came from outbreak area, had had direct or close

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contact with pigs or other infected animals, and had evidence of encephalitis as described above.[2]

The first serological test for Nipah virus infection was usually done on arrival to the ward, the second in a week's time and the third subsequently. The serological tests were performed based on solid phase immunoassay utilizing the IgM-capture ELISA for IgM and indirect ELISA for IgG. Antigen derived from Hendra virus infected with Vero E6 cells was used as the surrogate antigen in the initial testing but was switched over to Nipah virus infected Vero E6 cells. No significant difference in the sensitivity and specificity has been found between the two antigens used.

Statistical analysis was done using SPSS version 9.0 with the use of descriptive and independent students' t test.

RESULTS

One hundred and seventy six patients consisting of 142 Nipah encephalitis and 34 non-encephalitic Nipah virus infection fulfilled the inclusion criteria and were included in this study. For the encephalitis patients, 117 patients fulfilled the "serology" criteria, and another 25 patients fulfilled the "epidemiology" criteria. A comparison of the demographic features of Nipah encephalitis and non-encephalitic Nipah virus infection patients are listed in Table 1. As shown, other than a trend towards older mean age in Nipah encephalitis (37 years versus 32 years), the demographic features of the two groups are comparable. The mean duration of illness on admission was 4 days. The was no difference between the encephalitis and non-encephalitis groups (p=0.931)

For the IgM serology of those who fulfilled the "serology" criteria, the rate of positivity increased from 88% (130/147) in the first test, to 95% (98/103) in the second test and 95% (35/37) in the third test. The mean duration of illness for those with positive serology in the first test was significantly longer than those with negative serology (19 days versus 4 days, p<0.00). For the 17 patients whose first serology was negative, 93% (14/15) became positive on the second serology test. Most patients who had positive serology in the first test remained positive in the second test, similarly for the second to the third test. Of the 130 patients with positive serology in the first test, 95% (84/88) remained positive on the second test. Of the 98 patients with positive serology in the second test, 94% (34/36) remained positive on the third test. The mean duration of illness for the third IgM serology was 83 days, the longest was 228 days.

Table 1: Demographic features of patients with Nipah encephalitis and non-encephalitis Nipah virus infection based on serology criteria.

		Non-encephalitic Nipah virus infection (n=34)	Nipah encephalitis (n=117)	
Sex	Male	82%	86%	p=0.657
	Female	18%	14%	
Occupation	Pig farmer	71%	77%	p=0.245
	Lorry driver	0%	6%	
	Housewife	6%	9%	
	Others	23%	7%	
Mean age (years)		32	37	p=0.053
Race	Chinese	62%	68%	p=0.204
	Malay	6%	0%	
	Indian	12%	11%	
	Others	21%	21%	

Table 2: Daily rate of positive serum anti-Nipah IgM serology

	Days of illness													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Positive (%)*	50	75	100	71	83	100	94	94	100	100	100	100	100	100
No of patients*	4/8	6/8	4/4	12/17	25/30	13/13	17/18	17/18	13/13	9/9	8/8	6/6	7/7	5/5
Positive (%)#	44	67	40	60	84	93	94	94	100	100	80	100	100	100
No of patients#	4/9	6/9	4/10	12/20	26/31	13/14	17/18	17/18	13/13	9/9	8/10	6/6	7/7	5/5

* Nipah encephalitis and non-encephalitic Nipah virus infection based on "serology" criteria.

\# Nipah encephalitis and non-encephalitic Nipah virus infection based on"epidemiology" and "serology" criteria.

Table 3: Daily rate of positive serum anti-Nipah IgG serology

	Days of illness													
	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28	29-30
Positive (%)*	31	10	18	23	29	44	62	83	100	100	100	100	100	100	100
No of patients*	5/16	2/20	10/55	8/35	7/24	8/18	9/14	6/7	4/4	6/6	2/2	3/3	8/8	3/3	2/2
Positive (%)#	27	7	18	23	29	40	62	83	67	67	67	75	100	100	100
No of patients#	5/18	2/29	10/57	8/35	7/24	8/20	9/14	6/7	4/6	6/9	2/3	3/4	8/8	3/3	2/2

* Nipah encephalitis and non-encephalitic Nipah virus infection based on "serology" criteria.

\# Nipah encephalitis and non-encephalitic Nipah virus infection based on "epidemiology" and "serology criteria.

For the IgG serology of those who fulfilled the "serology" criteria, the rate of positivity increased from 33% (48/147) in the first test, to 76% (78/103) in the second test and 98% (41/42) in the third test. The mean duration of illness for those with positive serology was significantly longer than those with negative serology in the first test (34 days versus 6 days, $p=0.01$) and the second test (52 days versus 10 days, $p<0.02$). All patients who had positive serology in the first test remained positive on repeat testing in the second test. Similarly all patients who had positive serology in the second test remained positive on repeat in the third test.

There was no statistically significant difference in the rate of seropositivity between the Nipah encephalitis and non-encephalitic Nipah virus infection patients for IgM and IgG ($p=0.174$ and $p=0.240$).

The temporal relationship of the seropositivity is described in two different ways: daily rate of positive serology and cumulative seropositive response.

Daily rate of positive serology: Table 2 & 3 are the daily percentage of positive serology from the day of illness for IgM and IgG.

Cumulative seropositive response: Fig. 1 & 2 are the graphs of cumulative first seropositive response for serum IgM and IgG plotted against duration of illness for the Nipah encephalitis and non-encephalitic Nipah virus infection patients according to the "serology" criteria. For IgM, the graph was constructed from 144 plots, 130 plots from the first serology test and 13 from the second serology test. The cumulative 25%, 50%, 75% and 95% seropositive responses were seen on the 5th, 11th, 15th and 50th day of illness. For IgG, the graph was constructed from 99 plots, 48 plots from the first serology test and 51 plots from the second serology test. The cumulative 25%, 50%, 75% and 95% seropositive responses were seen on the 8th, 18th, 34th and 193rd day of illness.

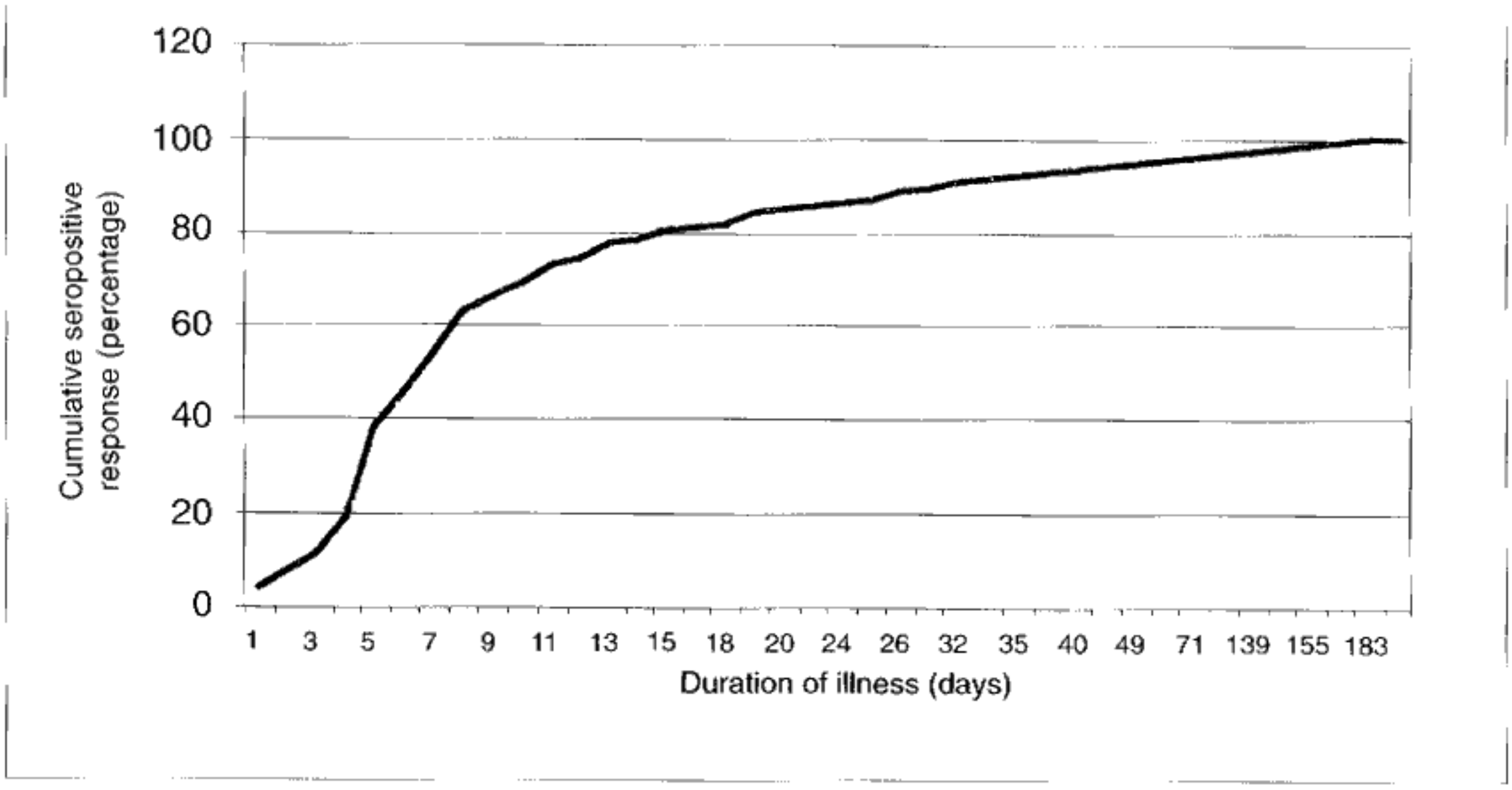

Fig. 1. Graph of cumulative first positive serum anti-Nipah IgM antibody response

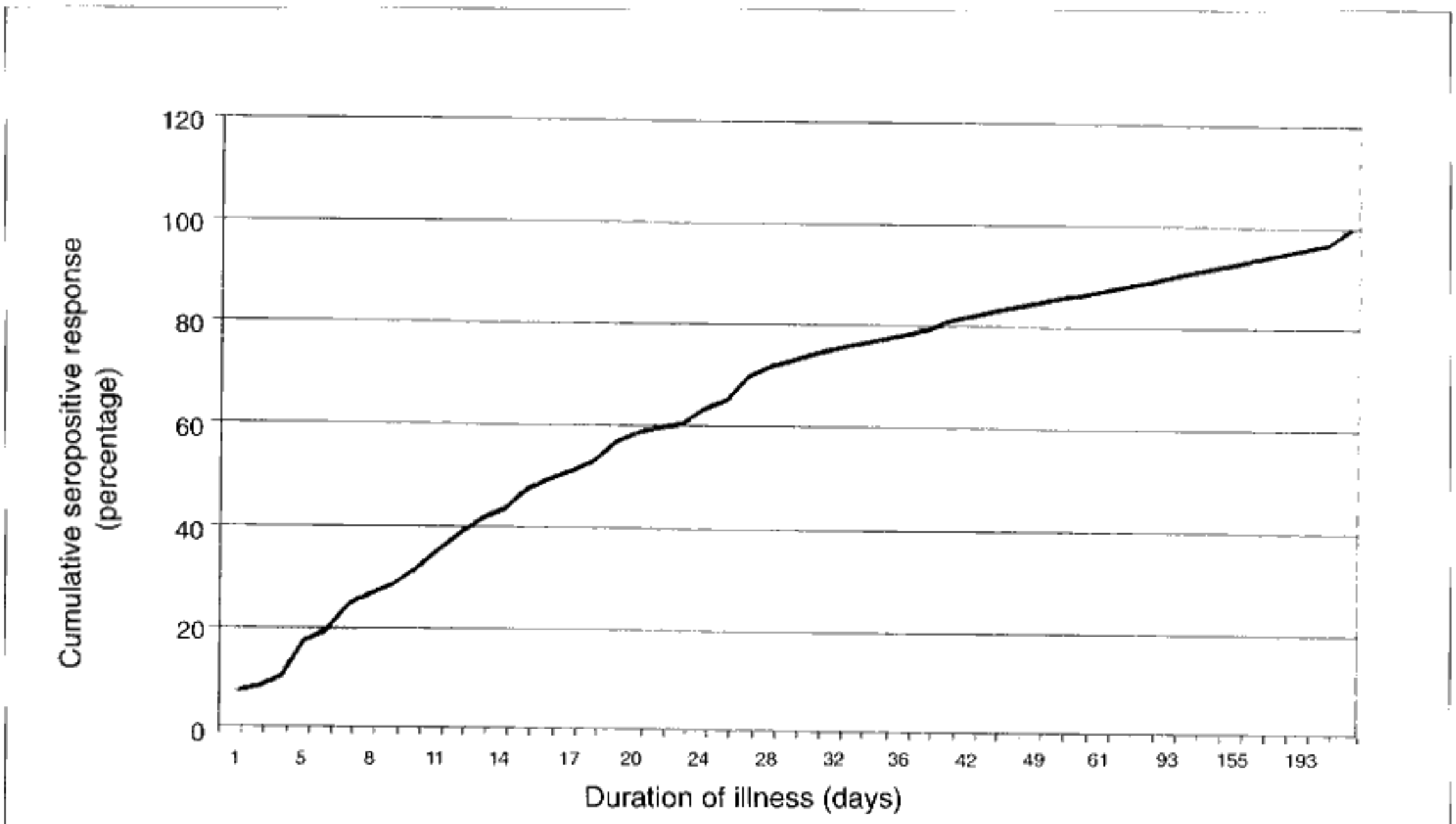

Fig. 2. Graph of cumulative first positive serum anti-Nipah IgG antibody response

DISCUSSION

The time-tested diagnostic test for a recent viral infection is to demonstrate a four-fold rise in the IgG antibody titers.[10] This requires a long wait of 10-14 days, often making the diagnosis retrospective. Due to its rapid and transient rise in antibody titer, detection of IgM has been used for the early diagnosis of various viral encephalitis, such as mumps, measles and rubella.[11-13] On the other hand, the use of serum IgM for the diagnosis of herpes simplex encephalitis is limited, due the reactivation of latent virus causing herpes labialis.[14] CSF IgM has been found to be highly sensitive in the diagnosis of Japanese encephalitis, with a positive rate of 67% in day one of illness, rising

to 100% on day 7.[15] As for this outbreak of encephalitis from Nipah virus, the causative agent was a novel virus occurring in communities previously not exposed to the infection. Thus, the classical serological dictum of a four-fold rise of IgG antibody titer in the acute and convalescence sera does not apply. A detection of IgM or IgG antibodies in the serum would be indicative of a recent Nipah virus infection.

As expected, the positive serum IgM response was seen earlier than serum IgG, 88% versus 33% in the first test on admission. The daily rate of positive serology listed in Table 2 showed that serum IgM serology was positive in 50% patients in day one of illness. The incubation of Nipah virus infection was short at 10 days to two weeks.[2,16] The serology was positive in 71% in day 4 which was the mean duration of illness on admission, and 100% by Day 9. The cumulative seropositive response for serum IgM (Fig. 1) however, showed a 75% positive response by day 16 and 95% positive response by day 50. The difference between the two is mainly due to the time gap of a week between the first test and the second, which was day 4 and day 11 of illness. As shown in Table 2, most patients would have seroconverted if the daily serum IgM have been done earlier. The cumulative seropositive response probably underestimated the sensitivity of the serum IgM for diagnosis of symptomatic Nipah infection.

As for serum IgG, the daily rate of positive serology was 10%-29% on day 1-10, and 100% after at day 17-18 (Table 3). The cumulative seropositive response (Fig. 2) showed a 75% positive response at day 34 and 95% positive response at day 193 of illness. The difference between the two is again mainly due to the time gap between the tests in the individual patients. The cumulative seropositive response probably underestimated the sensitivity of serum IgG for diagnosis of clinical Nipah infection.

In the earlier reports on the clinical, radiological and EEG features of Nipah virus encephalitis, the case definition was based on the "epidemiology" criteria, taking account of the epidemiology and clinical features without requiring a positive serology.[2,6,7] The reasons were that some patients died before the seroconversion. There was no significant differences in the demographics and clinical characteristics between patients with and without positive serology. Among those who did not have a positive serology, 61% had segmental myoclonus which was a distinctive feature of the encephalitis. There was no evidence of the presence of a concurrent epidemic of encephalitis due to other agents. With the culling of pigs, the epidemic rapidly died down, confirming that pig-related Nipah encephalitis was the only epidemic. Furthermore, the positive serology was seen in only 76% of patients, and a positive serology in CSF in only 31%.[2] Thus, a diagnostic criteria based on "serology" criteria would have underestimated the number of cases of Nipah encephalitis. In Table 2 & 3, the daily rate of positive serology was also estimated based on "epidemiology" and "serology" criteria. As shown, the IgM serology was positive on 44% in day one of illness, 60% in day 4 and 100% by Day 12. The IgG serology was positive in 7%-29% on day 1-10, and 100% by day 25-26

Thus, serum IgM is a sensitive test for the early diagnosis of symptomatic Nipah virus infection.

The persistence of the serology for IgG is as in other viral infection.[17] As for serum IgM, it remained positive in 35 out of 37 patients (95%) at the third serology test, which has a mean duration of illness of 83 days, the longest being 228 days. The positive serology for Serum IgM persisted for at least three months in most patients. In some patients, it persisted for longer than 7 months.

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