

Haemophilus influenzae type b antigenuria in children

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SUMMARY Detection of *Haemophilus influenzae* type b (Hib) antigenuria by latex agglutination has been shown to be sensitive, specific, and rapid. In children, antigenuria persisted for a mean duration of 10 days and a maximum of 18 days.

Antigenuria was demonstrated in 25 of 30 patients with Hib infection but not in 62 with other types of infection. In five children, antigenuria confirmed the diagnosis in the absence of bacteriological confirmation. In five other children, antigenuria was not found, but in this group the antigen was detected in another body fluid or Hib was recovered.

Haemophilus influenzae type b (Hib) is a common pathogen in children and occasionally in older patients. A total of 162 patients suffering from bacterial meningitis were admitted to this hospital between 1972 and 1977, and the causative agent was Hib in 68 of these. An exact and rapid bacteriological diagnosis of the organism in bacterial meningitis is important in the selection of appropriate antibiotic therapy, especially so as in recent years β -lactamase producing strains of Hib have been detected. Unfortunately, the direct smear (Gram stain) is not always helpful and may be misleading (Lewin, 1974; McCracken, 1976). Bacterial isolation may be delayed and may even be impossible if, as is not uncommon, children have been treated with antibiotics before admission to hospital (Converse *et al.*, 1973; Lewin, 1974; Mandal, 1976).

Specific bacterial antigen is present in the body fluids of patients infected with Hib and is detectable by simple, rapid techniques such as latex agglutination or counter immunoelectrophoresis (CIEP) (Newman *et al.*, 1970; Ingram *et al.*, 1972; Whittle *et al.*, 1974). In infants, urine is much easier to obtain than blood or cerebrospinal fluid, and antigenuria is prolonged during the course of the disease (Kaldor *et al.*, 1977). We therefore present an account of the detection, by latex agglutination, of Hib antigen in the urine of a series of patients admitted recently to Fairfield Hospital.

Material and methods

Two hundred and twenty-eight specimens of urine

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were obtained from 92 children aged 4 months to 14 years (48 boys and 44 girls) admitted to Fairfield Hospital between October 1975 and January 1978, who presented with signs and symptoms of infectious disease. Each specimen was tested for the presence of Hib antigen by a latex agglutination test. All specimens were boiled for 3 minutes before the test was performed. The method of agglutination and the preparation of sensitised latex particles have been described elsewhere (Kaldor *et al.*, 1977).

Briefly, for sensitising latex, commercially available *H. influenzae* type b antiserum¹ was used. Antibody-containing globulins were first precipitated with half-saturated ammonium sulphate. After suspension in glycine-buffered saline, they were dialysed against the saline buffer for removal of residual ammonium sulphate and then reacted with pooled, polymerised, human plasma to absorb possible non-specific antibodies to human plasma proteins. The end-titre of slide agglutination for the globulins was then obtained using doubling dilutions in glycine-buffered saline against appropriate bacterial suspension. Diluted to the titre, globulins were then mixed with an equal volume of 0.81 nm latex particles² and allowed to stand for 2 hours at 37°C. To prevent rheumatoid serum reacting directly with the uncoated latex surface, two volumes 0.5 g/dl of bovine albumin in glycine-buffered saline were added to cover remaining uncoated latex surfaces, and the suspension was left for an additional 30 minutes at 37°C. The latex thus prepared was found to be stable for

¹Hyland division of Travenol Laboratories Inc, Costa Mesa, California 92626, USA.

²Difco Laboratories, Detroit, Michigan, USA.

at least 12 months when kept at 4°C.

The agglutination procedure involved mixing 50 µl of specimen with 20 µl of sensitised latex on a glass tile. A positive and a negative control were tested in parallel, and the tile was then placed on a tile rotor or an ordinary shaker for 8-10 minutes, and the agglutination pattern was observed. Negative tests were repeated after 10- and 25-fold concentration of specimens with a Minicon¹ concentrator, while for positive reactions the titre of antigen concentration was obtained by serial dilution of the specimens.

Standard microbiological methods were used for the attempted isolation of viral and bacterial pathogens. Hib was looked for only in blood and cerebrospinal fluid (CSF). All patients suffering from Hib infections were treated from the time of diagnosis with chloramphenicol, with the exception of one child who was treated with ampicillin.

Results

A combination of clinical assessment by full-time staff physicians and serological and cultural techniques resulted in the classification of the 92 children into two groups:

(a) Sixty-two patients not suffering from Hib infections: pneumonia (22), otitis media (4), staphylococcal septicaemia (2), pneumococcal septicaemia (3), respiratory viral infection (9), croup (3), streptococcal throat infection (3), enteroviral infection (6), mumps (2), duo-virus gastroenteritis (3), varicella (2), meningococcal meningitis (1), and other suspected septicaemias (2). None of this group of patients has demonstrated a positive urinary latex agglutination even when after 25-fold concentration of specimens the test was repeated.

(b) Thirty patients considered to be suffering from Hib infections: Hib meningitis (20), Hib septicaemia (2), epiglottitis (7), and otitis media (1). The CSF and blood isolations and the presence of Hib antigenuria as detected by latex agglutination in this group of patients are presented in Table 1.

Table 1 *No. of patients with positive bacterial culture and/or Hib antigenuria*

	<i>No. of patients</i>			
	20	3	5	2
CSF or blood isolation	+	+	-	-
Antigenuria	+	-	+	-

The three culture-positive patients, in whom the presence of antigen in urine could not be demon-

strated, were suffering from bacterial meningitis, but in each case Hib antigen was detectable by latex agglutination in CSF or blood. In these three children, the CSF titres were 150, 12, and 8; only two children had serum for testing, and these titres were 32 and 10 respectively. In the first patient, who had the highest titres, urinary antigen could not be detected on consecutive days even with a 25-fold concentration.

Three of the five children with antigenuria and negative bacterial isolations were suffering from epiglottitis, and the fourth and fifth from otitis media and from previously treated purulent meningitis respectively. The two children in whom we failed to confirm Hib infection by either culture or latex agglutination techniques included a patient suffering from bacterial meningitis, whose CSF revealed Gram-negative rods in direct smear, and a child suffering from septic arthritis.

Urinary excretion of Hib antigen was followed in 15 patients, 12 with meningitis and three with epiglottitis, until urinary specimens became negative even after a 25-fold concentration. The findings for these patients are presented in the Figure.

In the 15 patients bacterial antigen was detected in unconcentrated urine for as long as 16 days with a mean of nine days after admission. Concentration of urine increased these times to 18 and 10 days respectively. Only two of the initial urine specimens in this series required concentration to demonstrate a positive latex agglutination, and one of these became positive in the unconcentrated urine on the second day after admission to hospital. As seen from Table 2 and the Figure, the antigen titre often rises on the second day after admission.

Table 2 *Arithmetic mean of urinary Hib latex titres calculated on all available, consecutive daily specimens after admission to hospital*

	<i>Day</i>				
	1	2	3	4	5
Hib latex titre	30.0	34.7	23.8	11.9	9.2

The height of the titre in urine seems to correlate with the height of the antigen titre found in the primary site of infection (for example, CSF or blood), and the highest we recorded in urine was 300.

As shown in Table 3, all patients suffering from bacterial meningitis had urinary antigen titres lower than in blood or CSF; in contrast, all children with epiglottitis showed a higher urinary Hib antigen titre than that found in blood.

¹Amicon Corporation, Texington, Mass 02173, USA.

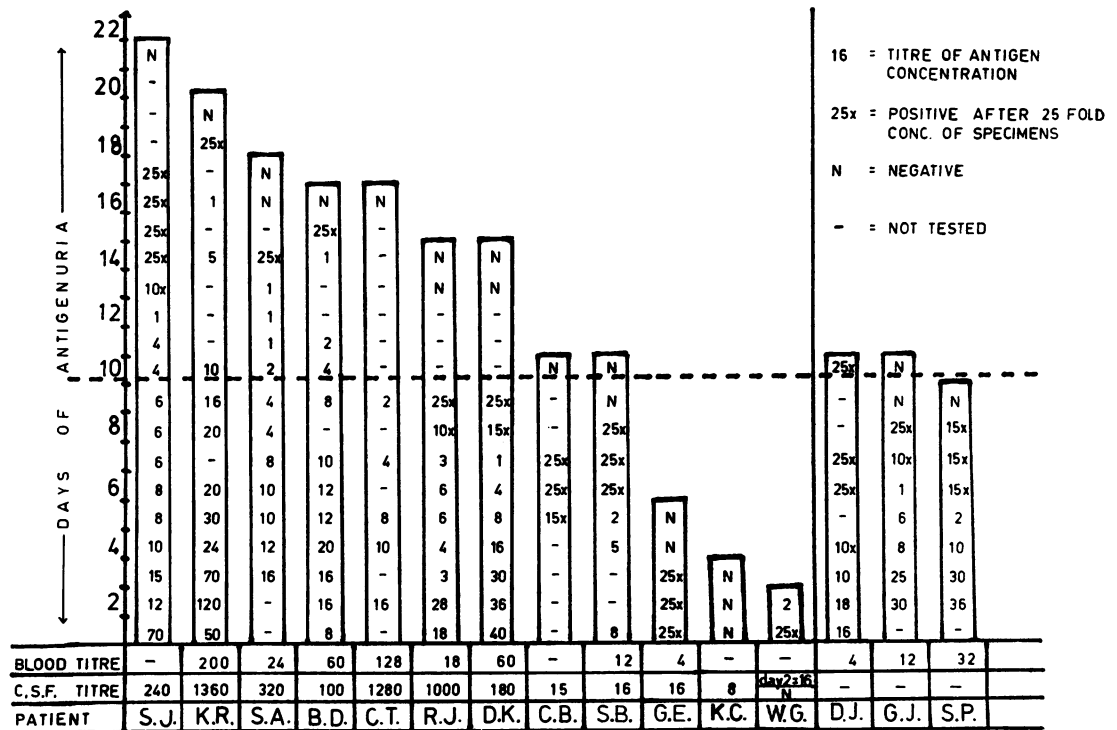


Figure Duration of antigenuria in 15 patients with Hib infections (12 with meningitis, 3 with epiglottitis). In patients WG and GE, admission specimens were found to be positive only after concentration: - - - mean duration of antigenuria in 15 patients. Antigen titres for serum and CSF are those found on admission.

Table 3 Arithmetic mean of Hib antigen titres found in CSF, blood, and urine in all patients

	Bacterial meningitis	Epiglottitis
CSF	390	-
Blood	129	7
Urine	43	24

Discussion

Our study shows that the detection of Hib antigen in urine by the sensitised latex method parallels the isolation of *H. influenzae* from blood and cerebrospinal fluid.

In our hands, the test is sensitive and was free of any false-positive results and, in addition to detecting antigenuria in 20 out of 23 patients in whom the disease was confirmed bacteriologically, it demonstrated the presence of antigen in the urine of another five patients with suspected Hib infections. Not one of the 62 patients without Hib infections had detectable antigenuria.

To date, most reports on bacterial antigen detec-

tion in urine have described counter immunoelectrophoresis and emphasised the need to concentrate the specimens (Shackelford *et al.*, 1974; Feigin *et al.*, 1976). Coonrod (1977) concluded that, as a result of its small molecular size, the capsular antigen of Hib in urine is more difficult to detect by CIEP than is the antigen in CSF or serum. It has been shown by Whittle *et al.* (1974), Kaldor *et al.* (1977), and Suksanong and Dajani (1977) that sensitised particle agglutination technique is more sensitive than CIEP in the detection of Hib antigen; hence the use of the latex agglutination procedure only occasionally necessitates concentration of urine specimens in the early stages of disease.

After the start of antibiotic therapy antigen titres rose above the initial titre and then declined during convalescence in five of nine children who had consecutive daily specimens from the date of admission. Shackelford *et al.* (1974) reported similar findings in their examination of sera from treated patients. In spite of the marked variation in antigen titre and of the duration of antigenuria, we did not observe the reported relationship between the titre of antigen and

the severity of disease (*Lancet*, 1976). We found the use of urinary specimens for the detection of Hib antigen especially useful in paediatric patients from whom it is always difficult to obtain serum. Large volumes of urine readily available for concentration may be used to detect and follow even low concentrations of antigen as the patients recover. The test is fast, reliable, cheap and simple to perform, and would be a valuable adjunct to conventional bacteriological methods, for after-hours work, or in surroundings where bacteriological facilities are not available.

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