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Antibody levels against *Streptococcus pneumoniae* and *Haemophilus influenzae* type b in a population of splenectomized individuals with varying vaccination status

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SUMMARY

In order to determine antibody levels against *Streptococcus pneumoniae* (pneumococcus) and *Haemophilus influenzae* type b (Hib) in a population of splenectomized subjects, 561 persons in a Danish county, splenectomized between 1984 and 1993 were identified. Two hundred and thirty-five were alive and 149 participated in the study. Each person donated a blood sample for antibody determination by ELISA. Though vaccine coverage among the 149 persons was 91% only 52% had 'protective' levels of pneumococcal antibodies. Despite recommendations for regular follow-up on pneumococcal antibody levels this had only been carried out in 4% of the subjects. Splenectomized subjects who needed pneumococcal revaccination were significantly more likely to have received their initial vaccination less than 14 days before or after splenectomy, as recommended, than those not requiring revaccination. Therefore, the timing of initial pneumococcal vaccination in relation to splenectomy seems to be important. All persons had Hib antibody levels higher than 0·15 μg/ml and 60% had levels higher than 1 μg/ml, which are the levels thought to provide short term and long term protection, respectively. In total, 37% of the 149 persons tested had pneumococcal and Hib antibody levels thought to correlate with protection from serious infections.

INTRODUCTION

Splenectomized subjects run an increased risk of serious infections such as meningitis and septicaemia caused by encapsulated bacteria, primarily *Streptococcus pneumoniae* and Hib [1].

The risk depends on the age of the person at the time of splenectomy, underlying diseases, and time elapsed since splenectomy. Although the risk decreases over time, it probably remains increased throughout life [2–4]. In 1978 a 14-valent pneumococcal vaccine was released for clinical use and recommended for vaccination of splenectomized subjects above the age of 2 years. This recommendation was based on studies showing that the vaccine engendered type-specific protection against invasive pneumococcal infections in healthy children and adults [5–6] and that splenectomized persons above the age of 2 years responded satisfactorily to vaccination [7]. In 1983 the 14-valent vaccine was replaced by an improved 23-valent vaccine. This vaccine covers approximately 90% of the pneumococcal types responsible for invasive infections in industrialized countries and gives protection of approximately 70% to splenectomized individuals, excepting those with severe immunocompromise [8]. Although pneumococcal vaccination of splenectomized individuals has been recommended for several years in different countries [9–11], studies have shown that this has been carried out in only a minority of patients [12, 13]. In

Denmark, vaccination of splenectomized individuals has been recommended since 1978 and proven efficacious in preventing invasive pneumococcal infections in splenectomized children when combined with empiric penicillin treatment in cases of fever [14]. In 1991 national guidelines were formulated for vaccination, revaccination and other prophylactic measures for splenectomized children and adults [15].

Less information has been obtained regarding the risk of Hib infection and its prevention by vaccination in splenectomized subjects [1, 16]. Protective antibody levels have been defined for normal individuals but a considerably higher antibody level may be needed in splenectomized individuals [17] due to less effective phagocytosis of bacteria [18]. Since 1985 and 1988 Hib polysaccharide and conjugate vaccines, respectively, have been available and recommended in the USA for use in splenectomized children [19] based on their protective efficacy against invasive Hib infections in small children and the observed increased risk of Hib infections in splenectomized individuals [1]. In Denmark Hib vaccination was included in the childhood vaccination program in 1992 and simultaneously was recommended for all splenectomized children aged 2–15 years.

The aim of the present study was to determine pneumococcal and Hib antibody levels in a well defined population of splenectomized individuals with varying vaccination status and to evaluate the level of current protection against invasive infections due to these bacteria [15].

MATERIALS AND METHODS

The study was carried out in 1995 and all persons who were alive at the beginning of this study, and who had a splenectomy between 1984 and 1993 in a Danish county (Nordjyllands Amt), covering approximately 10% of the Danish population of 5·1 million inhabitants, were identified. This was done by means of the patient computer system of the county containing inpatient and outpatient medical records and by means of registers at the Pathology Departments at the local hospitals containing information about all spleen examinations carried out during the period. During this period 561 persons were identified who had been splenectomized of whom 235 were alive at the beginning of the study. There were 108 females and 127 males with a mean age of 44·3 years (range 4–85 years) at the time of their splenectomy. The indications for splenectomy were: haematological illnesses in 63 persons (27%), trauma in 77 (33%), incidental (performed to achieve malignant tumour clearance) in 28 (12%), accidental (due to inadvertent intraoperative trauma to the spleen) in 47 (20%) and other indications in 20 persons (8%).

All patients received information about the study by mail and were invited to participate in the study. Participation implied, after written consent, donation of a blood sample for determination of antibodies against *Streptococcus pneumoniae* and Hib and completion of a questionnaire enquiring about their knowledge of their pneumococcal vaccination status and prevention of serious post-splenectomy infections [20]. One hundred and forty-nine patients participated. The mean age of these patients was 41·9 years (range 4–81 years). There were 14 children below the age of 15 years and 135 adults. Blood samples were sent to the Streptococcus Unit at the Statens Serum Institut for antibody determinations. All sera were absorbed for antibodies to the species-specific cell-wall polysaccharide (C-Ps (Statens Serum Institut)), since these antibodies are not protective and interfere with the determination of antibodies against the pneumococcal capsule [21]. Pneumococcal antibodies were determined against six pneumococcal types (1, 4, 7F, 14, 18C, 19F) using an enzyme-linked immunosorbent assay described earlier [21]. Antibody levels were calculated as a percentage of a standard serum and expressed as arbitrary antibody units [21].

Guidelines for pneumococcal vaccination and revaccination were formulated on the basis of a suggested protective type-specific pneumococcal antibody level of 300 ng N antibody per ml measured by RIA [22]. This level was translated to arbitrary ELISA values, since good correlation was found between results obtained by the two methods [23]. The way that the guidelines for vaccination and revaccination were formulated has been described before and is briefly summarized in Table 1 [24].

Antibodies against Hib were determined by an ELISA method. Briefly, polystyrene microtitre plates (no. 69620, Nunc, Denmark) were coated with 100 μl of a HbO-HA antigen (Praxis Biologics, Rochester, NY, USA) (1 μg antigen per ml of phosphate buffered saline (PBS), 0·1 M NaCl, 0·05 M phosphate buffer pH 7·4, filtered before use) to each well. To control wells 100 μl of phosphate buffered saline was applied. Plates were incubated for 90 min at 37 °C. Plates were then washed three times in washing buffer (PBS + 0·1% Tween 20). In order to ensure complete blocking, plates were kept filled with washing buffer from the last wash for 20 min before use. Serum

Table 1. *Guidelines for vaccination and revaccination based on pneumococcal antibody levels against individual pneumococcal types* and on geometric mean (GM) antibody levels against six pneumococcal types as a group in arbitrary ELISA units*

Number of types below 25	GM antibody level against six pneumococcal types as a group		
	< 25	25–40	⩾ 40
1	Revaccination	Subsequent antibody follow-up	Subsequent antibody follow-up
2	Revaccination	Revaccination	Subsequent antibody follow-up
3	Revaccination	Revaccination	Not relevant in the routine

* Types: 1, 4, 7F, 14, 18C, 19F.

samples were tested in eight final twofold dilutions, usually 1/25 to 1/3200. An in-house reference serum (SSI-pool), calibrated by means of the FDA Hib standard serum containing 70 μg antibodies per ml, was tested in eight final twofold dilutions starting from 1/100 to 1/12800 and was included on each plate. 100 μl of the samples were applied to the wells of the plates (except for the control wells). Plates were incubated for 60 min at room temperature, washed three times, and 100 μl of conjugate (goat anti-human Ig (P212, Dakopatts, Denmark)) diluted 1/2500 was applied to each well (except for the control wells). Plates were again incubated for 60 min at room temperature, washed six times and 100 μl of substrate (orthophenylenediamine (OPD) (Sigma, USA)) 10 mg per tablet (P8287) or 30 mg per tablet (P8412), were added to each well. The plates were incubated at room temperature on a microplate shaker (at low speed). After exactly 30 min the enzyme reaction was stopped by adding 100 μl of 1 M H_2SO_4 to each well. The optical densities of the developed colour were read at 490 nm on an automatic ELISA reader (Immunoreader NJ2000, Inter Med). Data were transferred to a computer for calculations. Antibody levels were calculated as μg antibody per ml by means of a computer programme [21]. If the Hib antibody level was below 1 μg/ml, vaccination with a Hib vaccine (Act-Hib, Pasteur Merieux) was recommended. Patients and their doctors were informed about the patient's pneumococcal and Hib antibody levels and received guidelines for vaccination and revaccination and other prophylactic measures [20].

The study was carried out in accordance with the Helsingfors Declaration II. The statistical methods used were the χ^2 test, Pearsons correlation test, Kruskal–Wallis test and Fisher's exact test. The study was reported to the National Register Office (no. 1994-1200-472) and was approved by the local Ethic Committee (no. 2-16-4-1-94(94/92)).

RESULTS

Pneumococcal antibody levels are given as geometric mean (GM) antibody levels against six pneumococcal types as a group or as recommendations for vaccination or subsequent antibody test, i.e. determination of pneumococcal antibody level, (see Materials and Methods) [24].

There were no differences in GM pneumococcal antibody levels nor in the number of patients who needed a revaccination or a subsequent antibody test, between the different groups of splenectomized persons (Table 2). Fifty-two percent of the 149 splenectomized subjects needed pneumococcal revaccination, whereas 31% had antibody levels which resulted in recommendations for a new antibody test after 10 years and the remaining 17% for an antibody test either 2 or 5 years later. One hundred and thirty-five splenectomized subjects were adults and information about pneumococcal vaccination was obtained from the medical records of 120; no information could be obtained from the remaining 15 adults.

Fifty-four of the 120 adults had received a pneumococcal vaccination less than 5 years previously, 57 between 5 and 10 years before and 9 adults more than 10 years ago (Table 3).

Fourteen children below the age of 15 years participated in the study. Six of the children were splenectomized due to haematological illnesses and eight following trauma. Five of the children had received a pneumococcal vaccination less than 5 years ago and only one of them (20%) needed a revaccination, whereas 5 out of 8 children (63%) who had received a pneumococcal vaccination more than 5 years ago did (data not shown). In the case of one child, no information about pneumococcal vaccination was obtainable. We found no correlation between GM pneumococcal antibody levels and each

Table 2. *Geometric mean (GM) pneumococcal antibody levels and recommendations for revaccination or subsequent antibody testing based on antibody determinations in 149 splenectomized individuals grouped according to the indication for their splenectomy*

Indication for splenectomy	Number of Patients	GM antibody level	Revacc.		Subsequent antibody testing within 2 years		5 years		10 years	
			n	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Haematological	44	31·6	22	(50)	3	(7)	7	(16)	12	(27)
Traumatic	47	39·7	24	(51)	1	(2)	6	(13)	16	(34)
Incidental	19	27·9	12	(63)	1	(5)	2	(11)	1	(21)
Accidental	26	26·0	12	(46)	2	(8)	3	(12)	9	(35)
Others	13	41·3	8	(62)	—	—	—	—	—	—
Total	149		78	(52)	7	(5)	18	(12)	46	(31)

Table 3. *Recommendations for revaccination or subsequent antibody test based on antibody determinations in 54, 57 and 9 splenectomized adults 0–5 years, 5–10 years and more than 10 years respectively after pneumococcal vaccination, grouped according to indication for splenectomy.*

Indication for splenectomy	Pneumococcal vaccination											
	0–5 years earlier (%)				5–10 years earlier (%)				> 10 years earlier (%)			
	Revacc.		Antibody test later		Revacc.		Antibody test later		Revacc.		Antibody test later	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Haematological	7	(54)	6	(46)	13	(59)	9	(41)	3	(75)	1	(25)
Traumatic	7	(41)	10	(59)	8	(62)	5	(39)		0	2	(100)
Incidental	6	(67)	3	(33)	3	(60)	2	(40)	2	(100)		0
Accidental	8	(62)	5	(39)	4	(44)	5	(56)		0		0
Others	2	(100)		0	4	(60)	4	(50)	1	(100)		0
Total	30	(56)	24	(44)	32	(56)	25	(44)	6	(67)	3	(33)

of the three factors: time elapsed since vaccination, indications for splenectomy and age of the splenectomized subjects (data not shown).

Among the 133 persons who received a pneumococcal vaccination 69 (52%) had a presumed protective antibody level (data not shown). The Combined Geometric Mean (CGM) antibody level among the 133 subjects was not significantly different from the CGM antibody level among the 16 subjects from whom no information about vaccination could be obtained.

Eighty-nine of the 133 pneumococcal vaccinated subjects (67%) were vaccinated after their splenectomy, mostly because of trauma and incidental reasons. The mean time interval between splenectomy and pneumococcal vaccination among the 133 persons was 23·8 days (range 1–270 days) when given before splenectomy and 78·3 days (range 1–1460 days) when given after splenectomy (data not shown).

Table 4 shows the relationship between the time of vaccination and the pneumococcal antibody level given as recommendations for vaccination or subsequent antibody test. A significantly lower percentage of patients ($P < 0{\cdot}05$) vaccinated more than 14 days before or 14 days after splenectomy needed revaccination compared with the patients vaccinated less than 14 days before or after splenectomy. There were no significant differences in the percentages of patients with Hib antibody levels above 0·15 μg/ml, 0·6 μg/ml or 1 μg/ml between the different groups of splenectomized individuals. All the patients had antibody levels above 0·15 μg/ml, whereas 78% and 60%, respectively of the patients had antibody levels above 0·6 μg/ml and 1 μg/ml (data not shown). There

Table 4. *Recommendation for revaccination or subsequent antibody test based on antibody determinations in 133 splenectomized subjects grouped according to the time of pneumococcal vaccination in relation to splenectomy*

Time of vaccination	Number of patients in group	Revacc.		Antibody test later	
		n	(%)	*n*	(%)
< 7 days before splenectomy	12	7	(58)	5	(42)
7–14 days before splenectomy	12	11	(92)	1	(8)
> 14 days before splenectomy	20	8	(40)*	12	(60)
< 7 days after splenectomy	23	12	(52)	11	(48)
7–14 days after splenectomy	36	23	(64)	13	(36)
> 14 days after splenectomy	30	14	(47)*	16	(53)
Total	133				

* Significantly lower compared to the groups of subjects vaccinated either less than 14 days before, or less than 14 days after splenectomy ($P < 0{\cdot}05$).

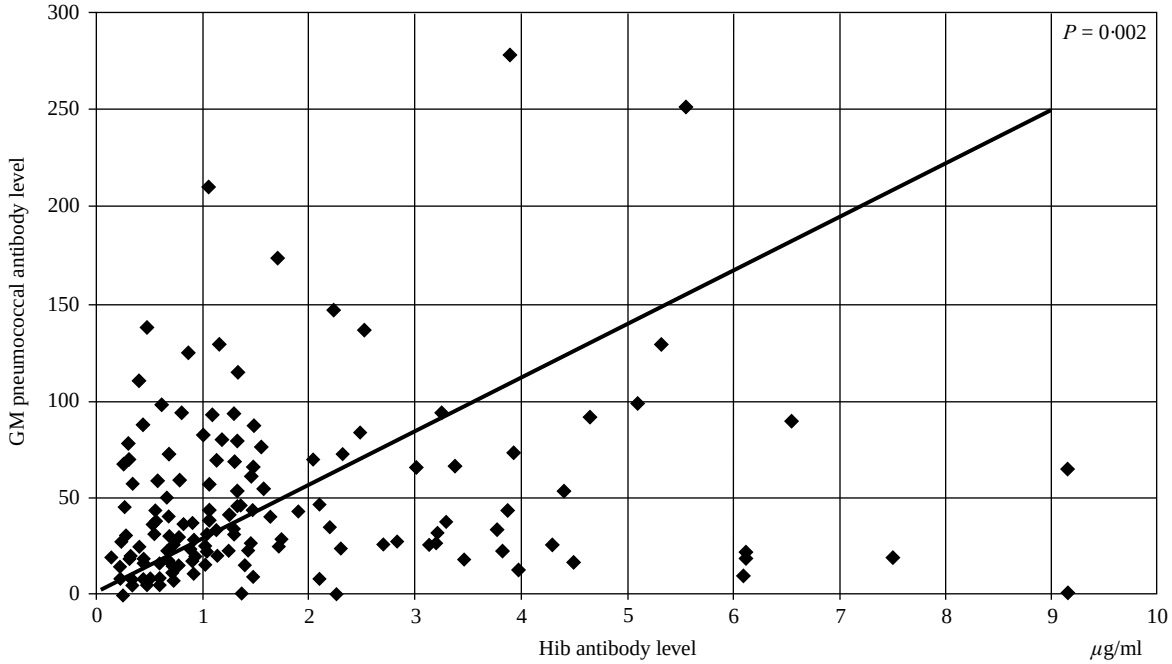

Fig. 1. Comparison of geometric mean (GM) pneumococcal antibody levels and *Haemophilus influenzae* type b (Hib) antibody levels in 149 splenectomized subjects.

was a significant correlation between GM pneumococcal antibody level and Hib antibody level in the 149 persons in the study (Fig. 1).

In 1992 Hib vaccination was introduced in Denmark and our study period ended in 1994. During this period, of nine children younger than 15 years who were included in the study, only three (33%) had received Hib vaccination. Twenty-seven percent of the patients were recommended vaccination with both vaccines, 23% with the pneumococcal vaccine only, and 13% with the Hib vaccine only, whereas 37% were currently protected and therefore not recommended any vaccination at the moment (data not shown). There were no significant differences in the percentages of subjects needing vaccination with both vaccines, only one vaccine or no vaccination at all between the different groups of persons based on the indications for splenectomy.

DISCUSSION

We found that 91% of the 149 splenectomized subjects who participated in our study had received a pneumococcal vaccination. This is a very high vaccine coverage compared with that found in other countries, where vaccination of splenectomized subjects is also recommended [12, 13]. Eighty-six out of the 235 splenectomized subjects who were alive at the time of our study chose not to participate. Although they did not differ from the rest of the group in respect of their indications for splenectomy, nor age, they may have represented a select group amongst whom pneumococcal vaccine coverage could have been lower. However, it appears that the recommendation to vaccinate splenectomized subjects has been largely complied with by Danish doctors.

According to the Danish vaccination recommendations [15], which take into account both the GM antibody level to a group of pneumococcal types and the antibody level against single pneumococcal types, 50% of the 149 splenectomized subjects in our study were currently protected against invasive pneumococcal infections. However, since we were able to measure pneumococcal antibody levels in only 149 out of 561 persons splenectomized during our study period, the number of subjects with protective antibody levels may have been overestimated since some of those who died may have had a low antibody level.

A significant problem is that the exact protective type-specific pneumococcal antibody level is not known. However, an antibody level of 300 ng antibody N per ml, measured by RIA, has been suggested as protective based on a study of antibody levels in 23 patients at the time of acquisition of pneumococcal bacteremia [22]. We translated this suggested protective RIA antibody level into type-specific ELISA antibody levels [23] on which we based our vaccination recommendations [15]. The recommendations for subsequent antibody tests were based on 10 years of studies of antibody levels in splenectomized persons. We found that approximately 50% of splenectomized children needed revaccination 5 years after their primary pneumococcal vaccination [25]. This time interval corresponds well with the results of the present study in which we found that 20% of children vaccinated 0–5 years ago and 63% of children vaccinated 5–10 years ago needed revaccination. In contrast, we found that 56, 56 and 67% respectively, of adults vaccinated 0–5, 5–10, or more than 10 years ago needed pneumococcal revaccination; these are higher percentages than expected, based on an earlier study in which 37% of 118 splenectomized adults needed pneumococcal revaccination 10 years after their primary vaccination [26]. There is no obvious explanation for this discrepancy apart from differences in splenectomy indications, age-distribution and time of vaccination in relation to splenectomy between the two studies. In the present study we found no correlation between GM pneumococcal antibody level and time elapsed since vaccination, and accordingly the percentage of adults needing a pneumococcal vaccination did not differ significantly with time elapsed since vaccination. This corresponds with results from a previous study in which we found that splenectomized adults in general lose approximately one third of the antibodies obtained by vaccination during the first 2 years after vaccination, after which the antibody level remains almost constant [27]. However, the response to vaccination and the persistence of antibodies after pneumococcal vaccination differs considerably between individuals. Therefore it might seem reasonable to offer an individual programme for antibody follow-up to all splenectomized persons. However, this is neither possible, for economical and practical reasons, nor necessary to prevent invasive pneumococcal infections in splenectomized persons according to an earlier Danish study [14]. Here we found that a programme for pneumococcal vaccination and regular antibody follow-up combined with instructions for empirical antibiotic prophylaxis in case of fever, prevented all cases of invasive pneumococcal infections in splenectomized children over a 10 year period [14]. Therefore, it seems reasonable to maintain the current recommendations for antibody testing for splenectomized children and similarly, to offer a 5 year antibody follow-up test to splenectomized adults. It is important to point out that these recommendations are for persons splenectomized as a result of trauma or illnesses which do not significantly affect the immune system. More frequently in immunocompromised patients an individual antibody follow-up test must be carried out.

Pneumococcal revaccination should not be given without prior determination of antibody since studies have shown that the response to revaccination is less pronounced and of shorter duration and carries an increased risk of more severe local and systemic side effects if the antibody level at the time of revaccination is high [28–31].

In order to obtain a significant antibody response to pneumococcal vaccination it is recommended in Denmark, to vaccinate at least 14 days before splenectomy, or in cases of non-elective splenectomies at least 14 days after the operation in order to avoid the immunosuppression caused by the surgical intervention [11]. We found that 18% of the splenectomized subjects were vaccinated less than 14 days before and 44% less than 14 days after splenectomy. The number of patients needing a revaccination was significantly higher among these patients than among patients who were vaccinated according to the recommendations. Thus it seems important to comply with the current recommendations for the time of pneumococcal vaccination in relation to splenectomy.

According to the current recommendations for vaccination and antibody testing [24], 27 subjects should have had an antibody test performed at the time of our study. However, an antibody test had been performed in only six (22%) of these cases and in only one (4%) was the antibody test performed at the recommended time. In order to increase this very low percentage it seems important to educate both doctors and splenectomized subjects regarding the important steps to be taken to prevent post-splenectomy infections, including regular antibody follow-ups.

In Denmark since 1992 the vaccination of all splenectomized children aged 0–15 years against Hib infections has been recommended. However, we found that only 33% of children younger than 15 years during the study period 1992–4 had received Hib vaccination. It is uncertain whether a Hib antibody concentration of 0·15 μg/ml, which is assumed to be protective in healthy unvaccinated persons, is protective in splenectomized individuals. An animal study showed that splenectomized rats needed four times as much antibody as non-splenectomized rats to be protected [17], probably due to less effective phagocytosis after splenectomy [18]. Since patients with various haematological illnesses are known to have a greater risk of acquiring post-splenectomy infections than patients with traumatic splenectomy [32], the protective Hib antibody level in these patients may be even higher than 0·6 μg/ml. We found that all of the 149 splenectomized persons in our study had Hib antibody levels higher than 0·15 μg/ml, whereas 78 and 60% had Hib antibody levels higher than 0·6 and 1·0 μg/ml, respectively. This was independent of the reason for splenectomy. This is in accordance with an earlier study showing that among healthy non-vaccinated adults, 96 and 50% had antibody levels above 0·15 and 1·0 μg/ml, respectively [33]. There seem not to be significant differences in Hib antibody levels between splenectomized and non-splenectomized subjects. We found a statistically significant correlation between Hib and GM pneumococcal antibody levels in the 149 persons. Hib conjugate and pneumococcal non-conjugated polysaccharide vaccines act as T-cell dependent and T-cell independent vaccines, respectively. Therefore the correlation between antibody levels induced by the two vaccines probably reflects individual capabilities to mount antibody responses to different antigenic stimuli. Since the exact protective Hib antibody level in splenectomized persons is not known, we chose to recommend Hib vaccination to all subjects in this study with an antibody level below 1 μg/ml. This included 59 subjects (40%) of whom two were children below the age of 15 (1%). This indicates that at least 60% of the splenectomized persons were currently protected against invasive Hib infection. In Denmark at present there are no recommendations for Hib vaccination of people above the age of 15 years. However, studies have shown that the risk of acquiring lethal sepsis in splenectomized adults is 2·7% which is 540 times more frequent than in the normal population [34]. Furthermore it has been shown that Hib is responsible for approximately 8% of these infections [1]. Therefore, it may be worth considering whether Hib vaccination should be offered to all splenectomized persons around the time of splenectomy, especially since it has been shown that administration of Hib vaccine is free of any serious side effects [35]. Whether a booster dose would be necessary some years later is not known and this would require further study as would the determination of the protective Hib antibody level in various groups of splenectomized individuals.

In conclusion 37% of 149 persons splenectomized during a 10-year period from 1984–93 were currently protected against serious infections due to both *Streptococcus pneumoniae* and *Haemophilus influenzae* type b.

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