

Persistent itching nodules after the fourth dose of diphtheria–tetanus toxoid vaccines without evidence of delayed hypersensitivity to aluminium

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Abstract

Studies in Gothenburg, Sweden, reported an exceptionally high rate of persistent itching nodules at the site of injection of aluminium containing vaccines, usually with positive epicutaneous tests to aluminium. When a new booster diphtheria–tetanus vaccine was introduced we performed a prospective cluster randomised active surveillance in 25,232 10-year-olds. Parental reports 6 months after vaccination with Duplex® or diTeBooster® were collected for 22,365 (88%) pupils in 851 schools. We identified 3–6 children per 10,000 with a local itching nodule persisting for at least 2 months. There were no significant differences between the vaccine groups. Contact allergy to aluminium was not detected. The findings support the use of the vaccine presently available in the Swedish vaccination program. Continued surveillance of persistent itching nodules and aluminium contact allergy is, however, warranted for vaccines containing pertussis toxoid and aluminium. © 2004 Elsevier Ltd. All rights reserved.

Keywords: DT-vaccine booster; Persistent local reactions; Aluminium contact allergy

1. Background

Recently an unexpectedly high frequency, 0.8%, of persistent itching subcutaneous granulomas at the injection site was described in 645 children vaccinated with aluminium hydroxide adsorbed with a monocomponent acellular pertussis vaccine, alone or in combination with diphtheria–tetanus toxoids (DT), DiTeKik®, but also after DT alone [1]. All vaccines were manufactured by Statens Serum Institut (SSI), Denmark. The lesions were first observed after subcutaneous injection but occurred also after intramuscular injection. Bergfors et al. [1] found a positive epicutaneous test to aluminium in 352 of 455 children with itching nodules. Similar persisting itching nodules and delayed hypersensitivity, or contact allergy to aluminium, have been re-

ported as rare events during hyposensitization with allergen extracts containing aluminium [2], and after administration of vaccines containing aluminium [3,4]. The Swedish DT vaccine Duplex®, SBL-Vaccin AB, has been used since 1986. Adverse events after the booster dose in children at 10 years of age are mild as shown in comparisons of the booster vaccination after different vaccines in primary vaccination schedules [5], and also as shown in a comparison of aluminium-adsorbed Duplex® and the same vaccine in a non-adsorbed form [6]. No persisting reaction after vaccination with this vaccine has been reported to the Swedish Medical Products Agency (MPA). diTeBooster® (SSI) has been used in Denmark since 1997 as a booster dose at 5 years of age, and was licensed in Sweden in February 2001.

When Duplex® was replaced by diTeBooster® as a DT-booster at 10 years of age the Expert Advisory Group on Vaccination at the Swedish National Board of Health requested a comparative study of the occurrence of clinically significant late local reactions in two DT-booster vaccines, both of which are used on a nation wide basis in Sweden and in Denmark, respectively.

We therefore conducted an active post marketing surveillance with an open randomised parallel group design with

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the following objectives:

- to estimate the proportion of late local reaction, characterized as an itching subcutaneous nodule and contact allergy to aluminium, and secondly
- to compare the proportion of other defined late local reaction between vaccines among children who received a booster dose as a single injection at 10 years of age of either Duplex® or diTeBooster®, after a primary series of Duplex® at 3, 5 and 12 months of age.

2. Materials and methods

2.1. Participants

The study was conducted in routine school health care in 896 schools in 88 selected suitable municipalities in Sweden, during December 2001 to June 2003. Participating schools were allocated to either vaccine group by a computerised randomisation procedure blocked by municipality in a 1:1 ratio.

Healthy school children attending fourth grade in school were recruited by written information to the parents shortly

before their scheduled DT booster. Medical contraindications were limited and followed the same criteria as used for a routine DT booster vaccination. At vaccination we included children born in 1991 who had received only three doses of DT vaccine, Duplex®, at 3, 5 and 12 months of age after informed written consent by the parent/guardian. The numbers of eligible children in participating schools are given in Fig. 1.

Children were excluded if they had received three doses of any diphtheria–tetanus–pertussis vaccine (DTP) at 3, 5 and 12 months of age or if they had participated in any of the Swedish trials of acellular pertussis vaccines including the previous pertussis study in Gothenburg and surrounding municipalities [7].

2.2. Vaccines and injections

The following two vaccines were used in the study: diTeBooster® from SSI, containing 6.25 Lf diphtheria toxoid, 6.25 Lf tetanus toxoid and 0.5 mg Al³⁺ as aluminium hydroxide per 0.5 mL dose, and Duplex® containing 7.5 Lf diphtheria toxoid, 1.8 Lf tetanus toxoid and 0.25 mg Al³⁺ as aluminium phosphate per 0.25 mL dose. School Health Nurses injected the vaccines intramuscularly in the deltoid

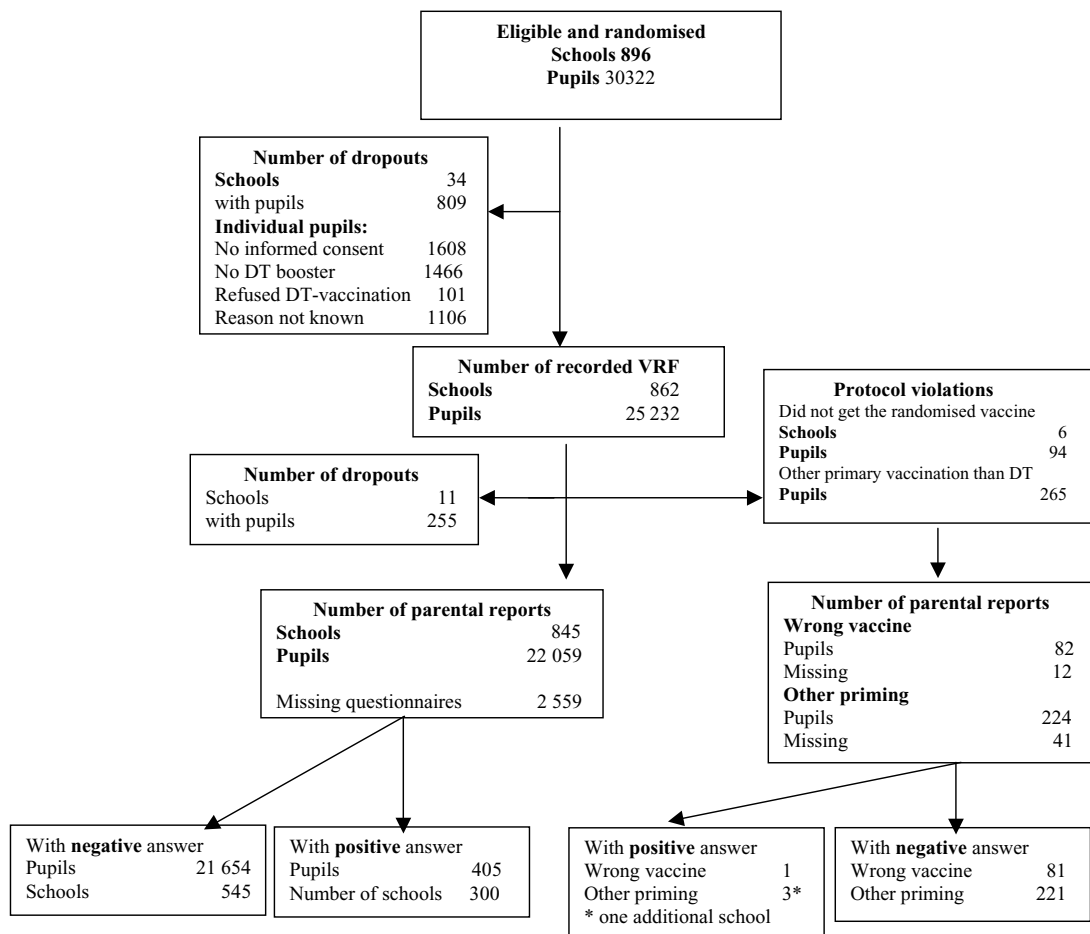


Fig. 1. Trial overview.

muscle using a Luer needle nr 16 size 0.6×25 (23 G $\times$ 1 in.) at an angle of 90° against the skin surface. The vaccine was administered using a muscle mass grasp between the thumb and fingers. Before withdrawing the vaccine the vial stopper was swabbed with 70% isopropyl alcohol. The injection site was swabbed with an alcohol wipe. If the body temperature was $\geq 38.0^\circ\text{C}$, or if the subject had an acute illness, vaccination was temporarily delayed.

2.3. Assessment of reactogenicity

The case definition for a persistent itching nodule was a subcutaneous nodule at the injection site and continuous or intermittent itching for more than 8 weeks after vaccination.

Six months after vaccination the School Health Nurses distributed a parental questionnaire about itching and a nodule at the site of injection. All positive answers were followed-up by the investigators asking structured questions about the nodule and itching in order to determine if the findings met the criteria set for a positive case to be investigated with an epicutaneous test. The parental questionnaire was validated by a blinded examination of randomly selected children by one of the investigators shortly after the questionnaires were completed.

Contact allergy was assessed with epicutaneous tests using the Finn Chamber technique and the IQ Chamber technique (Epitest Ltd Oy, Tuusula, Finland and Chemotechnique Diagnostics, Malmö, Sweden) on Scanpor (Norgesplaster A/S, Vennesla, Norge) which implies patch testing with the test preparation in aluminium chambers and polypropylene chambers, respectively. An aqueous solution of formaldehyde at 1.0% (w/v) (Chemotechnique Diagnostics), to exclude sensitivity to formaldehyde in the vaccine was tested in an IQ Chamber. Aluminium chloride hexahydrate in petrolatum at 2.0% (w/w) (Chemotechnique Diagnostics) was tested in a Finn Chamber. An empty Finn Chamber and petrolatum in an IQ Chamber were also tested. The test preparations were placed on the skin on the upper back under occlusion during 48 h [8]. Readings took place 3 and 7 days after application according to International Contact Dermatitis Research Group guidelines [9].

Children with symptoms meeting the primary case definition of a persistent itching nodule were tested epicutaneously. For each case one reference child without a history of itching nodules matched for sex, age and school and one reference child matched for sex and age from a school randomised to the other vaccine group in the same municipality were selected and the epicutaneous test was performed after informed consent.

The investigators also documented other adverse events spontaneously reported by School Health Nurses.

2.4. Statistical considerations

It was not judged feasible to use individual randomisation in a post-licensure study requiring a large study population

in many schools distributed in municipalities throughout the country. We therefore were advised to consider the effect of randomisation by school. Thus, according to Hayes and Bennett [10], sample size estimates were obtained for cluster randomisation for differences between proportions. We would need 479 clusters (schools), with an average of 30 individuals in each cluster, in each vaccine group ($n = 14,371$) to be able to detect a true difference (alpha 95%) between the vaccine groups with 90% power, assuming the rate of itching nodules in one group to be 0.01% and the rate in the other group to be 0.1. The coefficient of variation between clusters is assumed to be relatively high since the average number of children per cluster is relatively small. Using the same assumptions with Hayes' and Bennett's formula for individual randomisation, the sample size was estimated at 14,243 in 475 schools. If the power was set at 80%, cluster randomisation would require 358 schools with 10,740 children in each vaccine group.

The study was approved by all seven Research Ethic Committees in Sweden and by the MPA. The study was conducted according to ICH guidelines on good clinical practice with appropriate quality assurance for a post-licensure study in routine care.

2.5. Preplanned analysis

A plan of the primary analysis of results was made before the principal investigator and the Data and Safety Monitoring Board had any knowledge of the distribution of cases between vaccine groups. The plan stated:

In the analysis of safety most important is to utilize all available information. Therefore data will primarily be analysed including protocol violations in so called intent-to-treat analyses. When appropriate per protocol analyses will also be made.

Since randomisation was done for schools rather than for individual children the main analysis of children fitting the primary case definition will be done by estimating the relative risk of such events per participating schools randomised to the respective vaccine, with Duplex[®] as standard.

The selection of the primary outcome in this study was with necessity arbitrary. We therefore proposed to look at a few exploratory secondary outcomes to see if there are any signs of differences in relative risks between vaccine groups. Relative risks and 95% confidence intervals were estimated with the Duplex[®] group as standard.

3. Results

There were 896 schools with 30,322 pupils randomised into the trial. We have received reports from 862 schools out of 896 randomised schools (96.2%) (Fig. 1). From these 862 schools 25,232 vaccination reports have been entered into the database. According to the information submitted prior

to the study start there were 29,472 pupils in these schools. The extra children reflect the turnover of pupils over the period between study preparations and execution. According to the class lists of participating schools 3175 children have not been included in the study. Six schools with 59 children violated the protocol by using diTeBooster® rather than their randomised vaccine, Duplex®, Fig. 1. In addition 35 children in different schools did not receive the vaccine the school was randomised to. In some schools children who had received various whole-cell DTP vaccines in other countries were offered participation. Thus, there are 265 children with a primary vaccination violating the study protocol among the remaining 25,138 children in the database.

We have received follow-up questionnaires from 851 participating schools. We miss information from eleven schools with 255 pupils, who are considered as drop outs (Fig. 1).

We have received 22,365 follow-up questionnaires from parents from 851 schools (88.6% of 25,232). There were

21,956 reports with a negative answer on both questions concerning itching and a persisting nodule and 409 reports with at least one positive answer as shown in Fig. 1.

In Fig. 2 the evaluation of the positive parental reports is summarized. Out of 405 positive parental reports, that did not violate the study protocol, 70 were positive for both nodule and itching in the group primed with DT. At the investigator's follow-up five children fulfilled the protocol definition of a late itching nodule. In the remaining 65 cases the symptoms had occurred immediately after the immunisation with duration of a maximum of 3–4 weeks. Two of these immediate reactions involved the whole arm and four involved the whole upper arm and lasted 14–21 days. We have evaluated all 336 in the group primed with DT with a positive answer to only one question by phoning the parents. Among those 336, 171 had a positive response for nodule, and 162 had a positive response for itching. Among the children with a positive answer to only one question, four were found to fit the protocol case definition. Three children were

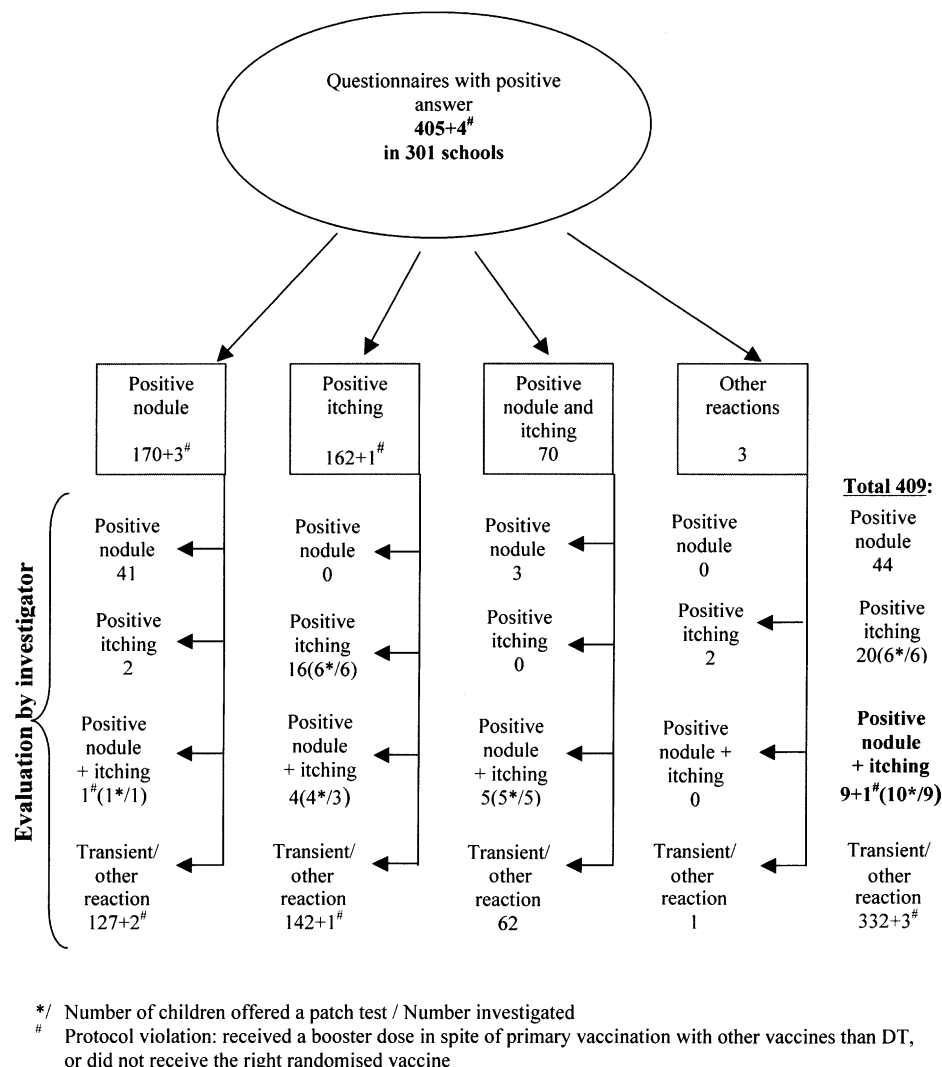


Fig. 2. Positive parental responses in 6-month questionnaire, and at the following evaluation by investigator (EN).

Table 1

Primary analysis of persistent itching nodule fitting the primary case definition according to the investigators' evaluation of the parental responses at the 6-month follow-up per school randomised to Duplex® or diTeBooster®

	Duplex®		diTeBooster®		Total		RR (95% CI) P-value
	Schools	Cases	Schools	Cases	Schools	Cases	
Included per protocol	413	3	431	6	844	9	1.92 (0.48 < RR < 7.61) 0.51
Protocol violations	6	–	1	1	7	1	
All	419	3	432	7	851	10	2.26 (0.59 < RR < 8.69) 0.34

No school had more than one such case. Schools included per protocol and schools that had violated the study protocol for all pupils are shown separately. Relative risks (RR) and 95% confidence intervals of RR are calculated on the number of schools with or without a positive case in schools randomised to diTeBooster® as compared to schools randomised to Duplex® (RR = 1).

evaluated due to other reasons. None of those fulfilled the criteria for the primary case definition.

We received 224 parental questionnaires out of the 265 children with a primary vaccination violating the study protocol. Among those there were three positive answers (Fig. 2). One of these children reporting only a nodule at the injection site was also found positive for itching at the follow-up call by the investigator.

As part of monitoring visits a blinded physical examination was carried out on 705 children shortly after parents had submitted the 6-month questionnaire. An independent comparison between the findings at the physical examinations and parental reports showed no discrepancies.

3.1. Primary analyses

There were 10 children fitting the primary case definition of a persistent itching nodule with at least a duration of 2 months after vaccination, Table 1. Three of these cases occurred in schools randomised to Duplex® and seven occurred in diTeBooster® schools. The intent-to-treat analysis of all events, including one child who had received a primary vaccination series with whole cell DTP, showed a relative risk of 2.26 (95% CI 0.59–8.69) for the diTeBooster® group

compared to Duplex®. The difference between the vaccines was not statistically significant, two-sided *P*-value 0.34. The per protocol analysis gave a similar unequivocal result.

There were no positive epicutaneous tests to aluminium among the investigated 9 cases and 17 controls, or in the 6 children with persistent itching tested outside the study protocol. One child in the diTeBooster® group had a faint but not a positive reaction to aluminium on the epicutaneous test. After the termination of the study a supplementary investigation with the same aluminium test preparations and aluminium chloride hexahydrate in petrolatum at 4% (w/w) was negative, thus no aluminium allergy was demonstrated.

3.2. Secondary analyses

Among 22,365 parental responses 48.1% concerned children in the Duplex® group and 51.9% children in the diTeBooster® group, Table 2 panel. There were somewhat more protocol violations among children receiving diTeBooster® (1.7%) compared to (1.1%) in children receiving Duplex®, mainly explained by the few schools that mistakenly used the available vaccine diTeBooster® rather than the randomised vaccine for the school (Fig. 1).

Table 2

Secondary analyses of individual parental responses for children who had received either vaccine

	Number of parental responses			RR (95% CI) P-value
	Duplex®	diTeBooster®	Total	
Included per protocol	10,638	11,421	22,059	
Protocol violations	114	192	306	
All	10,752	11,613	22,365	
Any positive parental response				
Included per protocol	190	215	405	1.05 (0.87 < RR < 1.28) 0.63
Protocol violations	2	2	4	
All	192	217	409	1.05 (0.86 < RR < 1.27) 0.68
Itching alone—verified by investigator				
All included per protocol	9	11	20	1.14 (0.47 < RR < 2.75) 0.95
Nodule alone—verified by investigator				
All included per protocol	21	23	44	1.01 (0.56 < RR < 1.83) 0.92

Protocol violations also include children in schools that had used the wrong vaccine. The number of “Any positive parental response” corresponds to data in Table 1. The number of “itching alone” and “nodule alone” are events noted as positive at the investigators' evaluation of positive parental responses at the 6-month follow-up. Relative risks are here based on the number of positive and negative responses for children who received diTeBooster® compared to children who received Duplex®.

Table 3

Positive findings at the evaluation of positive parental responses by vaccine group: onset and duration of defined local reaction and size of persisting nodules

Symptom	Duplex® (N = 10, 752)		diTeBooster® (N = 11, 613)		All (N = 22, 365)	
Itching nodule	3		7		10	
Onset <2 months	3		7		10	
Size nodule						
<5 mm	1		1		2	
5–10 mm	1		3		4	
10 mm	1		3		4	
Duration	Itching	Nodule	Itching	Nodule	Itching	Nodule
2–4 months			1	2	1	2
4–6 months	1				1	
6 months	2	3	6	5	8	8
Itching alone	9		11		20	
Onset						
<2 months	7		11		18	
2–4 months	1				1	
4–6 months	1				1	
Duration						
<2 months	3		2		5	
2–4 months			1		1	
4–6 months	1				1	
6 months	5		8		13	
Nodule alone	21		23		44	
Onset < 2 months		21		23		44
Size						
<5 mm		6		11		17
5–10 mm		7		9		16
10 mm		8		3		11
Duration						
2–4 months		1		3		4
4–6 months		1		1		2
6 months		19		19		38

The 409 parental responses that were positive to any of the three questions 6 months after vaccination were evenly distributed between vaccines (Table 2). Also regarding the 20 children with only persistent itching, and the 44 children with only a persisting nodule, the relative risks were close to 1 with modestly wide confidence intervals, and there were no statistically significant differences between the two vaccines, analysed for all children or *per protocol* when protocol violations were excluded. In general the symptoms were mild and of little concern to children and parents. A few children were treated temporarily with 1% hydrocortisone ointment.

Table 3 shows the duration, onset and size of the events noted as positive at the investigators evaluation of positive parental responses. No persistent nodule exceeded 10–15 mm at the time of follow-up. Among the nine children who had a persisting itching nodule none had remaining symptoms/lesions at the physical examination at the time of the epicutaneous test 12 months (10.5, 14) after the vaccination. The only child who fitted the primary case definition and was not tested had a minimal nodule and mild itching for 4–6 months. Fourteen of the nodules without

itching were still present at the time of follow-up at 8–12 months after the vaccination but had all become smaller.

No serious adverse event was reported. Spontaneous reports of other adverse events were registered. We have received such reports for 148 children (95/13,029 had received diTeBooster® and 53/12,203 had received Duplex®). In one child a cartilaginous exostosis of the proximal left humerus, i.e. close to the injection site, was noted six month after the vaccination. The child was referred to an orthopaedic surgeon who judged that the condition had pre-existed for several years and had become visible when it had grown beyond the growth zone towards the distal part of the deltoid muscle. A fine needle biopsy confirmed the diagnosis. There were no signs of a foreign body reaction nor atypical cells.

4. Discussion

Potential risks of aluminium adjuvant in vaccines have been on the agenda for many years. A useful summary was given at a US National Vaccine Program Office Workshop on vaccine adjuvants in Puerto Rico in May 2000: aluminium

has been used as a vaccine adjuvant for more than 70 years and has an established safety record with low incidence of reported adverse events. On the other hand there is a relative paucity of data on the detailed mechanism of action of aluminium adjuvants when used with different vaccines. Aluminium hydroxide containing vaccines have recently been implicated in a rare muscular disease, macrophagic myofasciitis, characterized by diffuse myalgia and aluminium containing cytoplasmic inclusions in macrophages in the deltoid muscle [11]. The observation suggested that aluminium might remain at the site of injection for a considerable time. However, published case series of macrophagic myofasciitis [12,13] have not included asymptomatic controls because of the invasiveness of the biopsy procedure, and the condition remains a poorly delineated histological finding of unknown clinical significance. Else, aluminium adjuvant has mainly been linked to immediate local reactions reported in increasing frequency by dose number. This phenomenon was particularly studied after the fourth and fifth dose of different acellular pertussis vaccines [14]. There was no clear dose response between local reactogenicity and aluminium content.

In Denmark contact allergy to aluminium was reported by Veien and co-workers. In at least 32 children after immunisation with diphtheria–tetanus–polio (Di-Te-Pol®) containing aluminium hydroxide as adjuvant [2,4,15]. Long time follow-up during 1–8 years in 21 of these children who had cutaneous granulomas for more than a year and aluminium allergy showed that the symptoms improved in 11 children, remained unchanged in 5 and cleared in 5 and that the course could not be correlated with the intensity of the initial aluminium patch test [16].

In the Gothenburg trials that sparked the present study an unexpectedly high incidence of persisting itching nodules together with contact allergy to aluminium were noted after vaccinations with aluminium containing vaccines [1]. Six of 645 cases occurred after the Danish DT alone. The authors do not state the rate of contact allergy for the different vaccines used. Therefore, it is not clear whether any contact allergy against aluminium was found after a series of the Danish DT alone. The by far highest rates of itching nodules were reported in the so called mass vaccination project when three doses of the acellular mono-component PT vaccine containing aluminium hydroxide were given after a primary series of two or three doses of the Swedish DT containing aluminium phosphate (1.7% of approximately 7200 children, and 1.2% of approximately 28,000 children, respectively). In our study we found a persisting itching nodule in a rate of 3–6 per 10,000 with no statistically significant differences between children, primed with three doses of Duplex®, when receiving a fourth dose of diTeBooster®, containing aluminium hydroxide, or Duplex®, containing aluminium phosphate. We recognise that the statistical power of the study did not permit us to show small differences between vaccines for rare events occurring at rates below 1 per 1000.

The validity of our observations is based on the active surveillance in a cluster randomised prospective design

with a high participation rate (88% of parents responded), whereas earlier post licensure studies mostly have had to rely on historical comparisons or case series without adequate control. Our study was not blinded for logistical and economical reasons within the limited time frame when both the new and “old” vaccine could be used in parallel. The open randomisation by school could cause reporting bias for two obvious reasons: the study was performed to rule out untoward effects of a new vaccine, thus nurses and parents could tend to report more suspected events for the new vaccine; and clusters of reported reactions would be more likely in schools randomised to the new vaccine. Our results suggest that the potential sources of bias did not influence our findings since no difference could be established between groups, and not more than one case fitting the primary case definition was identified in any school. The rate of only persistent itching, and the rate of only persisting nodules were practically identical for both vaccines. Also the rates of unspecific positive parental responses were very similar in both groups speaking against reporting bias. These findings suggest that we have not missed many potential cases that may have fitted the primary case definition of a persistent nodule, nor is it likely that many such events would have onset later than 6 months after the injection since practically all nodules were noted within 2 months after immunisation. In the Gothenburg study the median onset was at 3 months after vaccination with a range from 2 weeks to 5 years. However, since many of the cases in that study were reported retrospectively after widely published information in the media, the timing of onset seemed to be uncertain or not immediately recognised [1].

We did not detect any child with a positive epicutaneous test to aluminium and can only conclude that such events seem to be rare after these two vaccines given as a fourth dose after a primary series of Duplex®. Our results are in contrast to the Gothenburg study [1] and to case series in Denmark [2,4,16] where a large proportion of children with persisting nodules had a positive patch test to aluminium, using the same well-established patch test method [8]. However, we used a plastic chamber filled with petrolatum as a negative control, whereas in the Gothenburg study the patch tests were performed with two forms of aluminium without a negative control. The possibility of some false positive test results in the Gothenburg trial can therefore not be excluded, particularly in the symptom-free siblings who also had received the acellular mono-component PT vaccine in the mass-vaccination project and who were tested positive for aluminium [1].

We conclude that the study results support continued use of either vaccine in the Swedish vaccination programme. However, the present study indicates that late occurring itching nodules, albeit mild and with no sign of aluminium hypersensitivity, do occur after the fourth DT-dose at much higher rates than previously recognised from passive surveillance data. There is a difference in reporting 3–6 cases per 10,000 doses in an active surveillance and 1 per

1.3 million doses by passive reports for diTeBooster® to Danish authorities (personal communication M Stelfeld, SSI) or 0 reports for Duplex® in Sweden since 1986 (personal communication I Trolin, Swedish MPA). The large difference may partly be due to the possibility that only severe cases are likely to be reported in the routine reporting system.

Cited studies from USA and from Gothenburg, Sweden, had in common that local reactions were observed after multiple doses of aluminium containing vaccines that also contained acellular pertussis component. The Gothenburg study implicated vaccines from one manufacturer [1]. Perhaps more importantly the Gothenburg investigators studied one acellular pertussis vaccine containing only peroxide inactivated PT in a higher dose, 40 µg, than in two- to five-component acellular pertussis vaccines, containing 3.5–25 µg PT [17]. Booster doses containing PT is known to elicit a strong specific IgE production [18]. It is unlikely that a large protein like PT could cause contact allergy, but it is not known whether PT may potentiate the development of a delayed hypersensitivity against aluminium. Additional studies are warranted to better understand the pathogenesis and clinical significance of persisting local reactions in aluminium adsorbed vaccines and the possible relationship with aluminium delayed hypersensitivity after vaccination with PT. Contrary to the recommendation in a recent systematic review to not pursue any research on adverse effects of aluminium-containing vaccines [19], we consider that similar active surveillance of subsequent booster doses of PT-containing vaccines are warranted in children who previously have received primary series of aluminium adsorbed acellular DTP vaccines to obtain better estimates of the rate of persisting itching nodules and aluminium contact allergy within current immunisation programmes offering four or five doses of acellular DTP vaccines. Such studies should preferably be performed as a prospective comparison at the time of introduction of the new vaccines in the first cohort reaching the age of each booster dose, as part of recommendations for post licensure studies by the responsible drug regulatory agency.

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