Article

Effect on Diphtheria Immunity of Combined Tetanus and Diphtheria Booster Vaccination in Adults

S. Marlovits, R. Stocker, A. Efstratiou, K. Broughton, A. Kaider, V. Vécsei, H. Kollaritsch

Abstract The effect of a single booster injection of an adult formulation of a combined diphtheria–tetanus vaccine (Td) on diphtheria-specific immunity was evaluated. The booster injection, containing 2 IU diphtheria toxoid per dose, was given as part of the surgical wound management for adults with open soft tissue injuries. Diphtheria antitoxin concentrations were determined in serum samples from 534 patients (199 women and 335 men, aged 18–70 years) using an enzyme immunoassay and a tissue culture toxin neutralization assay. Seroimmunity against diphtheria toxin was classified at three levels: susceptibility, basic protection, and full protection against the toxic manifestations of the disease. Before vaccination, 27.1% of the subjects were susceptible to diphtheria, 26.5% had basic protection, and 46.4% were fully protected. Six weeks (minimum 25 days, maximum 98 days) after a single booster injection, 89.7% of the subjects achieved full protection against diphtheria, and only 3.9% had antitoxin levels below the protective level. The median increase from the prevaccination to postvaccination antitoxin concentration was found to be 14-fold (4.4–47; quartiles Q25 to Q75). The change in antitoxin levels after revaccination was higher in older age groups ($P < 0.001$), whereas neither sex ($P = 0.86$) nor the country of previous immunization with a different national immunization schedule ($P = 0.61$) had a significant influence on the revaccination effect. Systemic adverse reactions were rare, and local reactions of clinical significance were reported in only 1.9% of subjects.

S. Marlovits (✉), R. Stocker, V. Vécsei
University Clinic for Trauma Surgery, University of Vienna Medical School, General Hospital, Waehringer Guertel 18–20, 1090 Vienna, Austria
e-mail: stefan.marlovits@akh-wien.ac.at

A. Efstratiou, K. Broughton
WHO Collaborating Centre for Diphtheria and Streptococcal Infections, PHLS-Respiratory and Systemic Infection Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HAT, UK

A. Kaider
Department of Medical Computer Sciences, University of Vienna, General Hospital, Waehringer Guertel 18–20, 1090 Vienna, Austria

H. Kollaritsch
Institute for Specific Prophylaxis and Tropical Medicine, University of Vienna, Kinderspitalgasse 15, 1095 Vienna, Austria

Introduction

In the 1990s, the massive reemergence of diphtheria throughout the Newly Independent States (NIS) of the former Soviet Union marked the first large-scale diphtheria epidemic in industrialized countries in three decades [1, 2]. From 1990 to 1994, more than 140,000 new cases, with more than 4000 deaths, were reported [2]. The diphtheria epidemic in the NIS raised numerous concerns about the efficacy of diphtheria control programs and of the diphtheria vaccine itself [3]. Numerous factors appear to have contributed to the epidemic in the former Soviet Union, and arguably the most important factor for the diphtheria epidemic was the development of a large population of adults susceptible to the disease [4]. The decreased opportunity for naturally acquired immunity, along with the

waning of vaccine-induced immunity in the absence of routine adult vaccination, has resulted in a high proportion of adults susceptible to diphtheria [5]. Serologic surveys in the NIS, western Europe, and the USA indicate that 20–60% of adults aged ≥ 20 years are susceptible to diphtheria [6–12]. The importation of diphtheria into countries previously free from the disease has demonstrated the potential for the diphtheria epidemic in the NIS to spread to neighboring countries in Europe, the Middle East, Asia, and the USA [1, 7].

To improve diphtheria-specific levels of immunity within the adult population, public health advisory groups recommend that active vaccination against tetanus, administered as part of wound management for persons aged over 7 years, should involve the use of vaccines containing both diphtheria and tetanus toxoids, rather than the single antigen tetanus toxoid – i.e. Td, a formulation for adults containing decreased amounts of diphtheria toxoid with 1–5 Lf per dose [5, 13–16].

It was therefore essential to investigate the influence of one booster of the combined vaccine for injured adults on individual diphtheria immunity. We report here the effects of combining tetanus and diphtheria booster immunization as part of wound management on diphtheria-specific immunity in adults.

Patients and Methods

Study Subjects and Design. Patients from 18–70 years of age who presented to the emergency room at the University Hospital in Vienna over a 1-year period between July 1996 and July 1997 for treatment of open, soft tissue injuries (lacerations, abrasions, avulsions, and puncture wounds) were screened for tetanus immunity and for eligibility for inclusion in the study. Patients who stated that they had basic immunization against tetanus and that their last tetanus vaccination (booster or basic immunization) had been more than 5–10 years earlier were invited to participate. Among the 987 adults who were eligible, 558 gave written informed consent for initial interviews, follow-up, and collection of blood. The protocol and consent procedures were approved by the Ethical Board of the University of Vienna (code 96/142). A standardized medical record form was completed for each patient, in which the physician noted age and sex, country of previous immunization, whether the patient had been fully immunized against tetanus and diphtheria, and the number of years since the last vaccination. According to the national recommendation for the prevention of tetanus in injured patients in Austria (last tetanus immunization between 5 and 10 years with completed basic immunization), a combined tetanus and diphtheria vaccine for active booster immunization was used. Before vaccination and 6 weeks after (mean, 41 days; standard deviation, 11.29; minimum, 25 days; maximum, 98 days), blood samples from 534 (95.7%) patients were obtained for serological evaluation. Twenty-four (4.3%) patients were lost during follow-up: 20 patients refused consent, three patients moved to another town, and one patient died due to an accident.

Vaccine Used in the Study. The same batch of regular adult diphtheria–tetanus vaccine (L 1190), manufactured by Pasteur Mérieux Connaught (Pasteur Mérieux, France) was used in the study. Each dose of 0.5 ml contained 2 IU purified diphtheria toxoid, 20 IU purified tetanus toxoid, 1.25 mg aluminium hydroxide, 0.5% thimerosal, and buffered 0.9% sodium chloride solution. The vaccine was injected deep into the deltoid muscle.

Recording of Side Effects. Over the time period of 4 weeks, the patients were instructed to record the extent and duration of local and systemic reactions in a prospective, standardized fashion. Systemic reactions were assessed by body temperature and signs of malaise such as general discomfort, headache, nausea, and inability to work. Local reactions were assessed by the occurrence of pain, induration, redness, or swelling and were measured according to the area of the reactions (<5 cm, 5 to 10 cm, >10 cm) [17].

Serological Method. Serum samples were collected and stored at –20°C until all samples were tested together for diphtheria antitoxin antibody levels using a modified indirect enzyme immunoassay (EIA) as described previously [18–20]. Microtiter plates were coated with 0.25 Lf/ml nonadsorbed diphtheria toxoid (Swiss Serum and Vaccine Institute, Switzerland), diluted in carbonate–bicarbonate buffer at pH 9.6 to a final volume of 75 μl per well, and kept overnight in a humid box at 4°C. To each well, 75 μl of serum sample, diluted initially to 1:10 or 1:100 in PBS/Tween 20 at pH 7.5 with 2% bovine albumin, was added in 12-step serial dilutions in duplicates and incubated for 120 min at 38°C. Serial twofold dilutions of a standard positive human serum (Diphuman Berna, 150 IU diphtheria antitoxin per milliliter, Swiss Serum and Vaccine Institute) at a final concentration of 1 IU/ml and a pooled negative serum as a control were included in all assays. After three washings, 75 μl of alkaline phosphatase-conjugated goat antihuman IgG (Tago Immunologicals, code no. 2490, USA) in a 1:10,000 dilution was added and incubated overnight at 4°C. The plates were developed using beta-nitrophenyl phosphate disodium (NPP) substrate (Sigma Chemical, USA), diluted to 1 mg/ml in diethanolamine buffer at pH 9.8 (75 μl per well) at room temperature, and measured at 450 nm (Micro ELISA Auto Reader MR 580; Dynatech Instruments, USA). The absorbance was read when the first reference serum dilution gave an absorbance of 1. The antitoxin antibody levels were expressed in IU/ml and were calculated relative to the standard, assuming a linear relationship between log antibody concentration and log absorbance. Two determinations were carried out for each sample, and the mean value was used for further analysis.

In order to validate the performance of the EIA test on sera with low diphtheria antitoxin concentrations, 195 randomly selected serum samples were tested with a tissue culture toxin neutralization assay at the WHO Collaborating Centre for Diphtheria and Streptococcal Infections (London, UK) [8, 21, 22]. In a microtiter plate, doubling dilutions (final volume 50 μl) of serum and reference antitoxin internally calibrated with WHO reference antitoxin (1st International Standard, Statens Seruminstitut, Copenhagen, Denmark) were made in modified Eagle medium (MEM, Life Technologies, USA). Then, 50 μl of a 2.5×10^{-3} Lf/ml toxin solution in cell-culture medium was added to the serum and reference antitoxin dilutions, and the plate was incubated for 1 h in the dark at 37°C. After toxin neutralization, 50 μl of a suspension (2.5×10^5 cells/ml) of Vero cells in cell-culture medium was added to each well. Cell growth was checked by microscopy after 6 days of incubation in the dark at 37°C. Antitoxin concentrations in IU/ml were calculated by taking the last dilution of serum at which cells still grew and multiplying the dilution factor by the lowest concentration of reference antitoxin that neutralized the added toxin.

The degree of agreement in the determination of the antitoxin concentrations with EIA and in vitro neutralization assay was assessed using a weighted kappa coefficient [23]. The comparison of the determined antitoxin concentrations with EIA and the in vitro neutralization assay showed "very good agreement", with kappa =0.81. At a cut-off level of 0.01 IU/ml, agreement between the two methods was seen in 53 of 54 (98%) samples, with a sensi-

tivity of 98.15% (95% confidence interval [CI], 90.11–99.95) and a specificity of 100% (95% CI, 97.42–100.0%). In sera with antitoxin levels ≥0.01 IU/ml, perfect agreement was seen in 122 of 141 samples (87%; data not shown).

Definition of Antitoxin Levels. To classify seroimmunity against diphtheria toxin, antitoxin concentrations were classified at three levels: concentrations below 0.01 IU/ml indicate susceptibility to diphtheria; concentrations between 0.01 and 0.09 IU/ml indicate a basic protection against the toxic manifestations of disease; and concentrations ≥0.1 IU/ml give full protection [8, 13, 22, 24, 25].

Data Analysis and Statistics. For description, subjects were classified into five age groups [16]. The demographic factor "country of previous immunization" was defined according to the following four groups: Austria, western European countries, eastern European countries, and non-European countries. Before and after vaccination, the frequencies of the subjects in the defined serological groups "susceptible", "some protection" and "full protection" were determined. The median and quartiles Q25 to Q75 are given for the description of continuous variables: median (Q25 to Q75).

Log-transformed (log10) antitoxin concentration values were used for statistical analysis. The influence of sociodemographic factors (age, sex, and country of previous immunization) on the antitoxin concentration before and after vaccination was analyzed using linear regression models. Both univariate and multiple regression analysis were carried out. To test for a nonlinear age effect, a quadratic term for age was included in the regression models.

The effect of the vaccination was described using the differences (d) in the log-transformed antitoxin concentrations: log (y) –log (x), where x is the prevaccination antitoxin value and y the postvaccination antitoxin value. The conversion factor $(c = 10^d)$ describes the relative increase of the vaccination antitoxin concentration. A paired *t* test was used to analyze the change in the antitoxin concentration due to the vaccination. The influence of age and sex on the change of diphtheria immunity was also analyzed using univariate and multiple linear regression models.

Results

Immunity Status Prior to Vaccination. Before vaccination, 27.1% of the population studied were susceptible to diphtheria, 26.5% had basic protection, and 46.4% were fully protected (Figure 1, Table 1). A nonlinear effect toward decreasing immunity with increasing age was observed ($P < 0.001$). Susceptibility increased from 11% in the 18–24-year-old group to 46.3% in the 40–49-year-old group, and to 42.1% in the 50–70-year-old group. The median antitoxin level of 0.19 IU/ml (0.05–0.52) in the youngest age group decreased to 0.03 IU/ml (0–0.07) in the 50–70-year-old group (Table 3). A sex difference was found with respect to the protection against diphtheria: 32.2% of the women and 24.1% of the men lacked protective antidiphtheria immunity, with antitoxin concentrations <0.01 IU/ml (Table 1). The median antitoxin concentration in males was 0.12 IU/ml (0.02–0.32), compared with 0.05 IU/ml (0–0.21) in females ($P = 0.006$; Table 3). With regard to the country of previous immunization, no difference was found in the antitoxin concentrations ($P = 0.49$). The multiple linear regression analysis revealed that
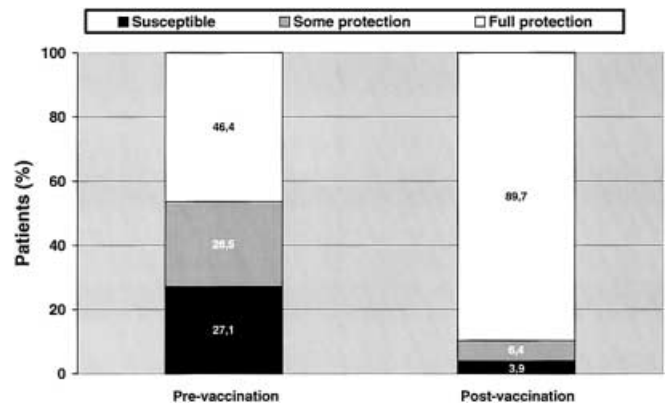

Figure 1 Diphtheria immunity of injured adults before and after revaccination with a combined tetanus–diphtheria vaccine at three levels (susceptible: <0.01 IU/ml; basic protection: 0.01–0.09 IU/ml; full protection: ≥0.1 IU/ml)

age ($P < 0.001$) and sex ($P = 0.004$) had a statistically significant independent influence on the diphtheria immunity level, whereas the influence of the country of immunization was not significant ($P = 0.72$).

Immunity Status After Booster Vaccination. After booster vaccination, 89.7% of the patients were fully protected, 6.4% had basic protection, and 3.9% were susceptible to diphtheria (Figure 1, Table 2). A statistically significant nonlinear age effect on immunity was still observed ($P < 0.001$). Full protection, with an antitoxin concentration of 0.1 IU/ml, was achieved by 99.3% of the subjects in the 18–24-year-old group. This decreased to 76.7% and 83.9% in the age groups 40–49 and 50–70, respectively. The highest proportion of unprotected patients was found in the 40–49-year-old group, with 11% of the subjects being unprotected. The median antitoxin ranged from 2.20 IU/ml (1.04–3.83) in the youngest age group to 0.86 IU/ml (0.11–2.53) in the 40–49-year-old group, and to 1.06 IU/ml (0.19–3.34) in the oldest age group (Table 3). A statistically significant sex difference in the antitoxin concentration was seen ($P = 0.005$), with 3.3% of males and 5% of females showing no protective antibody levels (Table 2). The median antibody concentration in males reached 1.99 IU/ml (0.73–3.93), compared with 1.23 IU/ml (0.34–2.95) in females (Table 3). The factor of the country of previous immunization was not statistically significant for the level of postvaccination immunity ($P = 0.37$). The multiple linear model revealed that age ($P < 0.001$) and sex ($P = 0.003$) were variables of independent statistical significance for protection after immunization, and the country of immunization was found not to be statistically significant ($P = 0.61$).

Effect of Vaccination. A minimum protective level of diphtheria antitoxin concentration was achieved in 96.1% of all subjects. The antitoxin concentration in the population studied increased from a median of

Table 1 Prevalence of diphtheria antitoxin levels before combined tetanus-diphtheria booster vaccination. Diphtheria antitoxin concentrations (IU/ml) are given at three levels (no protection: < 0.01 IU/ml; basic protection: 0.01–0.09 IU/ml; full protection: ≥ 0.1 IU/ml) for different age groups, gender, and the country of previous immunization

Variable	Percentage (n) of patients	Antitoxin antibody concentration (IU/ml)		
		< 0.01	0.01–0.09	≥ 0.1
Total	100 (558)	27.1 (151)	26.5 (148)	46.4 (259)
Age in years				
18–24	27.6 (154)	11.0 (17)	26.6 (41)	62.3 (96)
25–29	21.2 (118)	22.0 (26)	15.3 (18)	62.7 (74)
30–39	26.7 (149)	31.5 (47)	27.5 (41)	41.0 (61)
40–49	14.3 (80)	46.3 (37)	30.0 (24)	23.8 (19)
50–70	10.2 (57)	42.1 (24)	42.1 (24)	15.8 (9)
Sex				
Male	63.3 (353)	24.1 (85)	23.2 (82)	52.7 (186)
Female	36.7 (205)	32.2 (66)	32.2 (66)	35.6 (73)
Country of previous immunization				
Austria	78.0 (436)	27.8 (121)	24.5 (107)	47.7 (208)
Western European country	3.6 (19)	26.3 (5)	26.3 (5)	47.4 (9)
Eastern European country	15.9 (91)	26.4 (24)	38.5 (35)	35.2 (32)
Non-European country	2.5 (12)	25.0 (3)	16.7 (2)	58.3 (7)

Table 2 Prevalence of diphtheria antitoxin levels after combined tetanus–diphtheria booster vaccination. Diphtheria antitoxin concentrations (IU/ml) are given at three levels (no protection: < 0.01 IU/ml; basic protection: 0.01–0.09 IU/ml; full protection: ≥ 0.1 IU/ml) for different age groups, sex, and country of previous immunization

Variable	Percentage (n) of patients	Antitoxin antibody concentration (IU/ml)		
		< 0.01	0.01–0.09	≥ 0.1
Total	100 (534)	3.9 (21)	6.4 (34)	89.7 (479)
Age in years				
18–24	27.5 (147)	0.7 (1)	0.0 (0)	99.3 (146)
25–29	21.2 (113)	2.7 (3)	3.5 (4)	93.8 (106)
30–39	27.1 (145)	5.5 (8)	9.0 (13)	85.5 (124)
40–49	13.7 (73)	11.0 (8)	12.3 (9)	76.7 (56)
50–70	10.5 (56)	1.8 (1)	14.3 (8)	83.9 (47)
Sex				
Male	62.7 (335)	3.3 (11)	5.1 (17)	91.6 (307)
Female	37.3 (199)	5.0 (10)	8.5 (17)	86.4 (172)
Country of previous immunization				
Austria	77.3 (413)	4.4 (18)	5.6 (23)	90.1 (372)
Western European country	3.6 (19)	10.5 (2)	5.3 (1)	84.2 (16)
Eastern European country	16.9 (90)	1.1 (1)	10.0 (9)	88.9 (75)
Non-European country	2.2 (12)	0.0 (0)	8.3 (1)	91.7 (11)

Table 3 Effect of combined tetanus–diphtheria booster vaccination on diphtheria immunity. The median value and the quartiles (Q25 to Q75) of the diphtheria antitoxin concentration (IU/ml) before and after combined tetanus-diphtheria vaccination (x = prevaccination concentration, IU/ml; y = postvaccination concentration, IU/ml) and the difference (d) of the log transformed postvaccination and prevaccination values are given. The conversion factor (c) is defined as $10^d = 10^{\log(y) - \log(x)}$

Variable	Antitoxin antibody concentration (IU/ml) Median (quartiles)		Median (quartiles)	
	Prevaccinal (x)	Postvaccinal (y)	Difference (d = log(y) − log(x))	Conversion factor (c = $10^d = 10^{\log(y) - \log(x)}$)
Total	0.08 (0.0 to 0.29)	1.73 (0.54 to 3.53)	1.15 (0.64 to 1.67)	14.0 (4.4 to 47.0)
Age in years				
18–24	0.19 (0.05 to 0.52)	2.2 (1.04 to 3.83)	0.97 (0.52 to 1.48)	9.3 (3.3 to 30.1)
25–29	0.14 (0.03 to 0.37)	2.09 (0.8 to 4.35)	1.18 (0.62 to 1.62)	15.2 (4.2 to 41.7)
30–39	0.06 (0.0 to 0.21)	1.42 (0.33 to 3.33)	1.23 (0.70 to 1.68)	17 (5 to 47.9)
40–49	0.02 (0.0 to 0.09)	0.86 (0.11 to 2.53)	1.19 (0.73 to 1.76)	15.4 (5.4 to 57.5)
50–70	0.03 (0.0 to 0.07)	1.06 (0.19 to 3.34)	1.60 (0.96 to 2.03)	39.9 (9.1 to 107.2)
Sex				
Male	0.12 (0.02 to 0.32)	1.99 (0.73 to 3.93)	1.14 (0.70 to 1.61)	13.7 (5.0 to 40.8)
Female	0.05 (0.0 to 0.21)	1.23 (0.34 to 2.95)	1.15 (0.60 to 1.75)	14.0 (4.0 to 56.0)

510

0.08 IU/ml (0–0.29) before vaccination to a median of 1.73 IU/ml (0.54–3.53) after vaccination (Table 3). The conversion factor indicated a 14-fold (4.4–47) relative increase in the postvaccination antitoxin concentrations. In subjects aged 18–24 years, the median difference between prevaccination and postvaccination antitoxin concentrations (log transformed), at 0.97 IU/ml, increased to 1.60 IU/ml in the 50–70-year-old group, indicating a 9.3-fold (3.3–30.1) and 39.9-fold (9.1–107.2) increase in the antitoxin concentration, respectively. The influence of the factors of age and sex on the change in immunity due to the vaccination was estimated using linear regression models. The factor of age had a statistically significant influence on the change in immunity, indicating that at higher ages the change in antitoxin concentration was increased ($P<0.001$). In contrast, no sex differences in the effect of vaccination were observed ($P=0.86$). The median difference in males, at 1.14 IU/ml (0.70–1.61), was similar to the value of 1.15 IU/ml (0.60–1.75) in females (Table 3).

Adverse Reactions. Systemic reactions reported during the follow-up period were few and mild, consisting of fever >37.5°C (0.2%), headache (0.7%), nausea (0.4%), and inability to work (0.4%). Local reactions were more common but were also generally mild. Reactions of clinical significance, e.g. erythema and swelling with a diameter greater than 5 cm, were found in 10 of 534 vaccinations. In all cases, clinically significant reactions were transient and resolved without any specific medical attention or treatment.

Discussion

General recommendations for diphtheria immunization assume that lifelong immunity is necessary to prevent the recurrence of diphtheria [5, 6, 26, 27]. Children acquire high levels of diphtheria immunity as the result of infant immunization, which consists of a series of four doses of diphtheria toxoid [16, 28]. The level of immunity declines in late childhood and adolescence, depending on the schedule of immunization and the incidence of diphtheria [5, 26]. As diphtheria has become more rare and the number of carriers has diminished drastically, opportunities for acquiring or reinforcing natural immunity have been reduced [5]. In the absence of periodic administration of booster doses of diphtheria toxoid, adults become susceptible to diphtheria [1, 5, 6, 13]. To improve the level of diphtheria specific immunity within the adult population, the revaccination of injured patients using a combined diphtheria and tetanus vaccine (Td) instead of monovalent tetanus toxoid in cases of active immunization against tetanus is recommended [5, 13–16].

This study was designed to describe the effect of combined diphtheria and tetanus booster vaccination on diphtheria immunity within the adult population

and to evaluate whether combined revaccination as part of wound management could be performed without specific assessment of diphtheria immunity before the vaccination procedure. The probands were therefore selected according to their history of tetanus vaccination, and revaccination was carried out according to the national guidelines for the prevention of tetanus in injured patients. Subjects with completed basic immunization against tetanus and a last vaccination (booster or basic vaccination) between 5 and 10 years previously were included in the study. Information about past diphtheria immunization was obtained from 52.2% of the patients; 44.5% did not know if they had been vaccinated, and 3.3% stated that they had never received a diphtheria vaccination.

For epidemiological purposes, the minimum protective diphtheria antitoxin level is considered to be 0.01 IU/ml, providing a basic protection against the toxic manifestation of the disease. The higher level of 0.1 IU/ml is desirable for individual protection [4, 8, 14]. To achieve elimination and to prevent the spread of the disease by herd immunity, it is required that the minimum proportion with protective antibody levels should be 90% for children and 70–75% for adults [1, 5, 6].

Immunity against diphtheria is assessed by various techniques that differ in sensitivity, specificity, and feasibility [20, 29, 30]. For the present study, the time-effective and cost-effective EIA technique was found to have sufficient accuracy and was therefore used for all determinations of diphtheria antitoxin titers [18, 19, 31]. A number of reports have suggested that EIA may be less reliable for sera containing <0.1 IU/ml antitoxin, with a significant risk of false-positive interpretations of immunity [20, 32]. We therefore compared and verified the results of 195 randomly selected prevaccination serum samples in which lower concentrations were expected with the results of the in vitro neutralization assay, as recommended by previous studies [1, 20, 22, 30]. We found a high degree of agreement in the results, and for serum levels below 0.01 IU/ml, the sensitivity for the detection of false-positive determinations was very high (98.15%; 95% CI, 90.11–99.95).

In the present study, the overall proportion of susceptible persons before vaccination was 26.6%. This value is comparable with that in other studies showing that 20–60% of the adult population lacks protective serum antitoxin titers [6–12]. The rate of susceptibility increased with age, showing the highest value (46.3%) in the 40–50-year-old group. Among the female population, the overall prevalence of unprotected subjects (32.2%) was significantly higher when compared with that observed among males (24.1%). There have been conflicting reports in the literature stating that men are less protected than women, that there is little difference in immunity between the sexes; or that fewer women are protected than men [6, 8, 11]. This last observation

has been explained by booster immunization as a consequence of military service or by the administration of tetanus/diphtheria booster during treatment for traumatic injuries. No significant difference in the number of susceptible subjects was seen in relation to the country of previous immunization, with a prevalence of between 25% and 27.8%. Particularly among people from eastern European countries (those in this study having been born mainly in the former Yugoslavia), we observed no higher prevalence of unprotected patients. Another report identified an influence of the country of origin on diphtheria immunity and found especially that emigrants from the former USSR lacked protective antibody levels [12]. In the present study, no subjects from the former USSR were evaluated. In most European countries, general diphtheria vaccination became widespread around 1945–50, and similar vaccination schedules were used. The vaccines used and the differences in the national immunization schedules do not seem to be of significant importance for the prevalence of unprotected persons.

After the combined diphtheria and tetanus booster vaccination, the proportion of susceptible subjects was reduced from 27.1% to 3.9%. Antitoxin concentrations higher than 0.01 IU/ml were therefore achieved in more than 95% of the vaccinees. Secure individual protection with antitoxin concentrations >0.1 IU/ml was achieved in nearly 90% of the subjects. The median antitoxin concentration increased from 0.08 IU/ml before vaccination to 1.73 IU/ml after vaccination, indicating a median difference of 1.15 IU/ml, or a 14-fold increase. An elevation in the postvaccination titer of at least 4.4-fold was observed in 75% of the patients. Four percent of the vaccinees did not respond to the vaccination and showed postvaccination antitoxin levels of <0.01 IU/ml. This 4% corresponded well to the proportion of subjects who stated that they had never been vaccinated against diphtheria and who may have needed further diphtheria vaccination.

Following the booster vaccination, the proportion of susceptible subjects still increased with age, and when the antitoxin concentrations before and after vaccination were compared, the highest differences were found in older subjects. The median relative difference in the oldest age group of 1.60 IU/ml indicates a 40-fold increase in the antitoxin level compared with the other age groups, where the conversion factor attains only a 9- to 17-fold increase. Multiple regression analysis confirmed that in older subjects adjusted for sex and country of previous immunization, the effect of the vaccination on the change in antitoxin concentration increases. This result does not demonstrate a dependence of the protection rate and the efficacy of the vaccination relative to age. However, age does indicate generally a longer time interval since the previous vaccination. Therefore, with the waning of immunity over time, older people have lower antitoxin levels and

are more likely to be susceptible [11, 33]. These results indicate that the vaccine is highly effective in people with low antitoxin concentrations and that subjects with lower prevaccination antitoxin concentrations show a greater vaccination response.

Even after the booster vaccination, the number of unprotected females is still higher than the number of unprotected males. In this study, the effect of vaccination showed no sex-dependent difference. The median difference between the antitoxin level before and after vaccination in males, at 1.14 IU/ml, was similar to the value of 1.15 IU/ml in females, indicating a 14-fold median increase. We did not detect any statistically significant influence on the revaccination response in relation to the country of previous immunization.

In previous studies, the side effects of primary vaccination against diphtheria have been found to be more pronounced among adults than among children [6, 17, 34]. As a consequence, the dose of toxoid in the adult vaccine has been reduced. It was found subsequently that low doses (1–5 Lf) are sufficient both for primary vaccination and for revaccination of adults. In most countries, Td vaccine containing 2 Lf diphtheria toxoid is now recommended for adult revaccination every 5–10 years [6, 13, 14, 16, 20]. During the present study of 534 vaccinees, systemic reactions were observed in only 12 patients: one patient had a fever of more than 37.5 °C lasting for 3 days, and two other patients were unable to work. Reactions of clinical significance, such as local erythema and swelling (>5 cm in diameter), were observed in only ten patients. The analysis of diphtheria immunization using available patient histories revealed no relation between the occurrence of adverse reactions and the number of diphtheria vaccine doses received throughout the patient's life. Previous studies have demonstrated that diphtheria toxoid added to tetanus toxoid did not enhance side effects and that the manifestation of side effects after combined diphtheria–tetanus vaccination was mainly related to the tetanus component [17, 20, 35]. To evaluate the influence of the tetanus toxoid on the frequency of adverse reactions in the present study, we also determined the tetanus antitoxin concentration in those patients presenting with side effects. We detected high levels of tetanus antibodies in most of these patients. Further systematic analysis of all participating patients, together with statistical calculations, will provide conclusive data on the contribution of the tetanus toxoid to the occurrence of side effects.

This study provides information about the need and the efficiency of combining diphtheria revaccination with tetanus revaccination of injured adults in order to improve the level of diphtheria-specific immunity. Maintaining a high level of vaccination coverage in the adult population plays a key role in the control and elimination of diphtheria. Diphtheria immunity can be

improved markedly by a single dose of a combined diphtheria–tetanus toxoid during wound management for the prevention of tetanus. Revaccination of injured adults may be performed without specific assessment of diphtheria immunity before the vaccination procedure, and for public health purposes it is possible to obtain basic protection in more than 95% and secure individual protection in 90% of vaccinees. Such vaccination is also effective in older age groups with a long time interval since the last vaccination, and no sex-dependent difference in the efficacy is present. The improvement of diphtheria-specific immunity after combined revaccination is independent of the country of previous immunization. This intervention is simple, cost-effective, and is well accepted by patients. We hope that this report will contribute to the establishment of improved immunization policies and lead to the elimination of diphtheria throughout Europe.

References

1. Begg N: Manual for the Management and Control of Diphtheria in the European Region. The Expanded Programme on Immunization in the European Region of WHO, ICP/EPI 038 (B). WHO, Copenhagen (1994) pp 1–16
2. Anonymous: Expanded programme on immunization: diphtheria epidemic in the Newly Independent States of the former USSR, 1990–1994. Weekly Epidemiological Record (1995) 70:141–144
3. Vitek CR, Wharton M: Diphtheria in the former Soviet Union: reemergence of a pandemic disease. Emerging Infectious Diseases (1998) 4:539–550
4. Rakhmanova AG, Lumio J, Groundstroem K, Valova E, Nosikova E, Tanasijchuk T, Saikku J: Diphtheria outbreak in St. Petersburg: clinical characteristics of 1860 adult patients. Scandinavian Journal of Infectious Diseases (1996) 28:37–40
5. Galazka AM, Robertson SE: Diphtheria: changing patterns in the developing world and the industrialized world. European Journal of Epidemiology (1995) 11:107–117
6. Simonsen O: Vaccination against tetanus and diphtheria: evaluations of immunity in the Danish population, guidelines for revaccination, and methods for control of vaccination programs. Danish Medical Bulletin (1989) 36:24–47
7. Anonymous: Diphtheria acquired by U.S. citizens in the Russian Federation and Ukraine, 1994. Morbidity and Mortality Weekly Report (1995) 44:237, 243–244
8. Maple PA, Efstratiou A, George RC, Andrews NJ, Sesardic D: Diphtheria immunity in UK blood donors. Lancet (1995) 345:963–965
9. Comodo N, Bonanni P, Lo Nostro A, Tiscione E, Mannelli F, Tomei A: Low prevalence of diphtheria immunity in the population of Florence, Italy. European Journal of Epidemiology (1996) 12:251–255
10. Gupta RK, Griffin P Jr, Xu J, Rivera R, Thompson C, Siber GR: Diphtheria antitoxin levels in US blood and plasma donors. Journal of Infectious Diseases (1996) 173:1493–1497
11. Hasselhorn HM, Nubling M, Tiller FW, Hofmann F: Factors influencing immunity against diphtheria in adults. Vaccine (1998) 16:70–75
12. Low M, Almog R, Green MS, Ashkenazi S, Bercovier H, Katzenelson E, Ashkenazi I, Shemer J, Cohen D: Immune status against diphtheria among immigrants from the former USSR who arrived in Israel during 1990–1991. Infection (1998) 26:104–108
13. Ipsen J: Immunization of adults against diphtheria and tetanus. New England Journal of Medicine (1954) 251:459–466
14. Karzon DT, Edwards KM: Diphtheria outbreaks in immunized populations. New England Journal of Medicine (1988) 318:41–43
15. Dittmann S, Roure C: Diphtheria. Plan of action for the prevention and control of diphtheria in the European region (1994–1995). The Expanded Programme on Immunization in the European Region of WHO, ICP/EPI 038 (A). WHO, Copenhagen (1994) pp 1–21
16. Centers for Disease Control and Prevention. Immunization of adolescents: recommendations of the Advisory Committee on Immunization Practices, the American Academy of Pediatrics, the American Academy of Family Physicians, and the American Medical Association. Morbidity and Mortality Weekly Report (1996) 45:1–16
17. Bjorkholm B, Granstrom M, Wahl M, Hedstrom CE, Hagberg L: Adverse reactions and immunogenicity in adults to regular and increased dosage of diphtheria vaccine. European Journal of Clinical Microbiology (1987) 6:637–640
18. Melville-Smith M, Balfour A: Estimation of *Corynebacterium diphtheriae* antitoxin in human sera: a comparison of an enzyme-linked immunosorbent assay with the toxin neutralisation test. Journal of Medical Microbiology (1988) 25:279–283
19. Ambrosch F, Wiedermann G, Müller H: A new ELISA micromethod for the determination of tetanus antibodies. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene (1984) 258:173–182
20. Aggerbeck H: Vaccination against diphtheria and tetanus: assays, antigens, adjuvants and application. Acta Pathologica, Microbiologica et Immunologica Scandinavica (1998) 106, Supplement 81:1–40
21. Miyamura K, Nishio S, Ito A, Murata R, Kono R: Micro cell culture method for determination of diphtheria toxin and antitoxin titres using VERO cells, I: studies on factors affecting the toxin and antitoxin titration. Journal of Biological Standardization (1974) 2:189–201
22. Efstratiou A: Diphtheria. Laboratory diagnosis of diphtheria. The Expanded Programme on Immunization in the European Region of WHO, ICP/EPI 038 (C). WHO, Copenhagen (1994) pp 15–16
23. Cohen J: Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. Psychological Bulletin (1968) 70:213–220
24. Parish HJ, Edin MRCP, Wright J: The Schick reaction and circulating diphtheria antitoxin in man. Lancet (1938) 1:882–886
25. Bjorkholm B, Bottiger M, Christenson B, Hagberg L: Antitoxin antibody levels and the outcome of illness during an outbreak of diphtheria among alcoholics. Scandinavian Journal of Infectious Diseases (1986) 18:235–239
26. Jensen C: Antitoxin curve in children after active immunization with diphtheria anatoxin with special reference to the duration of the antitoxin immunity. Acta Pathologica et Microbiologica Scandinavica (1933) 10:137–158
27. Kjeldsen K, Simonsen O, Heron I: Immunity against diphtheria and tetanus in the age group 30–70 years. Scandinavian Journal of Infectious Diseases (1988) 20:177–185
28. Ajello F, Lampiasi AM, Candido G, D'Angelo C: Circulating diphtheria antitoxin levels in children aged 11–14 years in relation to the vaccinal history. Vaccine (1991) 9:502–504

29. Kriz B, Sladky K, Burianova-Vysoka B, Mottlova O, Roth Z: Determination of diphtheria antitoxin in guinea-pig sera by the Jensen and tissue-culture methods. Journal of Biological Standardization (1974) 2:289–295

30. Popovic T, Wharton M, Wenger JD, McIntyre L, Wachsmuth IK: Are we ready for diphtheria? A report from the Diphtheria Diagnostic Workshop, Atlanta, 11 and 12 July 1994. Journal of Infectious Diseases (1995) 171:765–767

31. Camargo ME, Silveira L, Furuta JA, Oliveira EP, Germek OA: Immunoenzymatic assay of anti-diphtheric toxin antibodies in human serum. Journal of Clinical Microbiology (1984) 20:772–774

32. Maple PA, George RC, Miller E, Morgan-Capner P, Hayward P: Diphtheria immunity in adults. Lancet (1996) 348:964

33. Galazka AM, Robertson SE, Oblapenko GP: Resurgence of diphtheria. European Journal of Epidemiology (1995) 11:95–105

34. Roberts JD, Roos LL, Poffenroth LA, Hassard TH, Bebchuk JD, Carter AO, Law B: Surveillance of vaccine-related adverse events in the first year of life: a Manitoba cohort study. Journal of Clinical Epidemiology (1996) 49:51–58

35. Trinca JC: Combined diphtheria–tetanus immunization of adults. Medical Journal of Australia (1975) 2:543–546