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Primary Hepatitis A vaccination failure is a rare although possible event: results of a retrospective study

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Abstract

A case of Hepatitis A occurred in a traveller in spite of a complete course of immunization with a combined HAV and HBV vaccine [Taliani G, Sbaragli S, Bartoloni A, Santini MG, Tozzi A, Paradisi F. Hepatitis A vaccine failure: how to treat the threat. Vaccine 2003;21(31):4505–6]. A retrospective study was performed to evaluate whether the failure was primary or could be attributed to a specific lot of vaccine or to its inadequate handling and/or storage. Two distinct populations of vaccinees were selected in a 1:2 proportion. The case group ($N=31$) included subjects who were vaccinated in the same period and with the same lot and batch of vaccine as the case. The control group ($N=62$) included subjects who received different lot and batch of the same vaccine as the case group. A persisting antibody response to HAV vaccine was found among all subjects (anti-HAV >20 mIU/ml). The overall anti-HBs seropositivity rate (anti-HBs >10 mIU/ml) was 74%, without significant difference between the case (77%) and the control group (73%; $P>0.05$). The reported Hepatitis A case can be attributed to a rare primary vaccine failure rather than to inefficacy of a specific lot of vaccine or to inappropriate vaccine handling or storage. Our study supports the indications for use of combined Hepatitis A + B immunization in travellers at risk for both infections, but stresses the need for information on correct hygienic behaviours while abroad.

Keywords: Hepatitis A and B combination vaccine; Vaccine failure; Protection; Travel

1. Introduction

Hepatitis A infection has a worldwide diffusion and represents a relevant public health concern. It is well known that one of the major risk factor for acquiring Hepatitis A infection is represented by travelling to highly endemic areas [2–4]. Although general hygienic measures play a basic role for prevention, the protection conferred by vaccination is of major importance and immunization against Hepatitis A is recommended for those persons who travel to areas of high or intermediate endemicity [5,6].

The existence of a combined Hepatitis A and Hepatitis B vaccine, approved since 1997 in Italy for both adults and children and since 2001 [7] by the US Food and Drug Administration (FDA) for persons aged 18 years or older (≥16 years in Europe) [8], gives the opportunity to protect against both hepatitis infections with a reduced total number of injections which ensures a higher compliance towards immunization practices. In addition, it implies minimal loss of time for travellers and health care workers (HCWs) and it provides a persistent immunity [9,10] comparable to the protection obtained from monovalent vaccines given separately.

Despite high vaccine effectiveness, at least four cases of primary vaccine failure were described in the international

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literature [1,11,12]. One of these cases occurred in a man who had travelled to an endemic area 2 years after completion of the vaccination course with three doses of combined HAV and HBV vaccine [1]. This case prompted us to perform a retrospective case-control study in order to examine whether the failure could be attributed to the use of a specific lot of vaccine or to inadequate handling and/or storage of the vaccine at the vaccination Centre.

2. Materials and methods

A retrospective study was performed to evaluate whether the failure was primary or could be attributed to a specific lot of vaccine or to its inadequate handling and/or storage.

All subjects included in the study were vaccinated at the "Centre for Travel Medicine and Migrations" of the Public Health and Hygiene Service, Local Health Agency, Florence. The selection of subjects (case group and control group) according to the inclusion criteria was performed through the database currently employed for the management of the vaccination service.

The first step was to identify the batches of HAV/HBV vaccine (Twinrix®, GlaxoSmithKline, Rixensart, Belgium) administered to the vaccine failure case. The first two doses belonged to the same batch (HAB 154 C6 – Expiry date: 30 November 2001) while the third dose belonged to another batch (HAB 168 B – Expiry date: 31 May 2002). The subject received a complete course of three doses, starting in November 1999 and he completed the vaccination course in July 2000.

In order to evaluate the immunogenicity of the batches used for the case, we recalled people who had been immunized in the same period (±45 days), with the same batches and with an identical schedule.

The control group consisted of vaccinees who had been administered different batches of the same vaccine. Necessarily, the study design implied that controls had received the vaccine in a different period. The chosen period corresponded to 12 months before vaccination of the Hepatitis A case.

2.1. Case group inclusion

Subjects of the so-called 'case group' were chosen among a population of 274 vaccinees who received the first dose between October 1999 and December 1999 (first dose of the case ±45 days). Among these, 195 subjects received the second dose between November 1999 and January 2000 (second dose of the case ±45 days), and 115 subjects received the third dose between June 2000 and August 2000 (third dose of the case ±45 days). Among these 115 identified subjects, we randomly selected 40 people (35% of the sample). Nine vaccinees refused to participate in the study, thus we finally obtained a serum sample from 31 randomly selected subjects which corresponded to 1/3 of the identified vaccinees (Fig. 1).

2.2. Controls inclusion

The rationale for choosing the control group was to assess the immunogenicity of a different batch of the vaccine administered in the same Centre with the same schedule as the Hepatitis A case. We identified two batches of Twinrix® that were used 12 months before the batches administered to the case and we chose controls among people who received the first two doses with the first identified batch (HAB 123 B6 – Expiry date: 31 January 1999) and the third dose with the sec-

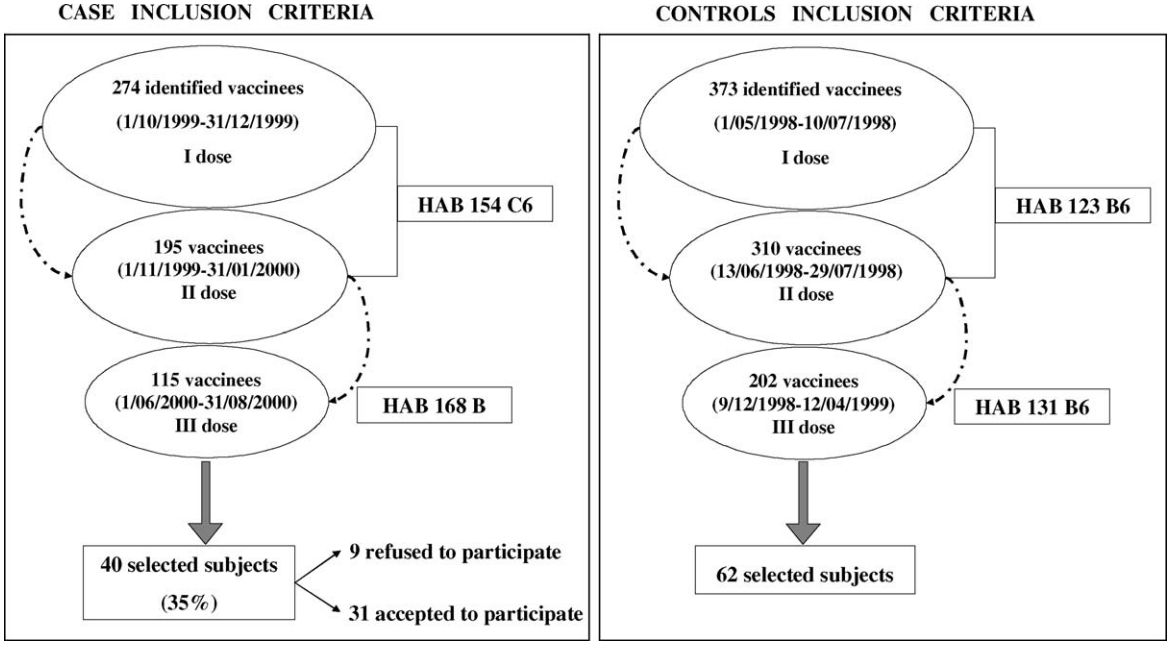

Fig. 1. Case and control inclusion criteria flow-chart. The batches of Twinrix® administered to the subjects are also reported.

ond batch (HAB 131 B6 – Expiry date 31 October 1999). A population of 373 people who received the first dose between May 1998 and June 1998 was identified. Among these subjects, 202 vaccinees were immunized with the second dose 1 month later and received the third dose after 6 months. Out of this sample we randomly selected 62 subjects (Fig. 1).

All subjects were contacted by phone. Written, informed consent was obtained from those vaccinees who accepted to participate in the study and to undergo blood testing. The blood samples, collected from September 2002 to March 2003, were frozen, stored in aliquots at −20 °C and tested simultaneously.

3. Laboratory methods and statistics

Qualitative/quantitative determinations of antibodies to HAV (ETI-AB-HAVK-3), to Hepatitis B core (anti-HBc, ETI-AB-COREK-2) and to Hepatitis B surface (anti-HBs, ETI-AB-AUK-3) antigens were performed with DiaSorin kits (DiaSorin s.r.l. Saluggia, Vercelli, Italy). All the assays are based on the ELISA technique (enzyme-linked immunosorbent assay). In particular, the method for quantitative anti-HAV determination is a competitive binding assay, the method for quantitative determination of anti-HBs is a direct, non-competitive sandwich assay, while the method for qualitative anti-HBc determination is a simultaneous competitive assay.

Samples with a concentration of anti-HAV greater than 20 mIU/ml were considered positive. Due to the objective of the study, no further determination of precise anti-HAV titre was performed (upper detection limit of the assay: 70 mIU/ml).

The cut-off value for anti-HBs was 10 mIU/ml. Only positive titres of anti-HBs (≥10 mIU/ml) were used in the calculation of the geometric mean titres (GMTs).

The GraphPad InStat Software (GraphPad Software Inc., San Diego, California) was used for statistical analysis of data. InStat gives descriptive statistics as mean, standard deviation, range and confidence interval for each group of values considered. The unpaired *t*-test was applied to compare the mean age of the two groups, assuming that data are sampled from Gaussian populations and that the two populations have the same variance (and thus the same standard deviation).

4. Results

The demographic characteristics of the two groups are reported in Table 1.

There were no statistically significant differences in the composition of the two groups as far as age or gender were concerned ($P > 0.05$ in both cases).

Due to the study design, the mean interval, in months, between administration of the last vaccine dose and anti-body testing was significantly longer among the control (45.2 ± 1.3) compared to the case group (28.2 ± 1.8).

Table 1
Demographic characteristics of case group and control group (*n*, number of subjects)

Group	*n*	Mean age (years)	Age range (years)		
Case					
Female	14	41.6	24–62	$P = 0.6826$	
Male	17	39.5	25–64		
Total	31	40.4	24–64		$P = 0.0876$
Control					
Female	31	34.8	20–63	$P = 0.4330$	
Male	31	37.0	24–60		
Total	62	35.9	20–63		

4.1. Anti-HAV

Determination of anti-HAV antibody titre showed that all vaccinees in both groups were anti-HAV positive (≥20 mIU/ml), which indicates a 100% rate of response to the vaccine. All serum samples belonging to the case group, and all but two samples in the control group (titres of anti-HAV: 50 and 64 mIU/ml, respectively) had anti-HAV titres ≥70 mIU/ml (Table 2).

4.2. Anti-HBV

The rates of anti-HBs ≥10 mIU/ml in the case and the control group were 77.4% and 72.6%, respectively ($P = 0.615$). Females were more frequently positive compared to males, among both the case and the control group, although the difference was significant only among controls, probably due to the small size of the case group (Table 2).

Stratification of anti-HBs seropositivity rate by age showed no difference within and between groups (Table 3).

Geometric mean titres (GMTs) of anti-HBs and 95% confidence intervals (95% CI) for case and control groups

Table 2
Seropositivity rate (SR) of anti-HAV and anti-HBs (*n*, number of subjects)

Group	*n*	Anti-HAV positive		Anti-HBs positive			
		n	SR (%)	*n*	SR (%)		
Case							
Female	14	14	100	14	100	$P = 0.5651$	
Male	17	17	100	10	58.8		
Total	31	31	100	24	77.4		$P = 0.6151$
Control							
Female	31	31	100	28	90.3	$P = 0.0017$	
Male	31	31	100	17	54.8		
Total	62	62	100	45	72.6		

Table 3
Seropositivity rate (SR) of anti-HBs stratified by age group (*n*, number of subjects)

	Case			Control		
	n	Anti-HBs+	SR (%)	*n*	Anti-HBs+	SR (%)
Age (years)						
<40	18	14	78	42	31	74
≥40	13	10	77	20	14	70

stratified by gender and age are reported in Table 4. Titres of anti-HBs in women were higher than in men, but this difference was significant neither in the case group ($P = 0.917$) nor in the control group ($P = 0.1978$). Besides, we compared GMT of cases and controls by age and even in this case no statistically difference was found.

All subjects belonging to the case group were negative to antibodies against HBc, while in the control group a 32-year-old-woman (anti-HBs titre 16,500 mIU/ml) and a 59-year-old-man (anti-HBs titre 59 mIU/ml) resulted anti-HBc positive (3.2%), which suggests the occurrence of HBV infection. We do not know the serologic profile of these two subjects at the time of vaccine administration, therefore we are not able to firmly discriminate between a condition of vaccine failure followed by HBV infection or vaccination of two subject who had spontaneously cleared HBV infection before vaccination.

5. Discussion

It is estimated that each year people who travel from Italy to foreign countries are about 18 million [13] and many of them leave to tropical areas where Hepatitis A and B are endemic.

Recently, an Italian epidemiological study indicated that among 17,039 cases of acute Hepatitis A observed during the period 1996–2000, 1519 (15.5%) reported recent travel to an endemic area as a risk factor, with an OR of 4.11 (95% CI 3.53–4.79) which was considerably higher than the OR of any other risk factor [14]. Consequently, vaccination against Hepatitis A infection is strongly recommended for travellers to endemic areas.

Our study was prompted by the observation of a vaccine failure represented by acute Hepatitis A in spite of a correct immunization scheme in a traveller returning from a hyperendemic country. By examining the seropositivity rates obtained in 31 vaccinees after administration of the same vaccine lot and batch during an identical time interval as the case, we found that the combined HAV and HBV vaccine induced a 100% immunization rate against HAV. Therefore, the occurrence of the failure was not attributable to the vaccine (production, storage, administration), but was most probably due to an episode of individual non-responsiveness to HAV immunization. This hypothesis was reinforced by the observation that also a different lot and batch of the same vaccine administered to control subjects induced an immunization rate of 100%. Seroprotection against HAV was fairly well detectable 32 and 48 months after administration of the last vaccine dose in the case and the control groups, respectively, with anti-HAV levels clearly above the threshold level of 20 mIU/ml in all vaccinees. These results are in accordance with previous studies reporting that protective levels of anti-HAV are maintained in all responder subjects 48 months after completion of vaccination course [15].

On the other hand, in our group of vaccinees, the administration of the combined HAV and HBV vaccine gave an anti-HBs seropositivity level well under 100% after the same time intervals, most appreciable among males. The lower rate of anti-HBs seropositivity among males after a correct immunization course suggests either a lower initial seroconversion rate and/or a more rapid loss of antibodies compared to females. This was previously reported in the international literature [16], but the large difference recorded in this study is probably due to the small sample size. Furthermore, a lower number of anti-HBs reactive subjects does not necessarily mean lack of protection against HBV infection in those subjects, because Hepatitis B vaccine induces a strong immunologic memory which is activated by the contact with the virus or a booster dose even after 10 years from primary vaccination [17]. It is interesting to note that we did not find any significant difference of response

Table 4
Geometric mean titres (GMTs) of anti-HBs and 95% confidence intervals (95% CI) for case and control groups stratified by gender and age (*n*, number of subjects)

	Case			Control		
	n	GMT	(95% CI)	*n*	GMT	(95% CI)
Gender						
Female	14	145.83	(62.66–339.63)	28	342.86	(181.13–648.63)
Male	10	136.66	(45.39–411.15)	17	179.33	(80.54–399.02)
Age (years)						
<40	14	164.53	(76.91–352.37)	31	305.9	(162.93–574.12)
≥40	10	115.43	(34.28–389.05)	14	200.88	(88.72–454.99)
Total	24	141.94	(76.56–263.63)	45	268.40	(164.82–437.52)

to HBV vaccine according to age. This result is in contrast with what is reported in a retrospective study showing that 18 months after vaccination with a combined HAV and HBV vaccine, the rate of anti-HBs positivity was significantly higher in people aged less than 40 compared to older people [18], the most probable explanation for this difference being related to the small size of our study population.

In conclusion, the present study showed that Twinrix® provides long-lasting antibody responses to HAV antigen among adults aged between 20 and 64 years, while low anti-HBs seropositivity rates detected in males in this study should be interpreted with caution and need further investigations. Although rare, primary vaccine failure to HAV vaccine is a possible event probably linked to individual inability to mount an appropriate, specific immune response to the vaccine components. Thus, vaccination may not be synonymous to absolute guarantee of protection and correct counselling, consisting in the recommendation of continuous adherence to the universal safety measures for the prevention of infections, should be strongly advised also to vaccinated people.

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References

[1] Taliani G, Sbaragli S, Bartoloni A, Santini MG, Tozzi A, Paradisi F. Hepatitis A vaccine failure: how to treat the threat. Vaccine 2003;21(31):4505–6.

[2] Franco E, Giambi C, Ialacci R, Coppola RC, Zanetti AR. Risk groups for Hepatitis A virus infection. Vaccine 2003;21:2224–33.

[3] Mele A, Stroffolini T, Palumbo F, et al., the SEIEVA Collaborating Group. Incidence of and risk factors for Hepatitis A in Italy: public health indications from a 10-year surveillance. J Hepatol 1997;26:743–7.

[4] Khuroo MS. Viral hepatitis in international travellers: risks and prevention. Int J Antimicrob Agent 2003;21:143–52.

[5] Steffen R, Kane MA, Shapiro CN, Billo N, Schollhorn KL, Van Damme P. Epidemiology and prevention of Hepatitis A in travellers. JAMA 1994;272:885–9.

[6] WHO. Public health control of Hepatitis A: memorandum from a WHO meeting. Bull World Health Org 1995;73:15–20.

[7] Spira AM. A review of combined Hepatitis A and Hepatitis B vaccination for travellers. Clin Ther 2003;25(9):2337–51.

[8] Anonymous. FDA approval for a combined Hepatitis A and B vaccine. Notice to readers: FDA approval for a combined Hepatitis A and B vaccine. MMWR Morb Mortal Wkly Rep 2001;50(37):806–7.

[9] Van Damme P, Van Herck K. A review of the efficacy, immunogenicity and tolerability of a combined Hepatitis A and B vaccine. Expert Rev Vaccines 2004;3(3):249–67.

[10] Van Damme P, Leroux-Roels G, Law B, Diaz-Mitoma F, Desombere I, Collard F, et al. Long-term persistence of antibodies induced by vaccination and safety follow-up, with the first combined vaccine against Hepatitis A and B in children and adults. J Med Virol 2001;65(1):6–13.

[11] Ellliot JH, Kunze M, Torresi J. Hepatitis A vaccine failure. Lancet 2002;359:1948–9.

[12] Junge U, Melching J, Dziuba S. Acute Hepatitis A despite regular vaccination against Hepatitis A and B. Deut Med Wochenschr 2002;127(30):1581–3.

[13] Ministero della Salute. http://www.ministerosalute.it/promozione/malattie/sezMalattie.jsp?label=inf.

[14] Ciccozzi M, Tosti ME, Gallo G, et al., the SEIEVA Collaborating Group. Risk of Hepatitis A infection following travel. J Viral Hepatitis 2002;6:460–5.

[15] Thoelen S, Van Damme P, Leentvaar-Kuypers A, Leroux-Roels G, Bruguera M, Frei PC, et al. The first combined vaccine against Hepatitis A and B: an overview. Vaccine 1999;17(13/14):1657–62.

[16] Averhoff F, Mahoney F, Coleman P, Schatz G, Hurwitz E, Margolis H. Immunogenicity of Hepatitis B vaccines. Implications for persons at occupational risk of Hepatitis B virus infection. Am J Prev Med 1998;15(1):1–8.

[17] Floreani A, Baldo V, Cristofoletti M, Renzulli G, Valeri A, Zanetti C, et al. Long-term persistence of anti-HBs after vaccination against HBV: an 18 year experience in health care workers. Vaccine 2004;22(5/6):607–10.

[18] Wolters B, Junge U, Dziuba S, Roggendorf M. Immunogenicity of combined Hepatitis A and B vaccine in elderly persons. Vaccine 2003;21(25/26):3623–8.