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Seroprevalence and vaccination coverage of vaccine-preventable diseases in perinatally HIV-1-infected patients

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Background. Even in the era of highly active antiretroviral therapy (HAART), HIV-infected subjects are at higher risk of complications from vaccine-preventable diseases than those uninfected. The current international guidelines strongly recommend that these patients should receive all the routine childhood vaccinations. Although these children represent an appropriate target for immunization, the available data indicate suboptimal coverage rates.

Methods. To evaluate seroprotection/seropositivity rates and vaccination coverage against the common vaccine-preventable diseases, all patients with vertically transmitted HIV-1 infection who attended San Martino Hospital were enrolled. Blood samples were collected for testing antibodies against diphtheria, tetanus, hepatitis A and B viruses by Enzyme-Linked ImmunoSorbent Assay and polioviruses by microneutralization test. In order to assess immunization coverage, retrospectively was recorded the vaccination history collecting data from Regional Immunization Database.

Results. A total of 39 perinatally HIV-1 infected patients were included in the study. At the time of serum was obtained, the mean age was 18,1 years (range: 6–28). The median CD4+ T-lymphocyte count was 702 cells/mm^3 (2–1476 cells/mm^3). Twenty-nine (74.4%) patients were found with HIV RNA load < 50 copies/mL. The proportion of subjects with protective anti-tetanus and anti-HBs were 43.6% and 30.8%, respectively. Seroprotection rates about 20% against rubella and measles were found, less than 20% against all the other antigens investigated. In particular, all patients resulted susceptible to mumps.

High immunization rates were observed for polio and HBV (100% and 92.3%, respectively) and suboptimal for diphtheria-tetanus (84.6%). For the other recommended vaccines the rates were generally low. None of the patients received varicella vaccine doses.

Conclusions. As in the HAART era the vertically acquired HIV infection has become a chronic treatable disease, the vaccine-induced long-term protection plays an increasingly significant role; despite good initial response to primary vaccination, subsequent decline and loss of detectable antibodies may be prevented by additional strategies for booster doses of vaccines in adolescents and young adults.

Introduction

Even in the era of Highly Active Antiretroviral Therapy (HAART), HIV-infected subjects are at higher risk of complications from vaccine-preventable diseases than those uninfected.[1-4] The current national and international guidelines strongly recommend that these patients should receive all the routine childhood vaccinations.[5-9] Although these children represent an appropriate target group for immunization, the available data indicate suboptimal coverage rates, partially due to the physician uncertainty regarding the time-course of the immune reconstitution, the optimal timing of vaccination after starting HAART, and the safety profile in such patients.[5,10-12]

Moreover, for HIV-infected individuals, appropriate strategies are crucial for identifying those susceptible to preventable infections and prone to severe complications, and for ensuring them adequate protective immunity through the specific immunization schedules in terms of timing and number of vaccine doses rather than undergoing revaccination upon failure of immunization.[13]

Recently, the Pediatric European Network for Treatment of AIDS (PENTA) provided guidance on how routine immunization should be modified for HIV positive children living in Europe, in order to optimize vaccination recommendations for this specific high-risk group.[5]

The aims of our study have been to describe the immunization coverage and to evaluate the seroprotection/seropositivity rates of

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the common vaccine-preventable diseases in perinatally HIV-1-infected children, adolescents and young adults followed up at the referral outpatient clinic of Infectious Diseases in the Liguria Region, Northern Italy.

Methods

Study design and population

This is a cross-sectional study in which we recruited all patients with vertically transmitted HIV-1 infection who attended San Martino Hospital, a regional reference hospital in Liguria.

Blood samples were collected for testing antibodies against diphtheria, tetanus, hepatitis A (HAV), hepatitis B (HBV) viruses and polioviruses. To define serologic evidence of immunity, prevalence of mumps seropositivity (correlate of protection have not been established) and prevalence of seroprotection for other antigens were estimated.

CD4 lymphocyte counts and HIV-1 RNA plasma level determinations were also performed. Other relevant clinical, serological, biochemical and laboratory parameters were registered from the medical records.

In order to assess immunization coverage, defined as the proportion of subjects who received all the required vaccine doses, as recommended by the Italian vaccination schedule, we retrospectively recorded the vaccination history collecting data from Regional Immunization Database.

All patients provided written informed consent according to local procedures of the clinical center and to the 1975 Declaration of Helsinki.

Serological assay for diphtheria, tetanus, HBV and HAV

The antibody status against diphtheria, tetanus, hepatitis A and B viruses was determined using commercial Enzyme-Linked ImmunoSorbent Assay (ELISA) kits, following the manufacturer's instructions: *Corynebacterium diphtheriae* NovaLisa TM (NovaTec Immunodiagnostics, GmbH), *Clostridium tetani* enzyme immunoassay NovaLisa TM (NovaTec Immunodiagnostics, GmbH), Eti-AB-HAVK PLUS (DiaSorin, Italy), Monolisa TM Anti-HBs PLUS (Bio-Rad, France) and HBsAb (Dia.Pro. Diagnostics Bioprobes Srl).

The antibody thresholds used as serological correlates for protection were defined as antibody concentrations ≥ 0.1 IU/ml for diphtheria- and tetanus-toxoids, ≥ 10 mIU/ml for anti-HBs level[5] and ≥ 10 mIU/ml for HAV.[14]

Assay for polioviruses antibody-neutralisation titres

Antibodies against polioviruses were measured by microneutralization assay using cell culture HEP-2 (Human Epidermoid cancer cells) formerly recommended for routine use in accordance with the World Health Organization (WHO) method.[15] 2-fold serial dilutions were performed after serum samples inactivation at 56 °C for 30 min, then were incubated for 1 h at 37 °C with a virus suspension (100TCID 50) of each virus in a 96 well microcultured plate. Subsequently, a cells suspension was added to each well and for the next six days the appearance of cytopathic effect (CPE) was examined, by a standard microscope. Cellular obliteration and plaque formation, indicated a low neutralizing antibody concentration; cells are protected where antibody titer was ≥ 1:8.[16] Then, the plates are fixed and serum absorbency was evaluated on an ELISA reader.

Statistical analysis

The baseline characteristics of the study population were compared using χ^2 and Fisher exact test and the differences in numeric variables were evaluated with ANOVA test for normalized distributed variables as appropriate.

All statistical analyses were performed using the software *Epi Info*®, created by the Centers for Disease Control and Prevention (CDC) in Atlanta (USA). A *P* value of less than 0.05 was considered to be significant.

Results

Seroprotection/seropositivity rates for the main vaccine-preventable diseases according to the baseline characteristics of the study population are shown in **Table 1**. A total of 39 perinatally HIV-1 infected patients were included in the study, 19 (48.7%) of which were males. At the time of serum was obtained, the mean age was 18,1 y (range: 6–28). The median CD4+ T-lymphocyte count and the median percentage of CD4+ T-lymphocyte were 702 cells/mm^3 (2–1476 cells/mm^3) and 32.4% (0–48.3%), respectively. The mean HIV RNA level at the same time was 24,634 copies/mL (2–564,300 copies/mL). Twenty-nine (74.4%) patients were found with HIV RNA load < 50 copies/mL. Thirty-seven (94.8%) were on HAART and 1 (2.5%) on monotherapy (Lamivudine).

The proportion of subjects with protective anti-tetanus and anti-HBs were 43.6% and 30.8%, respectively. Seroprotection rates about 20% against rubella and measles were found, less than 20% against all the other antigens investigated. In particular, all patients resulted susceptible to mumps (**Table 1**).

Coverage for recommended immunizations is reported in **Table 2**. A satisfactory profile for the mandatory vaccines was observed: high immunization rates for polio and HBV (100% and 92.3%, respectively) and suboptimal for diphtheria-tetanus (84.6%), even though the 15% of the patients not completed the immunization schedule. For the other recommended vaccines the rates were generally low. None of the patients received varicella vaccine doses.

Discussion

In the present study we estimated the immunization coverage and the prevalence of immunity among patients perinatally infected with HIV-1, relative to the vaccines included in the current Italian recommendations to the entire population and to some high risk groups only, including HIV infection.[7] With the exception of the satisfactory coverage for polio and HBV and suboptimal for diphtheria and tetanus (only about the 70% completed course of vaccination), immunization rates for other vaccines were generally low. However, our study population is not homogeneous, because patients recruited in this study represent

Table 1. Seroprotection/seropositivity rates of vaccine-preventable diseases, according to baseline characterics of the study population

	POS	NEG	TOT	P value
MEASLES				
Number (%)	8 (20.5)	31 (79.5)	39 (100)	
Mean age (range)*	19 (12–28)	17,8 (6–25)	18,1 (6–28)	0,55
Gender (% of males)	50%	48,4%	48,7%	0,62
VL < 50*	71%	87.5%	74,4%	0,32
CD4 > 500*	**100%**	**58,1%**	**66,7%**	**0,02**
CD4% ≥ 25*	100%	66,7%	73,7%	0,06
Nadir CD4 (mean) (range)	356,3 (14–917)	400,3 (4–1269)	391,23 (4–1269)	0,69
MUMPS				
Number (%)	0 (0)	39 (100)	39 (100)	
RUBELLA				
Number (%)	10 (26.6)	29 (74.4)	39 (100)	
Mean age (range)*	**15,1 (6–21)**	**19,1 (6–28)**	**18,1 (6–28)**	**0,02**
Gender (% of males)	50%	48,3%	48,7%	0,61
VL < 50*	80%	72,4%	74,4%	0,49
CD4 > 500*	80%	62,1%	66,7%	0,26
CD4% ≥ 25*	70%	75%	73,7%	0,53
Nadir CD4 (mean) (range)	520,2 (14–917)	346,8 (4–1269)	391,23 (4–1269)	0,08
POLIOVIRUS TYPE 1				
Number (%)	7 (17.9)	32 (82.1)	39 (100)	
Mean age (range)*	19,1 (13–23)	17,8 (6–28)	18,1 (6–28)	0,52
Gender (% of males)	42,9%	50%	48,7%	0,53
VL < 50*	57,1%	78,1%	74,4%	0,24
CD4 > 500*	42,9%	71,9%	66,7%	0,15
CD4% ≥ 25*	71,4%	74,2%	73,7%	0,61
Nadir CD4 (mean) (range)	284,3 (4–427)	414,6 (14–1269)	391,23 (4–1269)	0,26
POLIOVIRUS TYPE 2				
Number (%)	6 (15.4)	33 (84.6)	39 (100)	
Mean age (range)*	18,8 (13–23)	17,9 (6–28)	18,1 (6–28)	0,68
Gender (% of males)	33,3%	51,5%	48,7%	0,36
VL < 50*	66,7%	75,8%	74,4%	0,49
CD4 > 500*	50%	69,7%	66,7%	0,31
CD4% ≥ 25*	83,3%	71,9%	73,7%	0,49
Nadir CD4 (mean) (range)	284,2 (4–427)	410,7 (14–1269)	391,23 (4–1269)	0,30
POLIOVIRUS TYPE 3				
Number (%)	4 (10.3)	35 (89.7)	39 (100)	
Mean age (range)*	17,8 (13–23)	18,1 (6–28)	18,1 (6–28)	0,88
Gender (% of males)	50%	48,6%	48,7%	0,68
VL < 50*	50%	77,1%	74,4%	0,27
CD4 > 500*	50%	68,6%	66,7%	0,41

all subjects that vertically acquired HIV-1 infection in Liguria region, during a period of about three decades. This is to be taken into account in the interpretation of our results because the Italian immunization strategy has changed during time and new combined vaccine formulations have become available commercially. For example, only the 43% of our patients has been immunized

Table 1. Seroprotection/seropositivity rates of vaccine-preventable diseases, according to baseline characterics of the study population (continued)

	POS	NEG	TOT	P value
POLIOVIRUS TYPE 3				
CD4% ≥ 25*	75%	73,5%	73,7%	0,72
Nadir CD4 (mean) (range)	234,5 (4–324)	409,1 (14–1269)	391,23 (4–1269)	0,23
DIPHTERIA				
Number (%)	5 (12.8)	34 (87.2)	39 (100)	
Mean age (range)*	**12,0 (6–15)**	**18,9 (6–28)**	**18,1 (6–28)**	**0,00**
Gender (% of males)	60%	47,1%	48,7%	0,47
VL < 50*	80%	73,5%	74,4%	0,62
CD4 > 500*	100,0%	61,8%	66,7%	0,11
CD4% ≥ 25*	80%	72,7%	73,7%	0,60
Nadir CD4 (mean) (range)	453,4 (14–837)	382,1 (4–1269)	391,23 (4–1269)	0,59
TETANUS				
Number (%)	17 (43.6)	22 (56.4)	39 (100)	
Mean age (range)*	**16,4 (6–23)**	**19,4 (6–28)**	**18,1 (6–28)**	**0,03**
Gender (% of males)	35,3%	59,1%	48,7%	0,12
VL < 50*	88,2%	63,6%	74,4%	0,08
CD4 > 500*	**88,2%**	**50%**	**66,7%**	**0,01**
CD4% ≥ 25*	76,5%	71,4%	73,7%	0,51
Nadir CD4 (mean) (range)	388,6 (14–837)	393,3 (4–1269)	391,23 (4–1269)	0,95
HAV				
Number (%)	3 (7.7)	36 (92.3)	39 (100)	
Mean age (range)*	20,0 (17–25)	17,9 (6–28)	18,1 (6–28)	0,48
Gender (% of males)	66,7%	47,2%	48,7%	0,48
VL < 50*	100%	72,2%	74,4%	0,40
CD4 > 500*	66,7%	66,7%	66,7%	0,71
CD4% ≥ 25*	66,7%	74,3%	73,7%	0,61
Nadir CD4 (mean) (range)	473,7 (206–662)	384,4 (4–1269)	391,23 (4–1269)	0,59
HBV				
Number (%)	12 (30.8)	27 (69.2)	39 (100)	
Mean age (range)*	17,5 (12–25)	18,3 (6–28)	18,1 (6–28)	0,62
Gender (% of males)	33,3%	55,6%	48,7%	0,17
VL < 50*	83,3%	70,4%	74,4%	0,33
CD4 > 500*	83,3%	59,3%	66,7%	0,13
CD4% ≥ 25*	91,7%	65,4%	73,7%	0,09
Nadir CD4 (mean) (range)	409,3 (14–837)	383,22 (4–1269)	391,23 (4–1269)	0,78

Note: Statistically significant differences are shown in boldface type. VL, Viral Load; HAV, Hepatitis A Virus; HBV, Hepatitis B Virus; *At time serum was obtained

against pertussis, since the diphtheria-tetanus-acellular pertussis (DTaP) vaccine is available from the second half of the 90s.

However, these results are in line with the poor vaccination coverage reported in Italy in a sample of children with different chronic diseases, specifically type 1 diabetes, cystic fibrosis, Down syndrome, neurological diseases and HIV infection.[17] In particular, children with HIV infection were most likely to face delays in routine immunizations and significantly lower in completing DTP, polio and HBV vaccination course.[17]

Despite HIV infected children are prone to infectious diseases and the protection of such vulnerable population should be undertaken, immunization reluctance is a widely phenomenon described also in other European studies.[10-12] This is partially due to the concerns regarding safety of vaccines,[18,19] especially with live-attenuated vaccines, in severely immunocompromised individuals. Moreover, the increase of HIV viral load after vaccination, as result of T-cell activation and proliferation, therefore disease progression, represents reason of hesitancy.[5]

As in the HAART era the vertically acquired HIV infection has become a chronic treatable disease, the vaccine-induced long-term protection plays an increasingly significant role.[5]

In this study a poor prevalence of immunity to the common vaccine-preventable infectious diseases was showed among young patients perinatally infected with HIV-1. Low seropositivity/seroprotection rates were observed even in those who currently have their HIV infection well controlled (HIV-RNA ≤ 50 copies/mL and CD4 count ≥ 500 cells/mm^3) (data not shown). This finding is consistent with the suboptimal responsiveness and the rapid decline of immunity to childhood vaccinations described in HIV-1 infected children compared with healthy individuals,[13] also in the HAART era. In this regard, we should bear in mind that some patients of our study population were born before and others within the HAART era.

Timing of HAART initiation appears to be the major predictor of the persistence of protective immune response in vaccinated HIV-1 infected subjects.[13,20,21] Therefore, all vertically infected children should undergo HAART within the first year of life, before routine immunizations.[13,22]

Furthermore, several studies demonstrated that the stability over time of antibody levels varies between different vaccine antigens: in a cross-sectional study the presence of protective anti-HBs level was measured in only 1% of 69 HIV-infected children who had received HAART and had immune recovery at 5 y or more of age.[23] Bekker et al. showed that antibodies against live-attenuated measles, mumps and rubella vaccine (MMR) strains disappear in up to 40%, 38% and 11%, respectively, of 59 children and adolescents who were seropositive at baseline, despite immune reconstitution after HAART.[24] Children and adolescents previously immunized had waning protective antibody levels against DTaP[25] and conjugate pneumococcal vaccination.[26] The results of our study are in line with these evidences, even though their interpretation should be considered with some caution. The evaluation of immune protection exclusively by the antibody correlates could be inadequate, because none of these is sufficiently validated for immune compromised individuals.[13,27] Further immunological studies, exploring both T-and B-cell responses during vaccination, should be conducted with the aim to identify more specific serological markers of immune protection in this population.[13]

Anyway, our findings contribute to reinforce the importance of improving strategies for pinpointing susceptible individuals who, despite good initial response to primary vaccination, subsequent loss of detectable antibodies may be prevented by additional schedules for booster doses of vaccines. In this regard, the PENTA Vaccines Group suggest monitoring antibody status, useful in guiding the need for booster doses: children who have received a full course of vaccination prior to evidence of immunocompromisation should be tested for antibody titers when (around 4–6 y of age). In those with evidence of immunosuppression should be re-checked after approximately 5 y (at age 9–11 y)

Table 2. Immunization coverage by type of vaccine

	Immunized N (%)	CI 95%
Poliovirus	39 (100)	91.0–100.0
Diphtheria-Tetanus *Incomplete immunization schedule*	33 (84.6) *6 (15.4)*	70.0–92.0 *7.0–29.0*
Pertussis	17 (43.6)	29.0–59.0
Hepatitis B	36 (92.3)	79.0–97.0
Hepatitis A	4 (10.2)	4.0–23.0
Measles-Mumps-Rubella (1 dose)	23 (59.0)	43.0–72.0
Measles-Mumps-Rubella (2 doses)	8 (20.5)	10.0–35.0
Meningococcal C	3 (7.7)	2.0–20.0
***Haemophilus influenzae* type B** *Incomplete immunization schedule*	7 (18.0) *2 (5.1)*	9.0–33.0 *1.0–17.0*
Papillomavirus *Incomplete immunization schedule*	6 (15.4) *2 (5.1)*	7.0–30.0 *1.0–17.0*
Pneumococcal conjugate vaccine (PCV 7) *Incomplete immunization schedule*	2 (5.1) *1 (2.6)*	1.0–17.0 *0.0–13.0*
Pneumococcal polysaccharide vaccine (PPV23)	1 (2.6)	0.0–13.0

and again 5 y later, before shift to adult care (at age 14–16 y).[5] In detail, the guidance suggested anti-tetanus and anti-diphtheria testing every other fifth year and reinforcing vaccine in adolescent-/adulthood with 10-yearly booster dose of dTaP (adult formulation), every 3–5 y checking for MMR and revaccination in case of seronegativity for any component.[5] On the contrary, because serologic polio tests are not routinely available, booster dose of inactivated polio is recommended empirically for adolescents[5] and recent guidelines on immunizations for HIV-infected adults do not propose boosters into adulthood.[9] This aspect is crucial, especially before travel to endemic areas, considering the low seroprotection rates against polioviruses observed in young HIV-infected population[28] as well as only about the 15% of our patients presented protective immunity. In particular, we found a lower proportion of subjects with protective antibodies to poliovirus type 3 as compared with serotypes 1 and 2. Lower antibody levels to poliovirus type 3 in comparison with types 1 and 2 have been reported previously in HIV- infected[28] and HIV-uninfected children.[28-30]

In conclusion, this study underlines the optimization of selective strategies as the must for improving serological testing, in order to identify susceptible individuals. Therefore they are to be protected by supplementary strategies for booster doses, according to aligned schedules in terms of timing and number of vaccine doses. Furthermore, considering different protection needs in this such vulnerable population, it necessary to strictly adhere to the immunization policies with the aim of reaching adequate and long-term protection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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