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Ph.D. Thesis

PATHOPHYSIOLOGY, CLINICAL FEATURES, AND
MANAGEMENT OF CHILDREN WITH CHRONIC INFECTIOUS
DISEASES

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CHAPTER 1

BACKGROUND
1.1 Children with chronic diseases

1.11 Chronic viral hepatitis

Among human hepatitis viruses, hepatitis B (HBV) and C (HCV) viruses are able to persist in the host for years and thereby causing chronic hepatitis. Three hundred and seventy and 130 million people is estimated to be infected with HBV and HCV, respectively, worldwide (1). In endemic areas, HBV infection is often acquired perinatally or early in childhood and becomes chronic in a high proportion of cases. Universal vaccination of newborns has been effective in reducing the spread of infection. However, hepatitis B is still a social and health problem in underdeveloped areas where immunisation policies are unavailable, and even in developed countries, where the reservoir of infection is maintained by immigration and adoption. In some endemic areas children with chronic hepatitis B are also at risk for superinfection with the hepatitis delta virus (HDV), which worsens the prognosis of liver disease.

HCV is not a less important problem. The prevalence of circulating anti-HCV antibodies in the pediatric population averaged 0.3% in Italy in the early 1990s (2), but a national observational study suggest that the number of “new” pediatric infections dropped by approximately 40% in 2000-2004 compared with the previous 5 years (3). The lower prevalence of HCV in children reflects the disappearance of transfusion-related hepatitis and the reduced efficiency of mother-to-child (vertical or perinatal) transmission, although the latter form of transmission is currently responsible for most “new” infections in the developed world and contributes to maintaining the reservoir of infection worldwide (4-7). This favourable epidemiologic trend is balanced, however, by the strong tendency of HCV infection acquired early in life (either perinatally or following blood transfusions) to become chronic (8-14). In the absence of a specific vaccination, HCV infection remains a major global health problem and HCV-related end-stage liver disease is still the most frequent indication for liver transplantation in adult patients.

Chronic viral hepatitis acquired in childhood is a long-lasting process based on host-virus interaction, which may change over the years. A number of factors related to the virus (genotype, therapy), to the host (hormonal status, immunocompetence, therapy) and to the environment (alcohol, drugs, co-infections) affects the natural history of the disease, especially during adolescence and early adulthood. Strategies to improve the prevention and treatment of HBV and HCV infection, and the related liver disease in children, before the possible development of irreversible complications, should be investigated and implemented.
1.12 HIV infection

Countries most heavily affected, HIV has reduced life expectancy by more than 20 years, hampered economic growth, and deepened household poverty (UNAIDS. Data from: www.unaids.org/en/KnowledgeCentre/HIVData/GlobalReport/2008/2008_Global_report.asp). Mother-to-child transmission (MTCT) is the main source of pediatric HIV-1 infection. MTCT of HIV-1 mainly occurs around the time of delivery, but breastfeeding is an additional route of viral transmission and accounts for about one-third of pediatric infections in resource-poor Countries (15). In the absence of antiretroviral therapy, about 30% of women transmit the virus to their infants. The estimated number of perinatally acquired AIDS cases in the United States peaked at 945 in 1992 and declined rapidly with expanding prenatal testing and implementation of appropriate preventive interventions (16). At the end of 2007, there were 2 million children living with HIV around the world, an estimated 370,000 children became newly infected with HIV in 2007, and, of the 2 millions people who died of AIDS during 2007, more than one in seven were children. Every hour, around 31 children die as a result of AIDS.

HIV can affects a child’s life through its effects directly on the child, on that child’s family, and on the community within the child is growing up:
- Many children are themselves infected with HIV
- Children live with family members who are infected with HIV
- Children act as carers for sick parents who have AIDS
- Many children have lost one or both parents to AIDS, and are orphaned
- An increasing number of households are headed by children, as AIDS erodes traditional community support systems
- Children end up being their family’s principal wage earners, as AIDS prevents adults from working, and creates expensive medical bills
- As AIDS ravages a community, schools lose teachers and children are unable to access education
- Doctors and nurses die, and children find it difficult to gain care for childhood diseases
- Children may lose their friends to AIDS
- Children who have HIV in their family may be stigmatized and affected by discrimination
In the last 10 years, dramatic advances in medical management of HIV infection have followed the results of clinical trials of antiretroviral combination therapies in children. The use of antiretroviral therapy during pregnancy in HIV-infected women has resulted in a dramatic decrease in the transmission rate to infants, which is currently less than 2% in most high-income Countries (17). In parallel, the introduction of highly active antiretroviral therapy (HAART) has changed the natural history of HIV-1 infection and the life expectancy of HIV-1-infected adults (18,19) and children (20-26). Although in developed Countries children living with HIV infection are expected to live a long life, they still need to face major emotional burden, social stigma and global exclusion from the social contest (27). Being a child or an adolescent with HIV implies major problems in terms of management of multiple drugs, adherence to antiretroviral therapy, drug resistance, quality of life, frequency at school and social interactions with peers (28).

1.13 Immunization in at risk children

Vaccinations programs are one of the greatest public health interventions of the last century and have dramatically improved quality of life (29). Benefits of vaccination to the individual include partial or complete protection against infections and symptoms of illness, improved quality of life, and prevention of up to 3 million pediatric deaths per year worldwide (29,30). Benefits of a universal vaccination program to society include creation and maintenance of herd immunity, prevention of disease outbreaks, and reduced health care costs (30). Despite the availability of safe and effective vaccines and substantial progresses in reducing vaccine-preventable diseases, delivery to and acceptance of vaccinations by targeted populations are essential to further reducing and eliminating vaccine-preventable causes of morbidity and mortality (31). Children who are not vaccinated endanger public health representing a risk for other nonimmunized individuals, including subjects who cannot be immunized due to underlying health problems, and the small percentage of individuals in whom vaccination does not confer protection (29). They also contribute to increase health care costs (29).

Access to immunizations, prevalence of vaccine-preventable diseases, and vaccination rates varies by geographic area or country. Throughout the United States and European Countries, immunization rates of children and adults are rising, but coverage levels have not reached established goals (32). As a result of low immunization rates, vaccine-preventable diseases still occur as evidenced by the measles epidemic, the large number of annual cases of
varicella, pertussis, and hepatitis B, and the more than 50,000 annual deaths in adults from influenza or pneumococcal infections (33-36).

In an attempt to eliminate the risk of outbreaks of some diseases, governments and other institutions have instituted policies requiring vaccination for all people (compulsory vaccinations). For example, actual vaccination policies in most developed Counties require that children receive common vaccinations before entering school. In addition to compulsory vaccines, certain populations should receive additional vaccinations. Subjects with chronic medical conditions are at increased risk for severe complications related to vaccine-preventable infections, such as influenza and pneumococcal infections (34,37). In Italy, compulsory vaccines are generally administered in vaccination centers and complementary vaccinations are actively offered to children with chronic conditions and are included in the Essential Levels of Care (38). Despite long-standing recommendations to provide recommended vaccinations to children with chronic medical conditions, immunisation rates in these vulnerable populations remain poor (39). Several conditions hamper implementation of these vaccinations, including problems in identifying at risk children, ineffective organizational strategies and lack of awareness of disease severity or poor confidence by parents in specific recommendations (40,41). Often, the presence of a chronic condition is erroneously considered a contraindication rather than an indication to vaccination.

It is important to ensure that patients comply with the vaccination schedule to the extent possible, and to provide education to parents who may have concerns about pediatric vaccinations.
1.2 Goals of the thesis

In this PhD thesis, the organization and management of pediatric infectious diseases, with a perspective of public health, are investigated. Specific chronic diseases, as chronic viral hepatitis and HIV infection, have been selected as models to investigate the main aspects of prevention, management and treatment. The goal is to investigate the efficiency of organization and propose interventions with specific reference to treatment of infectious diseases, their direct and indirect results and how these conditions affect quality of life of at risk children and their families.

The final goal of this research is to provide strategies to optimize public health system.
### 1.3 References


2.1 Management of chronic hepatitis B and C in children

2.11 Rationale

HBV and HCV infections are the most common causes of infectious chronic disease worldwide, and are responsible of chronic hepatitis, cirrhosis, liver failure and hepatocellular carcinoma (HCC). Over the past two decades, the advent of universal immunization against HBV and the implementation of blood-donor screening for HBV and HCV have greatly decreased the rate of transmission; nevertheless, a considerable number of children and adults become chronically infected (1).

The risk of developing chronic HBV infection after acute exposure is roughly inversely proportional to the age of the individual. Newborns of HBeAg-positive mothers have a greater than 90% risk of becoming chronically infected with HBV, children and adolescents have a 25-50% risk, while only 5% of adults exposed to HBV develops chronic infection (2). Chronic hepatitis B is defined as persistence of HBsAg for longer than 6 months. In perinatally infected children estimated spontaneous clearance of HBV (i.e. loss of HBsAg and development of anti-HBs antibodies) occurs at a rate of 0.6% per year over the first decade of life, but the rate of clearance is higher, 1.8% per year, in patients infected as adolescents and adults (2). Of those children who remain infected, most develop “immune tolerance”, with HBeAg-positivity, high serum HBV DNA but normal ALT levels. During the course of chronic hepatitis B, clearance of HBeAg represents a key event, because it implies that the host is no longer immunotolerant and enters a low replication phase (2,3).

Among carriers with elevated ALT levels, the rate of clearance of HBeAg (seroconversion) averages between 8% and 12% per year. Seroconversion coincides with a decrease in serum viral load and biochemical and histological remission in the majority of cases (“inactive carrier state”) and may be followed by HBsAg seroclearance (2-4). Approximately 4% to 20% of inactive carriers have one or more reversions back to HBeAg. Among those who remain anti-HBe positive, 10% to 30% continue to have elevated ALT and high HBV DNA levels after HBeAg seroconversion (“HBeAg-negative chronic hepatitis B”). Clearance of HBeAg, whether spontaneous or after antiviral therapy, reduces the risk of hepatic decompensation and improves survival.

Children with chronic HBV infection are usually asymptomatic and have normal growth, physical examination results, nutritional parameters and development (5). During childhood, chronic hepatitis B is a mild disease associated with a low rate of cirrhosis (6). However,
decompensated liver disease and HCC have also been reported in children (7-9). Pediatric HCC has a poor prognosis, with a long-term survival rate of only 10-30% (8).

The goal of treatment for chronic hepatitis B encompass normalizing liver histopathology, suppressing viral replication and promoting immune-mediated clearance of HBV, and preventing the development of cirrhosis and HCC. Treatment options approved for children with chronic hepatitis B, interferon (IFN)-alpha, lamivudine and, recently, adefovir, do not achieve these goals in all treated patients (10-12).

The main source of HCV infection is now perinatal transmission from infected mothers. Transmission is higher in mothers with high titres of HCV RNA and who are HIV positive, with a transmission rates varying from 2-12% depending on maternal infectivity (13,14). Children constitute a small proportion of the HCV-infected population. This favourable epidemiologic trend is balanced, however, by the strong tendency of HCV infection acquired early in life to become chronic (15-17).

Chronically infected children are typically asymptomatic. Unlike with HBV, many patients with chronic hepatitis C have normal ALT levels despite necroinflammatory damage in the liver (18). Most children who are chronically infected with HCV have a milder disease with a more favourable natural course, compared to infected adults (16,19-22). Histological studies confirmed a prevalence of cirrhosis of 1.7% among 229 children with chronic HCV infection (20,23,24). Despite this favourable course, cases of HCV-infected children with cirrhosis who require transplantation have been reported (25-28). However, data about the severe course of pediatric HCV infection derive from studies performed in tertiary or quaternary referral centres and, consequently, do not provide an adequate perception of the real prevalence of worsening prognosis.

In spite of the indolent course in the majority of cases, a substantial number of children with chronic hepatitis C have been treated with IFN. An analysis of published trials of IFN therapy in children with chronic hepatitis C has shown a favourable effect of therapy in terms of sustained response, mainly for patients who are infected with HCV genotypes other than genotype 1 (29). To date, the approved treatment for HCV-infected children older than 3 years of age is combination therapy with IFN-alpha plus ribavirin for 1 year (30,31). Treatment should be discontinued in children who still have detectable HCV RNA after 6 months of treatment because of the unlikely change of achieving a sustained response under these circumstances (31). Studies of IFN-alpha plus ribavirin have reported promising results with regard to a sustained response rate (32-35), but the real impact of treatment on long-term outcome remain to be established. Moreover, antiviral therapy is expensive, and its efficacy is tempered by several adverse effects and impairments in the health-related quality of life (36).
The management of chronic hepatitis B and C in asymptomatic children is still a challenge. As antiviral therapies are now available for the treatment of both infections, the knowledge of natural history of chronic HBV and HCV infections and appropriate evaluation of potential treatment candidates is crucial.

We retrospectively investigated the long term outcome of chronic HBV and HCV infections in a large series of children who were observed regularly from childhood to young adulthood in a single care center. We compared treated and untreated children for clinical, biochemical, and virological outcomes.

2.12 Experimental procedures

Patients with chronic hepatitis B

All children with chronic HBV infection observed for more than 5 years during the period 1981-2005 at the Department of Pediatrics of the University “Federico II” were enrolled. At baseline, all patients underwent physical examination and liver function tests and were investigated for clinical history, risk factors for HBV infection, age at infection, clinical signs of liver disease, blood cell count, virological markers (i.e., HBsAg, antibody to HBsAg, HBeAg, anti-HBe, and serum HBV DNA levels), alfa-fetoprotein level, serum immunoglobulin level, and non-organ-specific autoantibody levels. Thereafter, all patients were monitored every 3-6 months by physical examination, liver function tests, virological tests, and alfa-fetoprotein level determination. At 6-12-month intervals, autoimmunity markers were determined for treated patients. Ultrasonographic examination of the liver, biliary tract, spleen, and portal vein was performed at 12-month intervals.

Data on IFN treatment were collected. Complete response to therapy was defined as a decrease in transaminase levels (within the normal range), an HBV DNA level less than $10^5$ copies/mL, clearance of HBeAg, and development of anti-HBe within 12 months after stopping treatment (37).

For untreated children, biochemical and virological aspects were monitored throughout the follow-up period. Inactive carrier state was defined as a detectable HBsAg level, an undetectable HBeAg level, a detectable anti-HBe level, a serum HBV DNA level less than $10^4$ copies/mL, and persistently normal ALT and aspartate aminotransferase levels. Resolved hepatitis B state was defined as previous HBV infection, with an undetectable HBsAg level, a normal ALT level, and an undetectable serum HBV DNA level (37).
Patients with chronic hepatitis C

All children (age, 2-18 years) who had antibodies to HCV (anti-HCV) present for 16 months and who attended the liver unit at our hospital (University “Federico II”) during 1986-2004 were enrolled. Patients who had concomitant systemic diseases or other causes of chronic liver disease were excluded from the study. For all patients, symptoms and health-related quality-of-life data were evaluated at each evaluation by clinical examination and an appropriate interview. Furthermore, all patients were evaluated at baseline for clinical history, risk factors for HCV infection, age at the time of infection, clinical signs of liver disease, liver function tests, complete blood cell count, serum HCV RNA level, HCV genotype, alfa-fetoprotein level, serum immunoglobulin level, presence of non-organ-specific autoantibodies and crioglobulins, levels of thyroid hormones. Thereafter, all patients were monitored every 3-6 months with physical examinations, liver function tests, virological tests, and determination of alfa-fetoprotein levels. At 6-12-month intervals, autoimmunity markers and hormone profiles were determined among treated patients. Ultrasound scanning of the liver, biliary tract, spleen, and portal vein was performed at 12-month intervals.

Laboratory procedures

At study entry and at each study visit, a serum sample was obtained from each patient and was stored at -80°C. Biochemical and virological tests were performed on fresh or frozen serum samples.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were evaluated using standard methods (upper limit of normal, 50 IU/L).

HBV markers (HBsAg, anti-HBsAg, HBeAg, and anti-HBe levels) were measured using commercial immunoassay kits (Abbott Diagnostics). Serum HBV DNA level was quantitatively investigated, depending on the time, with a commercial hybridization method (cut off value, 5 pg/mL; Abbott Diagnostics) or with a commercial PCR assay (Amplicor HBV-Monitor kit; Roche Diagnostic System).

The presence of anti-HCV was determined using a third generation ELISA (Ortho Diagnostic Systems). Quantification of HCV RNA level was performed by RT-PCR (COBASAmpliPrep/ HCV Monitor; Roche Molecular System; detection limit, 600 IU/mL of serum). In patients with serum HCV RNA levels less than the assay detection threshold, serum HCV RNA levels were determined using a PCR-based test (Amplicor; Roche Molecular System; detection limit, 50 IU/mL) (38). Genotyping was performed by analyzing PCR products with a reverse-hybridization assay (Inno LiPA HCV II; Innogenetics) (39).
**Histological evaluation**

Liver biopsy specimens, obtained after receiving informed consent from parents or legal guardians, were analyzed by the same liver pathologist, who was blinded to clinical and biochemical data. Specimens were scored with regard to hepatitis activity (grades, 0–18) and fibrotic changes (stages, 0-6), according to Ishak et al. (40).

**Statistical analysis**

All data are expressed as medians and ranges. Comparison of categorical variables was performed using the $\chi^2$ test or Fisher’s exact test, as appropriate. Comparison of continuous data was performed using the Mann-Whitney $U$ test and the Kruskal-Wallis test. A $p$ value of less than 0.05 was considered to be statistically significant.

**2.13 Results**

**Chronic hepatitis B**

One hundred eight consecutive patients (65 males; median age at last observation, 17.9 years; range, 6-34.2 years) observed over a period of up to 24 years were enrolled in the study. The course of chronic hepatitis B was evaluated for a median period of 12.1 years (range, 5-23 years).

A total of 57 liver biopsy specimens were obtained from 37 treated and 20 untreated children. Histological assessment revealed the presence of mild to moderate disease in most patients. In particular, 33 children (57.9%; 19 treated and 14 untreated; $p>0.05$) had minimal hepatitis, and 19 patients (33.3%; 14 treated and 5 untreated; $p>0.05$) had moderate hepatitis. Severe hepatitis was present in 4 patients (7.1%; 3 treated and 1 untreated; $p>0.05$), and features of micronodular cirrhosis were detected in one child (1.7%; an 8-year-old boy with unknown duration of disease who subsequently received IFN-alpha treatment).

During the observation period, 67 children remained untreated, and 41 were treated with IFN-alpha. After a median period of observation of 12.1 years (range, 5-23 years), HBeAg loss and serum HBV DNA clearance occurred in 43 untreated patients (69.3%) who were HBeAg positive at study entry and in 33 treated children (80%; $p>0.05$). In addition, 6 untreated patients (9.7%) and 4 treated patients (9.7%) became HBsAb positive at the end of the follow-up period.

No patient developed end-stage liver disease or hepatocellular carcinoma.
**Chronic hepatitis C**

A total of 67 children with chronic hepatitis C without other underlying systemic diseases (31 males, median age at liver biopsy 8.6 years, range 2-15; median duration of HCV infection 7.2 years, range 2-15) were enrolled.

On the basis of ALT levels during the first year of anti-HCV positivity, children were divided in 2 groups: those with hypertransaminasemia (100 patients, all of whom had detectable HCV RNA) and those with normal ALT values (25 patients). Of the 100 patients with hypertransaminasemia, 50 were treated with IFN during the period of observation.

At baseline, treated patients, untreated patients with hypertransaminasemia and untreated patients with normal ALT levels were comparable with regard to age, sex, clinical features, duration of HCV infection, and distribution of HCV genotypes.

All patients remained symptom free throughout the period of observation, with the exception of IFN-related adverse effects among the treated patients. No patient showed signs of hepatic decompensation.

**Treated patients**

Twenty-eight children had received IFN recombinant alpha-2b (5 MU/m² 3 times per week for 12 months), 9 children had received IFN recombinant alpha-2a (5 MU/m² 3 times per week, with durations of 6 months in the presence of a genotype other than 1b and of 12 months in presence of genotype 1b), and the remaining 13 children had received IFN alpha-lymphoblastoid (3 MU/m² 3 times per week for 12 months). The median duration of observation for treated children was 8.9 years (range, 4.9-14.4 years). Response to IFN treatment according to HCV genotype is reported in **figure 1**.
Figure 1. Flow diagram of the outcome in 50 treated children with chronic hepatitis C

50 treated children with chronic hepatitis C

- Genotype 1: 33 (66%) patients
  - Sustained virological response: 8 (24%) patients

- Genotype other than 1: 33 (66%) patients
  - Sustained virological response: 11 (65%) patients
Untreated patients

Median duration of observation was 9.2 years (range, 3.4-14.9 years) in 50 untreated patients with basal hypertransaminasemia and 7.9 years (range, 4.3-14.2 years) in 25 untreated patients with normal ALT levels at baseline.

The behaviour of ALT and HCV RNA in 75 untreated children at the end of observation period is reported in figure 2.

Figure 2. Flow diagram of the outcome in 75 untreated children with chronic hepatitis C
All children remained anti-HCV positive during the entire period of observation. At the end of the follow-up period, rates of HCV RNA clearance and ALT normalization were significantly higher in treated (38%) than in untreated children (12%; p <0.05).

Histological evaluation
A total of 64 patients (median age, 8.2 years; median duration of HCV infection, 6.9 years) underwent liver biopsy to assess the extent of liver damage and to guide management and treatment. Liver biopsy was performed for 47 of 50 treated children ≤6 months before commencement of treatment and for 17 of 75 untreated children. At the time of liver biopsy, all patients had viremia and hypertransaminasemia, with the exception of a girl who had a normal transaminase level. The median hepatitis grade was 4.4 (range, 1-8), and the median fibrosis stage was 1.6 (range, 0-4). No cases of cirrhosis (i.e., fibrosis scores of 5 or 6) were observed. The age of patients at the time of biopsy and the duration of HCV infection did not correlate with fibrosis score.

Twenty-one out of 50 treated children received a second liver biopsy at a median period of 5.5 years after the initial histological evaluation (range, 2-11.2 years). In 6 patients with sustained virological response, the hepatitis grade improved in all patients (from a median value of 5.5 to 2.2), and the fibrosis stage decreased in all children but 1 (83%), in whom fibrosis did not change (score 1). In contrast, of 15 non responders, the hepatitis grade improved in 4 children (26.7%); the fibrosis stage improved in 6 patients (40%), did not change in 5 patients (33.3%), and worsened in 4 cases (26.7%). In the group of non responders who had worsening fibrosis, only 1 HCV genotype 1b-infected obese child was found to have cirrhosis at the second biopsy, which was performed 7 years after the first biopsy and 13.1 years after the acquisition of HCV infection.

These data have been extensively presented and discussed in a previously published study (41).

2.14 Discussion

Children with chronic hepatitis B and C mostly remain symptom free, experience normal growth, and do not show clinical signs of chronic liver disease. A proper knowledge of natural history and identification of individuals who would benefit from therapy are mandatory. Our research contributes information regarding the long-term outcome of chronic hepatitis B and C acquired in childhood. During the entire period of observation, no child had decompensated liver disease or required liver transplantation.
Cirrhosis and HCC due to chronic HBV and HCV infection have been described to occur rarely during the pediatric age and more frequently during the second decade of life (7,9,25-28,42). In our series, only 1 patient with chronic hepatitis B had histological signs of cirrhosis, and none of the patients developed HCC. Because follow-up biopsies were not systematically performed in our study, it is possible that the progression to compensated cirrhosis could have been missed. However, strict investigation using ultrasonographic examination did not reveal features of severe liver damage in any of the patients. Among patients with chronic hepatitis C, histological evaluation revealed a morphologically benign disease in the majority of cases, a relatively slow progression of fibrosis and an extremely rare cirrhosis. The analysis of paired liver biopsies performed in a subgroup of children revealed a relatively slow progression of fibrosis. Unlike what has previously been reported (43), no linear correlation between duration of disease and progression of fibrosis was found in the present study. In fact, 2 of the 3 HCV-infected children with moderate fibrosis (score, 4) at the time of the first biopsy had a short duration of disease (2.1 and 2.5 years). On the other hand, the only patient for whom fibrosis was absent had a disease duration of 13.9 years. Therefore, the severity of liver disease does not seem to depend on the duration of HCV infection; instead, a host-virus interplay might be involved.

Several studies involving adults with chronic hepatitis B have shown that the long term outcome in patients after IFN-related HBeAg seroconversion is favourable in terms of HBV clearance, reduction of HCC, and prolongation of survival (44,45). Similarly, there is evidence that spontaneous seroconversion induces sustained remission in the majority of cases (4). Although it has been reported that IFN-alpha therapy accelerates HBeAg seroconversion in children (11), it is still not known whether faster seroconversion can modify the natural history of the disease. In our series, 66% of treated children and 55% of untreated patients with detectable HBeAg levels seroconverted to anti-HBe and had serum HBV DNA levels less $10^5$ copies/mL at the final evaluation; 8% of the patients achieved a detectable anti-HBsAg level. No advantage at final evaluation in terms of normalization of ALT levels, clearance of HBV DNA, and seroconversion was observed in treated patients compared with untreated, confirming that IFN-alpha therapy accelerates only seroconversion to anti-HBe. Although seroconversion seems to also confer favourable outcomes in children with HBV infection, Bortolotti et al. (46) reported 2 cases of HCC, 9 and 16 years after seroconversion. The possibility of spontaneous late reactivation and the occurrence of cirrhosis and HCC in patients with undetectable HBeAg and HBV DNA levels suggest that long-term surveillance is necessary for all patients with chronic hepatitis B, including inactive carriers and patients who do not have cirrhosis (47).
As for chronic hepatitis C, in our study the rate of spontaneous viral clearance in untreated children (12%) was lower than that reported by Vogt (45%) (21) and Locasciulli (26.8%) (22). This discrepancy could be related to the peculiar characteristics of patients included in those studies: the first included children who had post-transfusion HCV infection without clear evidence of chronic hepatitis, and the second evaluated patients who had leukaemia that was in remission and an atypical serological profile for HCV infection. Thus far, no predictive factor (including HCV genotype) of spontaneous viral clearance has been identified; it is likely that genetically determined immunological factors could be involved. Treated children with chronic hepatitis C had a rate of sustained virological clearance significantly higher than that for untreated children; in particular, this occurred in the presence of genotypes other than genotype 1. These findings confirm the previously reported favourable effects of IFN therapy (29). Presently, combination therapy using pegylated IFN and ribavirin has dramatically improved the sustained virological response among adults with chronic hepatitis C (30); preliminary studies have confirmed the efficacy of this therapy for children as well (48). Compared with previous studies (29), our study had a longer period of observation, both for treated and untreated patients. Despite the longer post treatment follow-up period, in our patients the rate of relapse was lower (8% vs. 22%). This discrepancy is probably attributable to the very strict response criteria used in our study, in which frequent determinations of serum HCV RNA level were performed.

A careful understanding of the natural history of HBV and HCV infections in children is important in making decisions regarding treatment. One of the most debated questions is whether treatment can modify the long-term course of chronic hepatitis B. According to current guidelines, children 2 to 17 years of age who are HBsAg seropositive for more than 6 months with persistent elevation of ALT levels >2xULN and evidence of active viral replication (positive HBeAg, HBV DNA levels >10^5 copies/ml or 20000 IU/ml in their serum) for more than 3 months should be considered for therapy (3). Treatment with IFN or lamivudine should be considered, but the side effects of IFN and the emergence of viral mutants mean that neither is ideal except for compassionate use. Adefovir is not sufficiently effective in children, but has the advantage of less viral resistance. Future therapies may hold more promising for HBV-infected children. Treatment for chronic hepatitis C obtains better results especially in presence of genotype other that 1. However, at the present time, all children with chronic hepatitis C do not seem to be reasonable candidates for such therapy, because treatment is expensive, prolonged and is associated to several adverse effects. Furthermore, in the vast majority of untreated children, no significant worsening is usually observed in our study.
In conclusion, this study indicates that chronic hepatitis B and C acquired in childhood are mild diseases with a slow progression of fibrosis. Considering the treatments available for chronic hepatitis B, it seems reasonable to not treat all children, but to treat only those with more-severe liver disease and/or with positive predictive factors of response. Treatment of children with chronic HCV infection has provided more promising results.
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   Is liver biopsy mandatory in children with chronic hepatitis C?

   Epidemiological profile of 806 Italian children with hepatitis C virus infection over a 15-year period.

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   Children with chronic hepatitis C: what future?
Long-Term Outcome in Children with Chronic Hepatitis B: A 24-Year Observation Period

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Background. Chronic hepatitis B seems to manifest as mild disease in children and young adults. However, data regarding the long-term course of hepatitis B in untreated and interferon-treated children are still scarce. This study investigates the long-term outcome of disease in a large series of untreated and treated children with hepatitis B virus (HBV) infection.

Methods. Clinical, biochemical, virological, and histological features were evaluated in children (age range, 2–18 years) with chronic HBV infection who did not have concomitant chronic systemic diseases other than HBV infection and who were admitted to the liver unit in the Department of Pediatrics at University “Federico II” (Naples, Italy) during the period 1981–2005.

Results. One hundred eight consecutive patients observed for up to 24 years were studied. During the observation period, 67 children remained untreated, and 41 were treated with interferon-α. After a median period of observation of 12.1 years (range, 5–23 years), hepatitis B early antigen loss and serum HBV DNA clearance occurred in 45 untreated patients (69.3%) who were hepatitis B early antigen positive at study entry and in 33 treated children (80%); the P value is not statistically significant. In addition, 6 untreated patients (9.7%) and 4 treated patients (9.7%) became hepatitis B surface antigen positive at the end of the follow-up period. Histological assessment, evaluated for 57 children, showed mild-to-moderate disease in 91.2% of cases of HBV infection. No patient developed end-stage liver disease or hepatocellular carcinoma.

Conclusions. Children with chronic HBV infection are symptom free, with morphologically mild liver disease. Considering that the overall long-term outcomes did not differ between treated and untreated patients, the real impact of therapy on the long-term course of HBV infection remains to be established. Additional studies are needed to confirm our conclusions.

Fifteen to sixty percent of patients with chronic hepatitis B (CHB) develop cirrhosis, with a significant risk for the complications of portal hypertension, liver failure, and hepatocellular carcinoma (HCC) [1, 2]. Age at onset of infection is an important factor affecting the outcome of hepatitis B virus (HBV) infection [3]. During the course of CHB, clearance of hepatitis B early antigen (HBeAg) represents a key event, because it implies that the host is no longer immunotolerant and enters a low replication phase [4, 5]. Serocconversion coincides with a decrease in serum viral load and biochemical and histological remission in the majority of cases and may be followed by HBV surface antigen (HbsAg) seroclearance [6–8].

During childhood, CHB is a mild disease associated with a low rate of cirrhosis [9]. However, decompensated liver disease and HCC have also been reported in children [10–12]. In an attempt to shorten the replicative phase of disease and obtain seroconversion to antibody to HBeAg (anti-HBe), IFN-α and lamivudine have been used to treat children with CHB [13–16]. Durable response rates associated with IFN-α therapy seem to be higher than those associated with lamivudine therapy, although IFN-α is associated with higher toxicity. Clearance of HBeAg has been reported in one-third of IFN-treated children, compared with 10% of untreated control subjects [13]. The benefits of IFN-α therapy seem to disappear during the follow-up period, considering that the cumulative HBeAg clearance rate over the years has not differed between treated and untreated patients [15]. With regard to lamivudine therapy, clearance of HBeAg and decrease in viral load...
have been reported in 23% of treated children, compared with
1% of untreated control subjects [16].

In contrast with chronic hepatitis C virus infection, in which a
sustained clearance of serum hepatitis C virus RNA is obtained
in 50%–60% of patients treated with pegylated IFN plus
ribavirin [17], in patients with CHB, a definitive clearance of
serum HBV DNA is more rarely observed in instances of la-
voratory response to therapy.

The ultimate goal of therapy is to prevent morbidity and
mortality related to the development of cirrhosis and HCC.
The parameters used to assess treatment response include
HBeAg clearance and decrease in serum HBV DNA level [18].
Data regarding differences between spontaneous serocon-
version to anti-HBe and treatment-induced seroconversion during
the long-term course of infection are still scarce.

The aim of this study was to provide information on long-
term outcome after spontaneous and treatment-induced
HBeAg seroconversion in patients with chronic HBV infection
acquired during childhood who were observed regularly from
childhood to young adulthood. For this purpose, treated and
untreated patients observed at the liver unit in the Depart-
ment of Pediatrics at University “Frederico II” (Naples, Italy)
over a 24-year period were retrospectively evaluated and com-
pared for the clinical, biochemical, and virological outcomes.

PATIENTS AND METHODS

Patients. All children with CHB (defined as the presence of
hepatitis B surface antigen [HBeAg], in serum for ≥6 months)
who were observed for ≥2 years and presented at the liver unit
in the Department of Pediatrics at University “Frederico II”
during the period 1981–2005 were enrolled in our study. Ex-
clusion criteria included presence of concomitant systemic dis-
ease, concurrent hepatitis C virus, hepatitis delta virus, HIV
infection, or other liver disease (e.g., α-1-antitrypsin deficiency,
Wilson disease, autoimmune hepatitis, cystic fibrosis, and celiac
disease–related liver damage).

At each evaluation, symptoms and health-related quality-of-
life data were recorded by clinical examination and appropriate
interview: growth was evaluated using standard height and
weight charting [19]. At baseline, all patients underwent phys-
ical examination and liver function tests and were investigated
for clinical history, risk factors for HBV infection, age at in-
fec tion, clinical signs of liver disease, blood cell count, viro-
logical markers (i.e., HBeAg, antibody to HBeAg, HBsAg, anti-
HBe, and serum HBV DNA levels), α-fetoprotein level, serum immunoglobulin level, and non-organ-specific autoantibody
levels. Serum aspartate aminotransferase and alanine aminotrans-
ferase (ALT) levels were evaluated using standard methods
(upper limit of normal, 20 IU/L).

Thereafter, all patients were monitored every 3–6 months by
physical examination, liver function tests, virological tests, and
α-fetoprotein level determination. At 6–12-month intervals,
autoimmunity markers were determined for treated patients.
Ultrasoundographic examination of the liver, biliary tract, spleen,
and portal vein was performed at 12-month intervals.

Liver biopsy specimens, obtained after receiving informed
consent from parents or legal guardians, were analyzed by the
same liver pathologist, who was blinded to clinical and bio-
chemical data. Specimens were scored with regard to hepatitis
activity (grades 0–18) and fibrotic changes (stages 0–6), ac-
cording to Ishak et al. [20].

Data on IFN treatment were collected. Complete response
to therapy was defined as a decrease in transaminase levels
(within the normal range), an HBV DNA level <10^5 copies/mL,
clearance of HBeAg, and development of anti-HBe within
12 months after stopping treatment [18].

For untreated children, biochemical and virological aspects
were monitored throughout the follow-up period. Inactive car-
rier state was defined as a detectable HBeAg level, an undetec-
table HBeAg level, a detectable anti-HBe level, a serum HBV
DNA level <10^4 copies/mL, and persistently normal ALT and
aspartate aminotransferase levels. Resolved hepatitis B state
was defined as previous HBV infection, with an undetectable
HBeAg level, a normal ALT level, and an undetectable serum HBV
DNA level [18]. This study was performed in accordance with
the Helsinki Declaration.

Laboratory procedures. At study entry and at each visit,
a serum sample was collected and stored at ~80°C. Biochemical
and virological tests were performed on fresh or frozen samples.
Viral markers (HBeAg, antibody to HBeAg, HBsAg, and anti-
HBe levels) were measured using commercial immunoassay kits
(ABBOTT Diagnostics). Serum HBV DNA levels were quantitatively
investigated, depending on the time, with a commercial hy-
bridization method (cutoff value 5 pp/mL; ABBOTT Diagnostics)
and with a commercial PCR assay (AmpliCor HBV Monitor Kit;
Roche Diagnostic System).

Statistical analysis. All data are expressed as median and
range or mean ± SD. Comparison of categorical variables was
performed using the χ² test or Fisher’s exact test. Comparison
of continuous data was performed using the Mann-Whitney U
and the Kruskal-Wallis test. A P value <.05 was considered to
be statistically significant.

RESULTS

One hundred eighty consecutive patients (65 male patients; me-
dian age at last observation, 17.9 years; range, 6–34.2 years)
observed over a period of up to 24 years were enrolled in the
study. The course of CHB was evaluated for a median period of
12.1 years (range, 5–23 years).

During the observation period, 67 children remained un-
treated, and 41 were treated with IFN-α; these patients had
been included in clinical trials performed at our department.
in previous years [21]. At baseline, treated and untreated patients were comparable with regard to demographic, clinical, and laboratory features (Table 1). All but 1 patient were white.

A total of 37 liver biopsy specimens were obtained from 37 treated and 20 untreated children. For treated patients, biopsy was performed before starting IFN-α therapy at a mean duration of HBV infection of 6.4 ± 2.9 years. The mean duration of disease at histological evaluation for untreated children was 7.2 ± 2.9 years (the P value is not statistically significant). Histological assessment revealed the presence of mild disease in most patients. In particular, 33 children (57.9%; 19 treated and 14 untreated; the P value is not statistically significant) had minimal hepatitis, and 19 patients (33.9%; 14 treated and 5 untreated; the P value is not statistically significant) had moderate hepatitis. Severe hepatitis was present in 4 patients (7.1%; 3 treated and 1 untreated; the P value is not statistically significant), and features of microsomal cirrhosis were detected in 1 child (1.7%; an 8-year-old boy with unknown duration of disease who subsequently received IFN-α treatment).

All patients were asymptomatic at presentation and remained symptom-free throughout the observation period, with the exception of IFN-α-related adverse effects among the treated patients. None of the patients showed abnormal growth. None of the patients showed signs of hepatic decompensation, and all of the patients had normal levels of albumin and international normalized ratio. Monitoring of α-fetoprotein levels and ultrasonographic findings did not reveal signs of HCC in any of the patients.

Treated patients. The median duration of follow-up for 41 IFN-treated children was 13.7 years (range, 5.4–20.7 years). On the basis of trials in which children had been included, 13 patients received recombinant IFN-α-2b therapy (5–10 MU/m², 3 times per week for 6–12 months), and 28 were treated with α-lymphoblastoid IFN (5 MU/m², 3 times per week for 6–12 months). No statistically significant difference was found between the 2 groups (divided according to different IFN-α treatments) with regard to sex, route of infection, age at the start of therapy, and duration of disease (data not shown); a statistically significant difference was found for basal ALT level, which was higher in patients who received α-lymphoblastoid IFN (ALT × normal value, 2.1; range, 1.3–7.9) than in those treated with recombinant IFN-α-2b (ALT × normal value, 1.5; range, 1.3–2.3; P = .035). Treatment was stopped before the end of the established period because of adverse events or significant elevation of transaminase levels in 4 patients. The median duration of posttherapy follow-up for 41 patients was 10.2 years (range, 3–15.3 years). Only 3 children had a posttherapy observation period that was a duration of <5 years (median, 3 years; range, 3–4 years).

A complete response to treatment occurred in 8 patients (21.9%; 6 treated with α-lymphoblastoid IFN and 3 treated with recombinant IFN-α-2b; the P value is not statistically significant). At the end of the observation period (figure 1), 23 patients (56%) achieved seroconversion to anti-HBe. An HBV DNA level <10⁴ copies/ml, and a normal ALT level (i.e., inactive carrier state). In 4 patients (9.8%) who achieved seroconversion to anti-HBe and normal transaminase levels, the HBV DNA level was 10⁴ copies/ml to 10⁵ copies/ml. HBV infection resolved in 4 patients (9.8%) who seroconverted to antibody to HBeAg. Six patients (14.8%) maintained detectable HBeAg and HBV DNA levels, as well as hypertransaminasemia. Two children (4.8%) seroconverted to anti-HBe, despite the persistence

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IFN-treated children (n = 41)</th>
<th>Untreated children (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M:F</td>
<td>36:15</td>
<td>29:9</td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td>6.7 (1.1–12.2)</td>
<td>6.7 (1.1–12.2)</td>
</tr>
<tr>
<td>Route of infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertical</td>
<td>14 (24.1)</td>
<td>12 (32.8)</td>
</tr>
<tr>
<td>Horizontal</td>
<td>15 (36.6)</td>
<td>13 (31.7)</td>
</tr>
<tr>
<td>Unknown</td>
<td>12 (29.3)</td>
<td>22 (55.0)</td>
</tr>
<tr>
<td>Age at diagnosis, median years (range)</td>
<td>5.9 (3.2–14)</td>
<td>4 (2.2–12)</td>
</tr>
<tr>
<td>ALT level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>5 (12.2)</td>
<td>18 (60.6)</td>
</tr>
<tr>
<td>Elevated</td>
<td>36 (87.8)</td>
<td>40 (37.1)</td>
</tr>
<tr>
<td>Median ALT level × ULN (range)</td>
<td>2 (1.4–7.9)</td>
<td>2.2 (1.1–26)</td>
</tr>
<tr>
<td>Detectable HBeAg and HBV DNA levels</td>
<td>41 (100)</td>
<td>47 (72.5)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, unless otherwise indicated. P > .05 for all variables. ALT, alanine aminotransferase; HBeAg, hepatitis B early antigen; ULN, upper limit of normal.

* Vertical transmission was defined as transmission of HBV infection from a mother with a detectable HBeAg level in the absence of other risk factors for HBV infection.
Figure 1. Flow diagram of the outcome in 41 treated children with chronic hepatitis B (CHB). Hepatitis B early antigen (HBeAg)-negative chronic hepatitis B (CHB) is defined as CHB in the absence of a detectable HBeAg level, and HBeAg-positive CHB is defined as CHB in the presence of a detectable HBeAg level. ALT, alanine aminotransferase; HBV, hepatitis B virus.

of elevated ALT levels and viral loads >10^5 copies/mL. This serological pattern was suggestive of HBeAg-negative CHB (i.e., CHB in the absence of a detectable HBeAg level). Two patients (4.8%) who achieved clearance of HBeAg and undetectable HBV DNA levels presented with mild ALT level abnormalities, in the absence of other known causes of liver damage.

Untreated patients. Sixty-seven untreated children were observed for a median duration of 10 years (range, 3–23 years). At baseline, 62 children had a detectable HBeAg level. Among this group, at the end of the observation period (figure 2), 30 patients (48.4%) became inactive carriers; 4 (6.4%) achieved seroconversion to anti-HBe, normal ALT levels, and HBV DNA levels of 10^3 copies/mL to 10^4 copies/mL; and 6 (9.7%) achieved clearance of HBeAg (i.e., resolved hepatitis B) and seroconverted to antibody to HBsAg. In contrast, 16 patients (25.9%) continued to have detectable HBeAg and serum HBV DNA levels, as well as elevated transaminase levels. Three patients (4.8%) had a detectable HBV DNA level in serum but an undetectable HBeAg level in serum (i.e., HBeAg-negative CHB). Three patients (4.8%) who achieved seroconversion to anti-HBe had mild ALT level abnormalities during follow-up, in the absence of viroemia. In this subgroup, coinfections, metabolic diseases, and autoimmunity were ruled out. Five untreated children were already HBeAg negative before first observation: 1 child experienced spontaneous HBeAg seroclearance during the follow-up period.

Seroconversion to anti-HBe in treated and untreated patients. No correlation was found between seroconversion and age, sex, and baseline ALT level. Median age at seroconversion (10 years; range, 3–22 years) did not differ between treated (median age, 10.3 years; range, 5–21 years) and untreated patients (median age, 9 years; range, 3–22 years; the P value is not statistically significant). The mean ALT serum level measured at the evaluation before seroconversion increased 1.5 times the value detected at the previous control evaluation (P < .001). No other significant predictive factors of seroconversion were identified. In addition, the long-term outcome of CHB was not significantly related to the different route of infection among 74 children with known sources of infection (table 2).

At the end of the follow-up period, 86 children (75.6%) had experienced HBeAg seroclearance. This clearance was associated with development of anti-HBe and an HBV DNA level <10^4 copies/mL in 74 patients. Twenty-two patients (20.4%) still had a detectable HBeAg level at the end of the follow-up period. The median duration of follow-up was 13.2 years (range, 5–23 years) for patients with undetectable HBeAg levels and 6.7 years (range, 5.1–20.4 years) for those with persistent detectable HBeAg levels (P = .01).

Among the treated children, throughout the observation period, 9 patients who experienced complete response maintained an undetectable HBeAg level in serum. In addition, 24 (73%) of 32 nonresponders seroconverted to anti-HBe ≥12 months after treatment withdrawal. Among the untreated children, 43 (69.3%) of 62 patients with a detectable HBeAg level at baseline experienced HBeAg clearance. At year 6 after the start of treatment or observation, the rate of HBeAg clearance in treated and untreated patients overlapped, becoming 63.4% among
Figure 2. Flow diagram of the outcome in 67 untreated children with chronic hepatitis B (CHB). Hepatitis B early antigen (HBeAg–negative CHB) is defined as CHB in the absence of a detectable HBeAg level, and HBeAg-positive CHB is defined as CHB in the presence of a detectable HBeAg level. ALT, alanine aminotransferase; HBV, hepatitis B virus.

treated patients and 62.7% among untreated children (the P value is not statistically significant); the final clearance rate of HBeAg did not statistically differ between treated and untreated patients (table 3). None of the patients who seroconverted to anti-HBe (either spontaneously or as a result of therapy) developed acute exacerbation or seroreversion to HBeAg during a median postseroconversion period of 7.2 years (range, 2.3–20 years).

DISCUSSION

This 24-year retrospective study contributes information regarding the long-term outcome of CHB acquired in childhood. None of the patients in our study became symptomatic or developed decompensated liver disease requiring liver transplantation. None of the patients died of liver-related causes. At the final evaluation, 65.8% of treated patients and 54.8% of untreated patients with detectable HBeAg levels seroconverted to anti-HBe and had serum HBV DNA levels <10^5 copies/mL; ~4% of the patients achieved a detectable anti-HBs level. With regard to seroconversion to anti-HBe in adults, several predictors have been identified [18]; in the present study, a significant increase in the serum ALT level was observed during the months preceding seroconversion.

Several studies on CHB acquired during childhood revealed a benign course of disease [7, 21, 22]. In a study including 52 children followed up for 3–22 years, Fujisawa et al. [21] reported a percentage of HBeAg clearance of 83.3% in untreated patients and 87.5% in treated patients; none of the patients achieved an undetectable HBeAg level. The authors explained that this lower rate of HBeAg clearance was a result of perinatal acquisition of HBV infection. In a recent longitudinal study involving 97 white patients with CHB, 91% of untreated patients and all treated patients without cirrhosis became inactive carriers; the HBeAg clearance rate was 79.5% [22].

Inactive carrier state seems to be stable for many years, as previously reported in Italian studies [22, 23]. In our cohort, patients were strictly followed up for a long period after seroconversion to anti-HBe. The serological profiles of our patients were stable for a median period of 7.2 years after seroconversion. It is notable that patients who did not experience seroconversion had a significantly shorter observation period than did patients who experienced seroconversion; therefore,
it is possible that a percentage of these patients will experience seroconversion at a later time. All inactive carriers had serum HBV DNA levels <10^6 copies/mL, but a group of these patients had detectable HBV DNA level by PCR. Other authors reported that a sizable percentage of patients with undetectable HBeAg levels had detectable HBV DNA levels by PCR [21, 22]. These low levels of viremia probably reflect a slow viral replication persisting for several years in patients with CHB, even after remission of liver disease and clearance of HBeAg. In our cohort, 4.6% of anti-HBe-positive patients experienced mild ALT level abnormalities during the follow-up period, in the absence of HBeAg seroreversion and increase in viral load. However, it is unlikely that the low HBV DNA level (<300 copies/mL) detected in this subgroup could be responsible for the slight ALT level elevation.

Several studies involving adults have shown that the long-term outcome in patients after IFN-α-related HBeAg seroconversion is favorable in terms of HBV clearance, reduction of HCC, and prolongation of survival [26, 27]. On the other hand, there is evidence that spontaneous seroconversion induces sustained remission in the majority of cases [28]. Although seroconversion seems to also confer favorable outcomes in children with HBV infection, Beriolotti et al. [22] reported 2 cases of HCC, 9 and 16 years after seroconversion. The possibility of spontaneous late reactivation and the occurrence of cirrhosis and HCC in patients with undetectable HBeAg and HBV DNA levels suggest that long-term surveillance is necessary for all patients with CHB, including inactive carriers and patients who do not have cirrhosis [29].

In our study, the development of HBeAg-negative CHB was not frequently observed both in untreated children and in treated children, being present in only 5 of 108 patients. This result is in contrast with results in earlier reports involving adults with CHB, in which the patients experienced HBeAg-negative CHB in a much higher proportion of cases [8]. Although we did not investigate the presence of an e-minus mu-

Table 2. Outcome of chronic hepatitis B virus infection in 74 children with overt route of infection.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Route of infection, no. (%) of patients</th>
<th>Vertical (n = 46)</th>
<th>Horizontal (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance of HBeAg and acquisition of anti-HBe</td>
<td>35 (76.1)</td>
<td>25 (90.3)</td>
<td></td>
</tr>
<tr>
<td>Persistence of detectable HBeAg levels</td>
<td>0 (10.6)</td>
<td>2 (7.1)</td>
<td></td>
</tr>
<tr>
<td>HBeAg-negative chronic hepatitis B</td>
<td>2 (4.3)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** HBeAg early antigen HBeAg-negative chronic hepatitis B is defined as chronic hepatitis B in the absence of a detectable HBeAg level. Anti-HBe, antibody to HBeAg.

To date, there have been no studies with a sufficiently long follow-up period to calculate the risk of progression towards serious liver disease in children with CHB. Cirrhosis and HCC due to chronic HBV infection have been described to occur rarely during the pediatric age and more frequently during the second decade of life [10-12]. In our series, only 1 patient had histological signs of cirrhosis, and none of the patients developed HCC. Because follow-up biopsies were not systematically performed in our study, it is possible that the progression to compensated cirrhosis could have been missed. However, strict investigation using ultrasonographic examination did not reveal features of severe liver damage in any of the patients.

A careful understanding of the natural history of HBV infection in children is important in making decisions regarding treatment. One of the most debated questions is whether treatment can modify the long-term course of CHB. To date, indications for treatment of children with CHB are still controversial, and current therapy has limited long-term benefits [30]. The goal of antiviral therapy is to reduce liver-related morbidity and mortality and to minimize the risk of transmission. Although it has been reported that IFN-α therapy accelerates HBeAg seroconversion in children [15], it is still not known whether faster seroconversion can modify the natural history of the disease. In our series, no advantage at final evaluation in terms of normalization of ALT levels, clearance of HBV DNA, and seroconversion was observed in patients treated with IFN-α, compared with untreated patients. In addition, the rate of

Table 3. Outcome of chronic hepatitis B virus (HBV) infection in treated and untreated children at the end of the observation period.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No (%) of IFN-treated patients (n = 41)</th>
<th>No (%) of untreated patients (n = 67)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance of HBeAg and acquisition of anti-HBe</td>
<td>27 (65.9)</td>
<td>38 (56.7)</td>
</tr>
<tr>
<td>Resolved hepatitis B</td>
<td>4 (9.7)</td>
<td>7 (10.3)</td>
</tr>
<tr>
<td>Persistence of detectable HBeAg levels</td>
<td>6 (14.9)</td>
<td>15 (22.9)</td>
</tr>
<tr>
<td>HBeAg-negative chronic hepatitis B</td>
<td>2 (4.8)</td>
<td>3 (4.5)</td>
</tr>
</tbody>
</table>

**NOTE:** HBeAg early antigen HBeAg-negative chronic hepatitis B is defined as chronic hepatitis B in the absence of a detectable HBeAg level. Anti-HBe, antibody to HBeAg.

* Five of 67 untreated children already had undetectable HBeAg levels at study entry.
HBeAg clearance was similar between the 2 groups, confirming that IFN-α therapy accelerates only seroconversion to anti-HBe. Compared with IFN-α, lamivudine is less expensive and well-tolerated, but the durability of response appears to be lower, and prolonged therapy is associated with increasing risk of lamivudine-resistant mutants, which may compromise the initial benefits and eventually determine a worsening of liver disease. Although viral clearance is important to reduce the risk of transmission, especially in a region where the vaccine is not extensively used, currently, the real impact of antiviral therapy seems to be scarce. However, it is not possible to exclude that, in the upcoming years, the use of new antiviral treatments (e.g., adenosvir, entecavir, and telbivudine) that have not yet been approved for use in children could modify the management of CHB during pediatric age.

In conclusion, the overall prognosis for CHB in vertically and horizontally infected patients seems to be favorable. On the basis of our data, IFN-α treatment did not significantly influence the long-term seroconversion rate among patients with chronic HBV infection acquired during childhood. These findings should be considered when selecting candidates for treatment and choosing antiviral agents.

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Potential conflicts of interest. All authors: no conflicts.

References

Is liver biopsy mandatory in children with chronic hepatitis C?

Raffaele Iorio, Antonio Verrico, Antonietta Giannattasio

Abstract

Liver biopsy is considered the most accurate means to estimate the necroinflammatory activity and the extent of fibrosis. However, histology evaluation is an invasive procedure associated with risk to the patient, risk of sampling error and diagnostic inconsistencies due to inter- and intra-observer error. On the basis of histological studies performed so far, chronic hepatitis C in children appears morphologically benign in the majority of cases. At the Pediatric Liver Unit of our university, a total of 67 children with chronic hepatitis C underwent liver biopsy. Liver biopsy was repeated 5.5 years after the initial histological examination in 21 children. On a total number of 88 liver biopsies, micronodular cirrhosis was detected only in one genotype 1b infected obese child. Since liver histology investigation of a child with chronic hepatitis C has few chances to highlight severe lesions, we question how liver biopsy helps in the management of children with chronic hepatitis C.

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Key words: Liver fibrosis; Cirrhosis; Natural history; Liver biopsy; Children


TO THE EDITOR

Liver biopsy is considered the most accurate means to estimate the necroinflammatory activity of a process and the stage of a disease involving the liver by assessing type and extent of fibrous together with recognition of architectural disturbance. The level of aminotransferase elevation does not adequately reflect the severity of the disease and methods measuring fibrosis-related molecules circulating in blood are not yet put into widespread use.

Liver biopsy represents, however, an invasive procedure associated with discomfort and risk to the patient. In pediatric age, it has been reported that liver biopsy is burdened with a 6.8% incidence of overall complications, a 2.4% incidence of major complications and a mortality rate of 0.4%[2]. Furthermore, histology evaluation carries risk of sampling error because of specimen fragmentation or inadequate length, and interpretation of the biopsy is subject to diagnostic inconsistencies due to inter- and intra-observer error.

In initial treatment trials of hepatitis C, liver biopsy was considered an important parameter to guide management and therapy, particularly at a time when response to treatment was low. As a consequence, it was desirable for patients with a more severe liver damage to receive a therapy with potentially serious side effects. In the vast majority of pediatric treatment trials of hepatitis C, patients were candidate for treatment only if liver histology was available. With the improvement in response rate to antiviral therapy, although reduction of fibrosis is still considered a common endpoint of clinical trials, the value of liver biopsy in management of patients with chronic hepatitis C has been questioned, advocating that this procedure may not be necessary for the initiation of therapy. Indeed, in the recent years, following the encouraging rates of response to combined therapy with pegylated-interferon and ribavirin, also patients with normal aminotransferases and minimal histological liver damage have been considered for therapy.

On the basis of the studies available so far, chronic hepatitis C in children seems to be a milder disease with a more favourable natural course when compared to hepatitis C virus (HCV) infection in adults. Recently, pediatric cases of severe chronic hepatitis C requiring liver transplantation have been reported, but in these reports no information was provided about either the total number of transplanted children for diseases other than HCV infection in the same period or the pediatric prevalence of hepatitis C in the area from which transplanted children derived. Furthermore, these data about the severe course of pediatric HCV infection obtained from studies performed in tertiary or quaternary referral centers, do not provide an adequate perception of the real prevalence of worsening prognosis. As a matter of fact, several histological studies have confirmed that in children chronic HCV infection is morphologically benign in the majority of cases, progression of fibrosis is relatively slow and cirrhosis is extremely rare. In an Italian-Spanish multicenter study, Guido et al. enrolled 80 children with chronic hepatitis C without underlying systemic diseases to study their liver histology. Liver biopsies were...
performed at a mean of 41.5 ± 11.9 mo after the first observation of increased transaminases. The authors reported a rate of 0.8% of cirrhosis. Vogt et al., in a study including German patients with a median duration of HCV infection of 20 years at histological evaluation, described only one child with micronodular cirrhosis (this patient was co-infected with hepatitis B virus) out of 17 who underwent liver biopsy. Jarrar et al., in an European multicenter retrospective study, analyzed 92 liver biopsy specimens from children with chronic HCV infection but no underlying systemic diseases. Histological analysis was performed on 77 to 17 years after the clinical diagnosis. Progressive liver disease was reported only in two HCV infected children (severe hepatitis in one case and cirrhosis in another).

At the Pediatric Unit of the University of the Federal (Naples, Italy), a total of 67 children with chronic hepatitis C without other underlying systemic diseases (31 males, median age at liver biopsy 8.6 years, range 2-15 years, median duration of HCV infection 7.2 years, range 2-15 years), underwent liver biopsy from 1986 to 2007. Percutaneous liver biopsies were performed after informed consent was obtained from parents or guardians in order to assess the extent of liver damage. Histological examination was performed by the same liver pathologist, who was blinded to biochemical and clinical data. Specimens were scored with regard to hepatitis activity (graded 0-18) and fibrotic stages (staged 0-6), in accordance with the methodology of Ishak et al. Thirty-two children acquired infection through blood transfusion, 22 through vertical transmission, and 8 through minor surgery. The route of infection was unknown in the remaining 5. Forty patients were infected with genotype 1, 16 with genotype 2, 3 with genotype 3 and 8 with other genotypes. At the time of histological evaluation, all patients had viremia (median serum HCV RNA 218000 IU/mL, range 40000-146700 IU/mL) and all but two had hypertransaminasemia (median alanine amino-transferase value 85 IU/L, range 40-350 IU/L). Liver biopsy was performed in 47 children before commencement of antiviral treatment and in 20 children who remained untreated. Liver biopsy was repeated 5.5 years (range 2-11.2 years) after the initial histological evaluation in 21 children. On a total number of 88 liver biopsies, micronodular cirrhosis was detected only in one genotype 1b-infected obese child at the second liver biopsy.

In conclusion, liver histology investigated of a child with chronic hepatitis C has few chances to highlight severe lesions. We question how the knowledge of histological assessment could affect the management of chronic HCV infection in children. Is it necessary to systematically perform liver biopsy in children with chronic HCV infection before starting therapy? Which information regarding the treatment will it add?

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Epidemiological profile of 806 Italian children with hepatitis C virus infection over a 15-year period

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Background/aims: To evaluate the epidemiological profile of Italian children with hepatitis C virus (HCV) infection over a 15-year period.


Results: Seven hundred and sixty four were Italian and 42 from foreign countries. Newly-diagnosed cases declined from 332 in 1995–1999 to 196 in 2000–2004, while the proportion of foreign children rose from 3% to 13%. Transfusion-transmitted infection disappeared after 1992. Maternal infection (with drug abuse in 63% of cases in the North) has become the most important mode of HCV diffusion throughout Italy and the exclusive source for all children infected in 2000–2004. The prevalence of HCV genotypes 3 and 4 increased and that of genotype 1b decreased significantly (p < 0.02). Male/female ratio was significantly lower among vertically infected (0.6) than in transfused children (1.3).

Conclusions: The number of children with newly-diagnosed HCV infection is declining in Italy and most post-transfusion cases are now young adults. Thus, foreign children could significantly contribute to the reservoir of pediatric infection in years to come. New infections result from maternal transmission and seem to privilege females and genotypes 3 and 4.

Keywords: Hepatitis C virus (HCV); Epidemiology of HCV infection; HCV infection in children; HCV vertical transmission

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Abbreviations: HCV, hepatitis C virus; HCV RNA, hepatitis C virus RNA; ANTI-HCV, antibodies to HCV; HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy.

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1. Introduction

Infection with hepatitis C virus (HCV) is a worldwide health problem [1]. In Italy the prevalence of HCV antibodies (anti-HCV) in the general population averages 3% with a considerable variability between Northern and Southern regions. After the disappearance of post-transfusion hepatitis [2], intravenous drug abuse and high-risk sexual behavior are maintaining the reservoir of infection in adults and groups of adolescents [3-6]. HCV infection is uncommon in children, because vertical transmission, which is responsible for most "new infections" in the Western World, has an efficiency of only about 5% [7-12]. In the early Nineties the estimated prevalence of pediatric infection in Italy was 0.3% [13] and multicenter studies were clearly needed to evaluate the epidemiological and clinical aspects of infection in this setting. A national Observatory for HCV infection and Hepatitis C in Italian children was consequently created in 1998 to take a retrospective-prospective census of HCV-infected children referred to tertiary care centers [14]. The analysis of data retrospectively collected from 1990 to 1998 confirmed the almost complete disappearance of post-transfusion hepatitis and a concomitant increase in the proportion of vertically-infected children. Maternal drug abuse was a prominent source of infection in Northern Italy, whereas transfusions and mothers with covert exposure were likely to be responsible for the majority of cases in the South. On the other hand, the map of HCV genotypes recently investigated in a sizable proportion of the same population [15] showed a declining prevalence of genotypes 1b and 2 and an increasing number of cases with types 3 and 4. Taken together these data suggest that rapid changes in the epidemiology of HCV infection may be underway among children in Italy. To evaluate the extent of these changes, which could influence the future burden of HCV infection, we investigated the epidemiological profile of a large cohort of anti-HCV positive children over a 15-year period. The specific purpose of this prospective/prospective study was to answer the following questions:

(a) Is the number of HCV infected children decreasing over the years?
(b) Did the map of putative exposure to HCV change during the survey?
(c) Are these changes correlated with the HCV genotype distribution?
(d) Are the putative changes related to the children’s geographic origin?

In addition, since maternal-infant transmission has become a major mode of HCV acquisition in the pediatric setting, we have investigated the time trends of related events such as: (a) maternal drug abuse, which is thought to promote transmission, and HIV coinfection which is known to facilitate the contagion; (b) mode of delivery and feeding which reflects the efficacy of counseling.

2. Materials and methods

2.1. Design of the Observatory

The Observatory was designed in 1998 by the Hepatology Group of the Italian Society of Pediatric Gastroenterology and Hepatology (SICEP, now SIGEP) for the purpose of recruiting consecutive anti-HCV positive children referred to tertiary care pediatric centers in Italy. The study was retrospective/prospective, based on the clinical records of children seen between 1990 and 1998 and on data collected at the initial visit in cases born between 1998 and 2004. It included children aged 6 months to 16 years, with no concomitant systemic disorders such as thalassemia, malignancy, autoimmune or metabolic disease, or confection with human immunodeficiency virus (HIV) or hepatitis B virus. Participation in the study was open, but the epidemiological data evaluation was restricted to centers meeting the following requirements: (a) survey conducted throughout the observation period; (b) referral area unchanged during the survey; (c) at least 10 subjects enrolled over the entire observation period.

2.2. Methods

The diagnosis of HCV infection relied on the detection of anti-HCV in serum by second- and third-generation commercial ELISA. HCV RNA seropositivity was required for the diagnosis in children born of infected mothers aged 18 months or less, given the possibility of passive antibody transmission from mother to newborn. HCV RNA was measured in fresh or well-preserved stored sera by polymerase chain reaction, using homemade or commercial qualitative assays. The putative time of exposure to infection was conventionally defined as follows:

(a) time of first transfusion, surgery or therapeutic injection for parenterally exposed children;
(b) time of birth for children whose mothers were known to be anti-HCV positive at delivery;
(c) time of first diagnosis of infection or liver disease in children whose maternal infection was not known at delivery;
(d) time of first diagnosis of infection or liver disease in children with unknown exposure.

A file was completed for each patient, recording age and sex, putative source of infection, mode of observation, maternal drug abuse, type of delivery and type of feeding. Privacy was assured by replacing patients’ names with codes and dates of birth in the database. Data were collected by the coordinating center in Padua, were checked for completeness and internal consistency, and amended by correspondence with the investigators. After checking for inconsistencies, the final database consisted of 806 consecutive subjects. This study was conducted in accordance with the principles of the Helsinki Declaration and of national laws, and was approved by the Ethical Committee of Padua Hospital/University.

2.3. Statistical data analysis

Quantitative variables were expressed as mean ± SD and analyzed using Student’s t-test, Wilcoxon’s rank sum test and one-way analysis of variance (ANOVA, Duncan’s multiple comparison). Qualitative variables were expressed as frequencies and percentages. The ε²-test was used as appropriate, all p values being two-tailed.
3. Results

Fifteen centers took part in the study: 9 in Northern, 4 in Central and 2 in Southern Italy. The minimum contribution per center was 10 cases, the maximum 175. Nine were referral centers for viral hepatitis although two centers (one in the North and one in Central Italy) had conducted a study on mother-infant HCV transmission, lasting one and two years, respectively, in the Nineties.

3.1. Demographic data and geographic distribution

Overall, 806 children were enrolled: 764 were of Italian origin and 42 were adopted or immigrant children from foreign countries. Table 1 summarizes the demographic features and the mode of recruitment of Italian children as a whole and by region of origin. Infected children were evenly distributed over Northern, Central and Southern Italy. Mean age at first visit was significantly lower (\( p < 0.001 \)) in Northern and Central than in Southern Italy. Only a minority of our patients presented with symptoms or signs of liver disease: maternal screening was the most common event bringing the children under our observation.

3.2. Distribution of cases by year of observation

Fig. 1 shows the distribution of the 806 cases by year of first visit at the referral centers. Overall there has been a 40% decrease in the number of children observed in the period 2000-2004 versus those recruited from 1995 to 1999. Conversely, the number of foreign children, though small, has increased significantly (\( p < 0.0001 \)) in recent years.

3.3. Distribution of cases by type of exposure and by year of putative exposure

Table 2 summarizes the distribution of the 764 Italian children based on the putative type of exposure to HCV in different periods during the survey. Blood transfusions – for the vast majority received during the first year of life – were the most frequent source of viral infection before 1990, while no transfusion-related cases were seen after 1992. Maternal transmission has thus become the prevalent route, responsible for more than 90% of cases observed since the year 2000. The proportion of mothers screened for anti-HCV before delivery raised from 48% (84/175) before 1995, to 98% (114/129) between 1995 and 1999, and to 98% (41/42) in most recent years. Male/female ratio was significantly (\( p < 0.001 \)) different among children of infected mothers.

Table 1

<table>
<thead>
<tr>
<th>Geographic origin of children</th>
<th>Total</th>
<th>North</th>
<th>Center</th>
<th>South</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>N cases</td>
<td>764</td>
<td>276</td>
<td>219</td>
<td>270</td>
<td>–</td>
</tr>
<tr>
<td>M/F</td>
<td>0.88</td>
<td>0.79</td>
<td>1.00</td>
<td>0.93</td>
<td>0.38</td>
</tr>
<tr>
<td>Mean age at first visit (±SD years)</td>
<td>5.8 (±4.6)</td>
<td>5.2 (±4.7)</td>
<td>5.5 (±4.7)</td>
<td>6.7 (±4.6)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Mode of recruitment: N cases (%)

- Symptomatic: 67 (8.5%)
- Maternal screening\(^a\): 324 (42%)
- Post transfusion screening\(^b\): 106 (15.5%)
- Screening for adoption: 23 (4%)
- Intercurrent disease: 174 (23%)
- Other/unknown: 115 (15%)

\( ^a \) \( p = 0.0001 \) South vs North; \( \text{p} = 0.0001 \) South vs Center.

\( ^b \) \( p = 0.0001 \) South vs North; \( \text{p} = 0.001 \) South vs Center.

\( ^c \) \( p = 0.04 \) South vs North; \( \text{p} = 0.04 \) South vs Center.

\( ^d \) \( p = 0.0001 \) South vs North and South vs Center.
Table 2
Type of exposure to HCV in relation to the putative time of exposure

<table>
<thead>
<tr>
<th>Type of exposure</th>
<th>Prevalent time of exposure</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfusion*</td>
<td>200 (55)</td>
<td>17 (10)</td>
</tr>
<tr>
<td>Other paternal exposure</td>
<td>40 (11)</td>
<td>10 (5)</td>
</tr>
<tr>
<td>Infected mother</td>
<td>62 (22)</td>
<td>123 (44)</td>
</tr>
<tr>
<td>Infected family contact</td>
<td>7 (2)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>26 (10)</td>
<td>24 (14)</td>
</tr>
<tr>
<td>Total</td>
<td>355</td>
<td>177</td>
</tr>
</tbody>
</table>

None of the children with a history of blood transfusions had received blood after 1992.

3.4 Distribution of cases by type of putative exposure

Table 3 shows the distribution of the potential sources of infection in the 764 Italian children, divided by geographic area of origin. Overall, the majority of children recruited in Northern and Central Italy had an HCV infected mother, whereas percutaneous exposure accounted for up to 50% of infections in Southern Italy.

3.5 HCV genotype distribution

Table 4 shows the distribution of HCV genotypes in relation to the geographic area of origin and the putative time of exposure. The prevalence of genotype 1b is decreasing, while that of types 3 and 4 is increasing. Overall, genotype 1 was the single most frequent genotype throughout the survey, with a prevalence rate of 67% in children investigated up to 1999 and of 42% in those observed from 2000 to 2004.

3.6 Events related to maternal infection

(a) Table 5 shows the distribution of HCV infected children whose mothers were drug abusers and non-drug abusers, in relation to geographic origin, time of exposure and maternal HIV co-infection.

(b) Information on type of delivery (vaginal or caesarean) was available for 261 non-HIV infected mothers. The frequency of caesarean delivery increased significantly over the years, from 17% among mothers who delivered before 1990 to 28% at the end of the Nineties, to 42% from 2000 to 2004 (p = 0.018). The prevalence of formula-fed children is also increasing, from 24% before 1990 to 42% at the end of the Nineties, to 44% from 2000 to 2004, although the trend does not reach statistical significance (p = 0.20).

Table 3
Prevalence of putative types of exposure to HCV infection in the whole cohort and in the different geographical areas

<table>
<thead>
<tr>
<th>Type of exposure</th>
<th>Geographic origin</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>North</td>
</tr>
<tr>
<td>Transfusion*</td>
<td>217 (28)</td>
<td>63 (23)</td>
</tr>
<tr>
<td>Other paternal exposure</td>
<td>53 (7)</td>
<td>18 (6.5)</td>
</tr>
<tr>
<td>Infected mother</td>
<td>409 (54)</td>
<td>175 (22.5)</td>
</tr>
<tr>
<td>Infected family contact</td>
<td>11 (1)</td>
<td>4 (1.5)</td>
</tr>
<tr>
<td>Unknown</td>
<td>74 (10)</td>
<td>13 (6.5)</td>
</tr>
<tr>
<td>Total</td>
<td>764</td>
<td>278</td>
</tr>
</tbody>
</table>

* Blood transfusion South vs North, p = 0.008; South vs Center, p = 0.007.

Table 4
Prevalence of putative types of exposure to HCV infection and in the different geographical areas

<table>
<thead>
<tr>
<th>Type of exposure</th>
<th>Prevalent time of exposure</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfusion*</td>
<td>200 (55)</td>
<td>17 (10)</td>
</tr>
<tr>
<td>Other paternal exposure</td>
<td>40 (11)</td>
<td>10 (5)</td>
</tr>
<tr>
<td>Infected mother</td>
<td>62 (22)</td>
<td>123 (44)</td>
</tr>
<tr>
<td>Infected family contact</td>
<td>7 (2)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>26 (10)</td>
<td>24 (14)</td>
</tr>
<tr>
<td>Total</td>
<td>355</td>
<td>177</td>
</tr>
</tbody>
</table>

* p = 0.0001 before 1990 vs 1990–1994.

* p = 0.0001 before 1990 vs other time periods.

* p = 0.0001 before 1990 vs 1990–1994; p = 0.0001 before 1990 vs 1995–1999.

* p = 0.0001 before 1990 vs 1990–1994; p = 0.0001 before 1990 vs 1995–1999.
Table 4

Distribution of HCV genotypes in 402 children in relation to area of origin and time of putative exposure

<table>
<thead>
<tr>
<th>Geographic origin</th>
<th>1a</th>
<th>1b</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>15 (22)</td>
<td>56 (35)</td>
<td>28 (18)</td>
<td>26 (16)</td>
<td>12 (3)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Center</td>
<td>30 (22)</td>
<td>47 (35)</td>
<td>21 (15)</td>
<td>25 (18)</td>
<td>8 (6)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>South</td>
<td>25 (15)</td>
<td>83 (59)</td>
<td>37 (22)</td>
<td>9 (5)</td>
<td>5 (3)</td>
<td>9 (5)</td>
</tr>
<tr>
<td>N</td>
<td>0.16</td>
<td>0.01</td>
<td>0.32</td>
<td>0.01</td>
<td>0.17</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Time of exposure

* Genotype 1b; p = 0.01, South vs North; p = 0.01, South vs Center. Genotype 3; p = 0.001, South vs North; p = 0.001, South vs Center.


3.7. Features in the 42 foreign children

Male/female ratio was 1.1 and mean age at first observation was 5.7 ± 3.6 years. Twenty-two children had been recruited after post-adoption screening. 26 had no known source of infection, 8 had an infected mother and 8 had had blood transfusions or unsafe injections. HCV genotype 1 was detected in 12 (66%) of 18 cases tested.

3.8. The burden of infection at the end of the survey

During the survey, 106 (21%) out of 509 children with available clinical data were treated with standard protocols using recombinant IFN either alone or, in a few cases, combined with ribavirin. Most treated children, independent of the geographic area of origin, had been included in therapeutic trials. The response rate (i.e., percentage of patients with sustained HCV RNA seroclearance 6 months after withdrawal of therapy) was 35%. Of the 37 responders, 62% were infected with HCV type 2 or 3. By the end of 2004, 328 (40%) children were over 16 years of age.

4. Discussion

Several reports on adults in the Western World suggest that the epidemiology of HCV infection is changing, both in the general population and in selected risk groups, due to intercurrent socio-sanitary events, such as HCV screening of blood donors, HIV and HCV prevention campaigns, changing patterns of drug abuse and efficient therapy for eradicating the infection [1,16–22]. These events may conceivably have influenced the pattern of HCV spread in childhood too, but no much information is available in the pediatric literature [14,15]. This prospective/retrospective multicenter study updates and extends the epidemiological information obtained by an Italian Observatory in a large pediatric population referring to 15 pediatric centers in Italy between 1990 and 2004. Several changes seem to have taken place if we consider our sample in three different 5-year time brackets from 1990 to 2004 and the geographical distribution of cases.

The number of HCV-infected children seen in Italy from 2000 to 2004 is more than 40% lower than in the 5 years before. After the disappearance of post-transfusion hepatitis, other events may also contribute to this finding, i.e., the aging of women infected by past transfu-
sions or covert exposure, the changing modes of drug abuse, HAART therapy in HIV-infected pregnant women and the efficacy of treatment with IFN and ribavi

...rvin in women of childbearing age. The pattern of risk factors has also changed significantly. In this study having an infected mother has become the single most important risk for Italian children, including 100% of those born in the 2000s. Overall 69% of infected mothers were known to be anti-HCV positive at delivery and, based on national data [8,12,22,24] it can be assumed that 63–76% of them were HCV RNA seropositive.

These considerations suggest that most children had been likely infected in utero or at birth, although vertical transmission can be regarded as an infrequent event in HIV seronegative mothers. On the other hand postnatal infection by breastfeeding cannot be excluded but is likely to be rare and the role of maternal infection in the family setting remains speculative. In a recent study in the general Italian population Del Corno et al. [25] reported a higher risk of infection in offspring exposed to HCV seropositive mothers in comparison with offspring not exposed. Minola et al. [36], however, investigating the families of 285 consecutive HCV infected adults found that the overall rate of infection for offspring was low (2.3%).

The map of HCV genotypes is also changing. The present study confirms the finding of our recent paper [15] showing that the prevalence of genotypes essentially linked to drug abuse (types 3 and 4) is increasing, while the prevalence of genotype 1b is declining. Several studies have assessed the epidemiological aspects of hepatitis C in Italian adults [16-18,27-29] and some authors [19] identified a clear North-to-South gradient of increasing anti-HCV prevalence, suggesting the existence of two well-characterized transmission patterns: one in older individuals from Southern and Central Italy, especially attributable to the use of disposable syringes for health care practices, the other in young adults in Northern Italy, linked to drug abuse and immigration. Our results are in keeping with these considerations: children in Southern Italy were significantly older at their first visit, 40% had a history of transfusions, and 37% had infected mothers, often with no history of drug-taking, whereas 62% of the cases in Northern Italy had an infected mother with a history of drug abuse in 68% of cases. The prevalence of females was significantly greater among children with infected mothers than among those with a history of transmission, which is consistent with a large European study on mother-to-infant HCV transmission [30] showing that girls were twice as likely to be infected as boys. The authors hypothesized that genetic and hormonal factors might make females more susceptible to HCV infection.

In recent years national [31] and international [32] guidelines have discouraged universal HCV screening for pregnant women due to the low rate of mother-to-child transmission and the lack of any treatment options for preventing newborn infection. Screening could be cost-effective in a high risk population, such as pregnant women with a history of drug abuse in Northern Italy: the diagnosis of infection would allow counseling, antiviral treatment soon after delivery and early screening of the child. Jhaveri et al. [33] estimate that the medical costs of chronic HCV infection in children will be significant over the next 10 years and underline the advantages of early diagnosis and treatment. Our clinical observations support this view, showing that severe liver disease may develop during the first two decades of life [34].

Both national guidelines [35] and the international literature [36] encourage non-HIV coinfected mothers to deliver vaginally and breastfeed their children. In our series, the proportion of infected mothers delivering by cesarean section increased during the survey, but similar figures [37] have been recorded in the general population in Italy, suggesting that factors other than HCV have influenced mode of delivery. The proportion of breastfed children was 55% at the end of this survey, as compared to about 90% of children of uninfected mothers [38]. This discrepancy seems to emphasize the limited efficacy of official guidelines.

What is the contribution of pediatric infection to the pool of adults with chronic hepatitis C? Our data and previous reports [31,32] suggest that the burden of HCV infection acquired early in life is not only a matter of concern for the pediatrician. By the end of 2004, a consistent proportion of our patients were over 16 years, thus entering follow-up in adult health care facilities. In parallel with the increasing number of foreign children living in Italy [39], the proportion of immigrants or adopted children in our study rose from 8% in the Nineties to 15% in 2000–2004, thus adopted or immigrant children might contribute significantly to maintaining the reservoir of infection in years to come.

Our study is not without limitations. First of all, there is the retrospective collection of data in older patients. Due to the mode of recruitment in tertiary care centers, this population might also suffer from a selection bias, preventing the generalizations of our data. It is worth noting, however, that only a minority of cases presented with symptoms of liver disease, while most children were referred after a serological screening. Each participating center maintained the same referral area over the years and only two conducted a study on mother-infant HCV transmission in the mid-nineties. This relatively homogeneous mode of recruitment in the retrospective and prospective parts of the study, the consistent size of the sample distributed in three geographical areas and the substantial agreement between our data and the epidemiological trends seen in Italian adults [17,18] lead us to believe that our sample may be representative of the population of otherwise healthy HCV-infected children in Italy.
In conclusion, our data show a 40% reduction in the number of children with HCV infection reared in the years 2000–2004 by comparison with the previous 5 years. These findings indirectly suggest that the incidence of pediatric infection is decreasing in Italy. The source of infection is now almost exclusively represented by HCV infected mothers, a still consistent proportion of whom admit to drug abuse, especially in the North. Prevention campaigns and counseling for young people about illicit drug use, screening of women at risk and subsequent consideration for antiviral treatment may limit the spread of infection. Adopted or immigrant children from endemic countries could significantly contribute to the reservoir of pediatric infection in Italy in years to come.

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In the above paper the following persons also contributed to the Italian Observatory for HCV Infection and Hepatitis C in Children: Anna Maccabruni, Dept Infectious Diseases, Policlinico S. Matiá, Pavia, Nadia Guisotti, Dept. Infectious Diseases, Azienda Ospedaliera, Padua, Fiorella Palli, Pediatric Clinic, University, Modena, Alessandra Buja, Dept. of Hygiene, University, Padua.

References

participation in physical activity by those patients with reduced exercise capacity does not necessarily require "strict monitoring" by a cardiologist. In our study, we performed maximal symptom-limited treadmill testing in 37 individuals with NAFLD without adverse events, despite the fact that most patients manifested several comorbidities. Obviously, appropriate management of comorbidities will clearly reduce the risk of an adverse event during physical activity participation of substantial exertion. Requiring cardiological supervision for physical activity participation adds another potentially significant barrier (and expense) for these patients to adopt and maintain a regular exercise program.

We certainly agree that our lack of appropriate control groups (nonobese, albeit with abdominal obesity, one with both insulin resistance [IR] and NAFLD and the other with only IR) is a limitation of this study. However, the intent of this initial study was not to determine whether objective measures of health-related fitness and physical activity differ with severity of NAFLD. Furthermore, in practice, it would be quite difficult to include such control groups of matching size for the following reasons: (1) nonobese patients with IR and NAFLD are relatively uncommon and (2) patients with IR without NAFLD frequently lack histological confirmation.3,4

Indeed, reduced lean mass, small cross-sectional muscle fiber area, and increased triglyceride deposition in skeletal muscle in conjunction with dysfunctional/insulin resistance can result in reduced cardiovascular fitness. However, it is unknown whether these adaptations take place as a result of reduced physical activity, NAFLD, or both.

We agree entirely that for exercise to be successfully implemented as a therapeutic strategy there is a strong need for individualized exercise prescription. As such, we have recently submitted a manuscript for publication that reviews evidence for exercise training as a therapy for NAFLD and also presents the principles for prescribing exercise as a therapeutic intervention. Future studies will aim to better determine the relative contributions of IR, adiposity, and NAFLD to reduced fitness.

References


Children with Chronic Hepatitis C: What Future?

To the Editor:

We read with interest the study by Goodman et al. in which hepatic histological features of 121 treatment-naive children with chronic hepatitis C (CHC) were investigated. The authors reported that inflammation was minimal in 43% of the patients, mild in 17%, moderate in 38%, and severe in only 3% of patients. As for fibrosis, five (4%) patients had bridging fibrosis and two (2%) cirrhosis, whereas the remaining had a stage of fibrosis between 0 and 2 according to Ishak. Although the sample of children with bridging fibrosis or cirrhosis included only seven patients, a significant correlation between inflammation and fibrosis was found. There was a weak correlation between duration of infection and fibrosis, although the authors concluded that the positive correlation of inflammation with duration of infection and fibrosis suggested that children with CHC will be at risk for progressive liver disease as they age and possibly acquire other comorbid risk factors. Furthermore, Goodman et al. compared their histological findings with large published pediatric series. A previous study of ours regarding the long-term outcome of disease in a large series of children with CHC was not considered in our study. We not only confirmed that CHC in children is morphologically mild in most cases, but it also showed that fibrosis progression is relatively slow and cirrhosis extremely rare. During a 18-year period, no child had decompensated liver disease or required liver transplantation. In addition, no linear correlation between duration of disease and progression of fibrosis was found. Two of three children with moderate fibrosis (stage 3) at the time of the first biopsy had a short duration of disease (2.1 and 2.5 years). On the other hand, the only patient with absence of fibrosis had a disease duration of 13.9 years. Therefore, the severity of liver disease did not seem to depend on the duration of hepatitis C virus (HCV) infection. As for the long-term outcome of CHC in adults, it has been reported that 5%-20% of adults developed cirrhosis over periods of 20-25 years, whereas the majority of patients had a favorable course. In another study in children infected as young military recruits, the frequency of cirrhosis after 50 years was only 4%. No linear correlation was found between duration and severity of disease. Furthermore, it seems that higher degrees of fibrosis were related with age at infection being more frequent in patients over 265 years regardless the duration of infection. Finally, it has been shown that the majority of fibrosis progression occurred in patients aged 30 years or older.

In conclusion, on the basis of the majority of studies concerning the long-term outcome of HCV infection in children and adults, we think that the conclusion of Goodman that chronically HCV-infected children will be at risk for end-stage liver disease as adults is not adequately supported by results observed so far.

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References


Reply:

We thank Iorio et al. for their interest in our work and for making us aware of their study, which included some information on liver biopsies in 64 children. Their findings support those of our and other series, namely that chronic hepatitis C is not as frequent in children as previously estimated. By design, our own study excluded children with decompensated cirrhosis, so if anything, our collection of liver biopsies could have been biased toward more severe disease. None of the patients in the study by Iorio et al. initially had cirrhosis, but it is noteworthy that 5 of 29 children (17.2%), 2.1-2.7, and 13.6 years, already had stage 4 fibrosis, which is defined as marked bridging fibrosis. Furthermore, one child in that series, who was initially stage 0, progressed to stage 5 (compensated cirrhosis) on subsequent biopsy after failing antiviral therapy. Although not observed in the reported large series, there are even reports of hepatocellular carcinoma following hepatitis C in adolescents. All observers agree that a minority of patients with hepatitis C will progress to cirrhosis and its complications, although there are known risk factors for progression. It is impossible to know for certain which patients will progress. The fact that some children already have advanced fibrosis, even with a short duration of disease and with no obvious clinical differences from the rest, can only lead to the conclusion that an unknown proportion of others will eventually have the same outcome if there is not an effective therapeutic intervention.

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References


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2.4 Liver steatosis in children with chronic hepatitis B and C

2.41 Rationale

Hepatic steatosis is defined as fat deposition in the liver, with more than 5% of hepatocytes containing fat deposits. Hepatic steatosis may contribute to liver disease increasing sensitivity to oxidative stress and cytokine-mediated hepatic damage (1). The causes of fatty liver in children are enumerable as compared to adults and can be divided into hepatic and non-hepatic (2,3). Chronic hepatitis B and C have been frequently associated with hepatic steatosis. The frequency of steatosis in adults with chronic hepatitis B ranges from 18 to 76% while in chronic hepatitis C it is between 31 and 72% (4-8).

While the pathogenesis of hepatic steatosis in chronic hepatitis B seems to be related to metabolic factors, in chronic hepatitis C viral factors (HCV genotype 3) play a role, in addition to obesity, dyslipidaemia and insulin resistance (6,8). Evidences supporting the direct steatogenic role of HCV include the positive correlation between the presence and severity of steatosis and the viral load and the reduction of steatosis in presence of a sustained response to antiviral therapy (clearance of serum HCV RNA) (9-11). Hepatic steatosis impacts on the degree of inflammation and fibrosis and may influence the long-term prognosis of chronic hepatitis C (8,12,13).

Data on the prevalence and significance of hepatic steatosis in children with chronic hepatitis B and C are scarce. Furthermore, the role of host-viral interactions might be better understood in children since they haven’t so many co-factors responsible for steatosis such as alcohol intake, dyslipidaemia, insulin resistance, type 2 diabetes or metabolic syndrome as adults have.

Aim of this research was to investigate the prevalence of histological evidence of hepatic steatosis at liver biopsy in pediatric patients with chronic hepatitis B and C. Further, we investigate the correlation between hepatic steatosis and clinical, viral, metabolic and histological factors.
2.42 Experimental procedures

Patients
A total of 120 consecutive otherwise healthy children with chronic HBV infection (56 patients) or chronic HCV infection (64 patients) who underwent liver biopsy for diagnostic purposes at the Pediatric Liver Unit of the University Federico II were enrolled. Diagnosis of chronic hepatitis B was based on the presence of HBsAg in serum for 6 months or longer. Diagnosis of chronic hepatitis C was based on the presence of serum anti-HCV antibodies and HCV RNA lasting more than 6 months. Exclusion criteria included the presence of concomitant systemic diseases, concurrent infection with major hepatotropic viruses or HIV, or presence of other liver disease. Patients with thyroid diseases, malnutrition, diabetes, dyslipidaemia, exposure to hepatotoxic drugs or alcohol were also excluded from the study. None of the patients had received antiviral treatment before liver biopsy.

The database and the medical records were used to retrieve the following information: demographic data, primary risk factors for HBV or HCV infection, year of infection, clinical features with particular regard to the presence of hepatomegaly, splenomegaly, signs of hepatic decompensation, anthropometric and laboratory data. As for anthropometric data, height, weight and body mass index (BMI), calculated as Kg/m$^2$, were evaluated at the time of liver biopsy. Overweight was defined by BMI $\geq 90^{th}$ percentile for age and obesity by BMI $\geq 95^{th}$ percentile for age, according to CDC standards (14). For each patient the following laboratory evaluation was performed at or near the time of liver biopsy: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, albumin, prothrombine time, international normalized ratio, blood cells count, fasting plasma glucose, insulin, cholesterol and triglycerides serum levels, as well as viral markers (for children with chronic hepatitis B: HBsAg/HBsAb, HBeAg/HBeAb, serum HBV DNA; for children with chronic hepatitis C: serum HCV RNA). For patients with chronic hepatitis C who had received antiviral therapy after liver biopsy, the correlation between the presence of hepatic steatosis and response to therapy was also analyzed. A sustained response to treatment was defined as absence of serum HCV RNA after 24 weeks following the end of therapy.

Patients were included in the present study if anthropometric, clinical, laboratory and histological data were complete.
**Laboratory procedures**

Liver function tests, fasting glucose, cholesterol and triglycerides were measured by standard laboratory methods. Biochemical and virological tests were performed on fresh or frozen serum samples.

HBV markers (HBsAg/HBsAb, HBeAg/HBeAb) were measured using commercial immunoassay kits (Abbott Diagnostics, Chicago, IL, USA). Serum HBV DNA was quantitatively investigated, depending on the time, with a commercial hybridization method with a cut-off value of 5 pg/ml (Abbott Diagnostics, North Chicago, IL, USA) or with a commercial PCR assay (Amplicor HBV-Monitor kit, Roche Diagnostic System, NJ, USA).

Anti-HCV antibodies were tested by an enzyme-linked immunosorbent assay (ELISA, third-generation). HCV RNA was measured by quantitative RT-PCR method (Amplicor Monitor, Roche Diagnostics) with a detection limit of approximately 600 IU/ml serum. HCV genotyping was performed with a second-generation reverse hybridization line probe assay (Inno-Lipa HCV II, Innogenetics) following the manufacturer’s protocol.

Insulin resistance was calculated from fasting plasma insulin and glucose by the homeostasis model assessment (HOMA) method proposed by Matthews (15).

**Histological evaluation**

Liver specimens were formalin-fixed and paraffin embedded for histological evaluation. Histological analysis of the liver sections was performed by two experienced pathologists blinded to the clinical data. Liver histology was evaluated according to Ishak scoring (necroinflammation score: 0-18, fibrosis score: 0-6) (16). Hepatic steatosis was graded semiquantitatively by determining the percentage of affected hepatocytes according to the Brunt classification: grade 0 (<5% of hepatocytes involved), grade 1 (5–33% involved), grade 2 (34–66% involved), grade 3 (>66% involved hepatocytes) (17,18).

**Statistical analysis**

Statistical analysis was performed using a statistical program package (GraphPad Instat 3 Software). Descriptive data were expressed as median and range or mean and standard deviation (SD). Clinical, laboratory and histological differences between groups were assessed using the Mann-Whitney test (continuous variables) or chi-square test (categorical variables). Correlation analysis was performed by the Spearman test as appropriate. For all tests, results with p values less than 0.05 were considered statistically significant.
2.43 Results

Children with chronic hepatitis B

All 56 patients (median age 8.1 years, range 2.2-17.3) were symptom free and none of them have decompensated liver disease. All patients were HBsAg positive at the time of liver biopsy, 48 (85.7%) patients were HBeAg positive at the time of liver biopsy, 3 (5.4%) patients had already obtained spontaneous seroconversion to HBeAb, in 5 (8.9%) cases HBeAg status was unknown. All but two patients had detectable HBV DNA in serum at the time of liver biopsy. In children with detectable viremia, median serum HBV DNA was 104710 copies/ml (range 330-854660).

Fifty-five (98.2%) patients had fibrosis at liver biopsy while one child had no fibrosis; the stage of fibrosis was 1 in 19 (34.6%) children, 2 in 30 (54.5%) cases, 3 in 3 (5.4%) cases and 4 in one (1.9%) child. Two (3.6%) patients had histological evidence of micronodular cirrhosis (fibrosis score 5).

Fifty-four patients had no steatosis at liver biopsy; steatosis was present only in 2 (3.6%) children (grade 1 in both cases). Features of patients with steatosis are reported in table 1.

Twelve (22.2%) of 54 patients without steatosis and both (100%) patients with steatosis were overweight (6 and 1, respectively) or obese (6 and 1, respectively) (p=0.059). Relative BMI did not statistically differ between the two patients with steatosis (126.4±1.2) and 12 overweight/obese children without steatosis (125.7±12.3).

Mean relative BMI was significantly higher in 2 patients with steatosis compared with the whole group without steatosis (p=0.04).

No significant difference between patients with and without steatosis was found with regard to demographic parameters (age, sex, route of HBV infection) and biochemical features (fasting glucose, triglycerides, cholesterol, ALT values, GGT values). Further, serum HBV DNA levels, HBeAg positivity and histological features did not significantly differ between the two groups.

Children with chronic hepatitis C

All 64 patients (median age 8.3 years, range 2.5-14) with chronic hepatitis C were symptom-free and none of them have a decompensated liver disease. Among the studied children, 16 (25%) had hepatic steatosis on liver biopsy specimen. In particular, steatosis was mild in 10 (62.5%) patients and moderate in the remaining 6 (37.5%) children. None of the patients had signs of severe steatosis. Steatosis was macrovesicular and located in the periportal area rather than in the centrilobular area. Histopathologic features of nonalcoholic steatohepatitis...
such as perisinusoidal and perivenular fibrosis and ballooned hepatocytes were absent. Features of children with steatosis are reported in table 1.

Clinical, laboratory and histological features at liver biopsy were compared in children with and without steatosis and no difference was found between the two groups. The most represented HCV genotype was 1b in both groups (24 children without and 8 with steatosis). Five (10.4%) children without steatosis and 3 (18.7%) with steatosis were overweight, while 5 (10.4%) patients without steatosis and 3 (18.7%) with steatosis were obese (p>0.05).

Although no significant difference in the necroinflammatory and fibrosis scores was found between children with and without steatosis, it is to note that 3 (18.7%) out of 16 patients with hepatic steatosis had a fibrosis score > 2 with respect to 1 out of 48 patients without steatosis (2.1%, p<0.05). It is also to note that these 4 patients were the sole subjects having a more severe degree of fibrosis (fibrosis score 3 or 4), while in the remaining 60 children the fibrosis score ranged from 0 to 2.

Non-parametric correlation analysis showed that the severity of steatosis, expressed by steatosis score, significantly correlated with both ALT (r=0.27, p<0.05) and GGT (r=0.25, p<0.05). No relationship was found with relative BMI, HOMA or duration of HCV infection (p>0.05).

Only 3 children (2 without steatosis and 1 with steatosis) had GGT value over the normal range for age (1.7, 2.2 and 1.7 times the upper normal value, respectively), 4 children (3 without and 1 with steatosis) had cholesterol serum level upper the normal value (1.2, 1.05, 1.08 and 1.05 times the upper normal value, respectively), while no patient had hypertriglyceridemia.

Forty-seven children (34 patients without and 13 with steatosis) received a course of IFN therapy at standard dosage after liver biopsy. A sustained response was observed in 18 (53%) out of 34 treated patients without hepatic steatosis and in 3 (23%) out of 13 treated children with steatosis (p>0.05).
Table 1. Features of children with chronic hepatitis B and C and presence of steatosis at liver biopsy

<table>
<thead>
<tr>
<th></th>
<th>Patients with chronic hepatitis B and steatosis (n=2)</th>
<th>Patients with chronic hepatitis C and steatosis (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>2 (100%)</td>
<td>7 (44%)</td>
</tr>
<tr>
<td>Median age in years (range)</td>
<td>9.3 (5.6-13)</td>
<td>7.6 (2.7-13.6)</td>
</tr>
<tr>
<td>Route of infection:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Vertical transmission</td>
<td>2 (100%)</td>
<td>6 (37%)</td>
</tr>
<tr>
<td>- Others</td>
<td>0</td>
<td>10 (93%)</td>
</tr>
<tr>
<td>HCV genotype:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1</td>
<td></td>
<td>10 (63%)</td>
</tr>
<tr>
<td>- Others</td>
<td></td>
<td>6 (37%)</td>
</tr>
<tr>
<td>Mean relative BMI±SD</td>
<td>132±9</td>
<td>112.7±15.4</td>
</tr>
<tr>
<td>Median ALT values (IU/L (range)</td>
<td>133 (102-164)</td>
<td>117 (50-300)</td>
</tr>
<tr>
<td>Median GGT values (IU/L (range)</td>
<td>20 (14-26)</td>
<td>17 (8-79)</td>
</tr>
<tr>
<td>Median cholesterol levels (mg/dl) (range)</td>
<td>192 (174-210)</td>
<td>146 (116-201)</td>
</tr>
<tr>
<td>Median triglycerides levels (mg/dl) (range)</td>
<td>95 (68-123)</td>
<td>62 (35-99)</td>
</tr>
<tr>
<td>Median fasting glucose levels (mg/dl) (range)</td>
<td>85 (78-93)</td>
<td>72 (70-90)</td>
</tr>
<tr>
<td>Median insulin (mU/ml) (range)</td>
<td>-</td>
<td>10.15 (4.8-22)</td>
</tr>
<tr>
<td>Median serum HBV DNA (copies/ml) (range)</td>
<td>231494</td>
<td>-</td>
</tr>
<tr>
<td>Median serum HCV RNA (IU/L) (range)</td>
<td>-</td>
<td>220000 (40000-587000)</td>
</tr>
<tr>
<td>Histology:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Necroinflammatory score</td>
<td>4</td>
<td>4 (2-7)</td>
</tr>
<tr>
<td>- Fibrosis score</td>
<td>1</td>
<td>2 (1-4)</td>
</tr>
</tbody>
</table>

SD= standard deviation
2.44 Discussion

This study shows a very low frequency of liver steatosis in chronically HBV-infected children, while a higher prevalence was found in children with chronic hepatitis C. Recently, histologically proven steatosis has been found in 18% of 233 adults with chronic hepatitis B and it was mild in the majority of cases (4). Another study showed a percentage of steatosis higher (76%); however, it included patients with alcohol consumption, a well-known risk factors for steatohepatitis (7). The prevalence of histological evidence of hepatic steatosis found in our HCV-infected children is similar to that reported in adults, accounting for about 25% (6).

In HBV infection steatosis appears to be unrelated to virological factors. In previous studies on adults patients, only metabolic parameters (such as fasting glucose and overweight) were correlated with the presence of steatosis at liver histology while no correlation was found with HBV DNA titre and HBeAg status (4,7). In a double-transgenic mouse model expressing the HCV coding sequence and the HBV X gene-encoded regulatory protein HBx it has been showed that steatosis was more prevalent and severe in presence of HBx protein expression (19). Nevertheless, the direct steatogenic role of HBV has not yet been demonstrated. In our population of HBV-infected otherwise healthy children no correlation was found between viral factors and presence of steatosis. Hepatic steatosis was only associated with higher BMI. However, the number of children with chronic hepatitis B and steatosis was too small in order to evaluate if viral factors might play a role in determining liver steatosis.

In adults with chronic hepatitis C, two distinct mechanisms are involved in the development of hepatic steatosis. In patients infected with HCV genotype 1, metabolic factors of the host, such as obesity and metabolic syndrome, play a major role in intracellular lipid accumulation while in HCV genotype 3 infected adults steatosis seems to be viral-induced (6,8,20). At variance with these data, our results suggest a direct association between genotypes other than 3 and hepatic steatosis, since children with steatosis infected with genotypes non-3 had similar relative BMI and percentages of overweight and obese subjects with respect to patients without steatosis. Furthermore, according to a previous report (21), our data show that the degree of insulin resistance, expressed by HOMA, was not associated with hepatic steatosis. This observation is noteworthy in view of the debated role of HCV infection in the establishing of an insulin resistance state. It remains unexplained the reason why in children and not in adults genotypes non-3 are associated with hepatic steatosis in absence of metabolic causes. It would have been very interesting to explore the behaviour of hepatic steatosis in genotypes non-3 infected children who achieved a sustained response to IFN therapy, but for
ethical reasons a follow-up biopsy was not performed in these subjects. Owing to the low prevalence of genotype 3 in Italian children, in the present study only 3 patients were infected with this genotype, so the relationship between genotype 3 and steatosis was difficult to evaluate in this context. Therefore on the basis of the present data, we can not exclude a direct association between genotype 3 and steatosis.

As for the relationship between steatosis and severity of liver damage, it has been reported that adults with chronic hepatitis C and steatosis show a more severe liver disease in terms of necroinflammation, fibrosis and aminotransferases serum levels (6). We found no relationship between steatosis and activity of necroinflammation in liver histology, and the median serum level of transaminases did not differ between children with and without steatosis. It is to note that in the group of children with steatosis ALT serum levels significantly correlated with degree of steatosis. Furthermore, children with steatosis showed a significantly more severe fibrosis (fibrosis score > 2). It remains to be shown to what extent steatosis itself, or steatosis acting synergistically with other co-factors, contributes to the severity of fibrosis.

Analysis of treatment outcomes in children with chronic hepatitis C indicated that, although response to IFN was not significantly related to presence of steatosis, children with steatosis were less likely to achieve sustained response to therapy. This result, in agreement with previous studies in adults with chronic hepatitis C (9,22), suggests that steatosis might induce a mechanism of resistance to IFN treatment.

In conclusion, our research shows that steatosis in children with chronic hepatitis B is a rare event which seems to be related to the host rather than the effect of virus. On the basis of these findings, we think that the detection of liver steatosis in a non-obese child with chronic hepatitis B can not be explained with HBV infection but must be carefully investigated to rule out the other multiple causes of pediatric fatty liver. In children with chronic hepatitis C hepatic steatosis is a frequent finding and, differently from adults, genotypes other than 3 may be associated with steatosis independently from the presence of classical metabolic risk factors.
2.5 References


2.6 Publications


Is HCV infection associated with liver steatosis also in children?

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Background/Aims: Prevalence and significance of steatosis in children with chronic hepatitis C are not well defined. We analysed the prevalence of steatosis in children with chronic hepatitis C and its relationship with clinical, laboratory features and response to interferon.

Methods: Sixty-four consecutive children with CHC undergoing liver biopsy were retrospectively evaluated.

Results: Twenty-five percent of children showed mild to moderate steatosis. Only one child was infected by genotype 3. Body mass index did not significantly differ between children with and without steatosis. Although no significant difference in necroinflammatory and fibrosis scores between children with and without steatosis was found, 3 (18.7%) of 16 patients with steatosis and only one (2.1%) of 48 patients without steatosis had a fibrosis score >2 (P < 0.05). Forty-seven children (13 with steatosis) received interferon after liver biopsy. A sustained response was observed in 3 (23%) children with steatosis and in 18 (53%) without steatosis.

Conclusions: Histological evidence of steatosis is detectable in a quarter of children with CHC. Differently from adults, genotypes other than 3 may be associated with steatosis independently from classical metabolic risk factors. Children with steatosis seem to have more severe fibrosis and lower rates of sustained response to interferon therapy compared to children without steatosis.

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Keywords: Liver steatosis; Children; Liver fibrosis; Chronic hepatitis C

1. Introduction

Hepatic steatosis, defined as lipid accumulation in the hepatocyte cytoplasm, is a common histopathological finding demonstrable in liver biopsies of 31–72% of adults with chronic hepatitis C (CHC) [1–3]. Adult patients with hepatitis C virus (HCV) genotype 3 infection are more likely to have steatosis than those infected with other genotypes [3,4]. The pathogenesis is complex and both host and viral factors are involved [3–6]. It has been hypothesized that steatosis may be induced by metabolic factors (e.g. overweight, diabetes and hyperlipidemia) in genotype 1-infected patients, while it is primarily virus-induced in genotype 3-infected patients [5]. Interestingly, in adults only body mass index (BMI) and HCV genotype 3 were found to be independently related to steatosis in multivariate analysis [7]. The direct role of this genotype has been recently suggested by the positive relationship between the presence and severity of hepatic steatosis and HCV RNA load, while no association has been found with the other HCV genotypes [3,5,6]. Furthermore, sustained response to interferon (IFN) is accompanied by improvement or disappearance of steatosis in patients infected with HCV.
genotype 3; this phenomenon did not occur with other genotypes [4,8,9].

Recent studies have pointed out insulin resistance as an additional factor capable of influencing the degree of both steatosis and fibrosis in CHC [10,11]. Other studies have suggested that steatosis appears to be associated with more rapid progression of hepatic fibrosis and may influence the long-term prognosis of CHC [2,3]. Furthermore, steatosis was found to be the only independent factor associated with progression of fibrosis in multivariate analysis [12]. It has been also reported that adults with steatosis are less likely to achieve a response to IFN [8,9].

Currently there are scant data on the prevalence and significance of hepatic steatosis in children with CHC. Furthermore, the role of host-viral interactions might be better understood in children since they have not so many co-factors responsible for steatosis such as alcohol intake, dyslipidaemia, insulin resistance, type 2 diabetes or metabolic syndrome as adults have.

We retrospectively investigated the prevalence of histological evidence of hepatic steatosis in a group of consecutive children with CHC undergoing liver biopsy and compared clinical data, laboratory features and response to IFN therapy between patients with and without steatosis.

2. Patients and methods

2.1. Patients

A total of 64 consecutive otherwise healthy children (29 males) with CHC, undergoing liver biopsy for diagnostic purposes over a 12-year period at our Department of Paediatrics, were enrolled in this retrospective study in order to evaluate the prevalence of histological evidence of steatosis. In all cases diagnosis of CHC was performed in presence of serum anti-HCV antibodies and HCV RNA lasting more than 6 months.

All patients were investigated in order to assess their risk factors for HCV infection, clinical features, anthropometric parameters, presence of conditions associated to hepatic steatosis, liver function tests, serological markers of HCV replication.

Furthermore, in patients who received antiviral therapy after liver biopsy, the correlation between the presence of hepatic steatosis and response to therapy, evaluated according to conventional criteria, was also analyzed.

All patients had negative tests for hepatitis B surface antigen and HIV antibodies. None of them had autoimmune hepatitis, genetic liver disease, celiac disease, thyroid diseases, malnutrition, diabetes, dyslipidaemia, exposure to hepatotoxic drugs or alcohol.

The database and the medical records were used to retrieve the following information: demographic data, primary risk factors for HCV infection, year of infection, clinical features with particular regard to the presence of hepatomegaly, splenomegaly, signs of hepatic decompensation, anthropometric and laboratory data. As for anthropometric data, height, weight and BMI, calculated as kg/m², were evaluated at the time of liver biopsy. Overweight was defined by BMI ≥ 90th percentile for age and obesity by BMI ≥ 95th percentile for age, according to CDC standards [13]. Laboratory data of all children, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), alkaline phosphatase, albumin, prothrombin time, INR, blood cell count, fasting plasma glucose, insulin, cholesterol and triglycerides serum levels, as well as presence of HCV RNA in serum and viral load, performed at or near the time of liver biopsy, were retrieved from the clinical records. In all patients who had received antiviral therapy after liver biopsy a sustained response to treatment was defined as absence of serum HCV RNA after 24 weeks following the end of therapy.

Patients were included in the present study if anthropometric, clinical, laboratory and histological data were complete.

The study was performed in accordance with the Declaration of Helsinki. Patients' parents or legal guardians provided written informed consent.

2.2. Serum assays

Assays were carried out on plasma samples or fresh serum samples. Anti-HCV antibodies were tested by an enzyme-linked immunoassay (ELISA, third-generation). HCV RNA was measured by quantitative RT-PCR method (Ambion Monitor, Roche Diagnostics) with a detection limit of approximately 600 IU/ml serum. HCV genotyping was performed with a second-generation reverse transcription line probe assay (Inno-Lipa HCV II, Innomogenics) following the manufacturer’s protocol. Insulin resistance was calculated from fasting plasma insulin and glucose by the homeostasis model assessment (HOMA) method proposed by Matthews [14].

2.3. Histological evaluation

Liver specimens were formalin-fixed and paraffin embedded for histological evaluation. Liver histology was evaluated according to Ishak scoring (microfibration score: 0-18, fibrosis score: 0-6) [15]. Steatosis was graded semi-quantitatively by determining the percentage of affected hepatocytes and the following scoring system was employed: grade 0, <5%; grade 1, 5-33%; grade 2, 34-66%; grade 3, >66% [16].

2.4. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 12.0, SPSS Inc., Chicago, Illinois). Descriptive data were expressed as median (range) or mean (standard deviation, SD). Clinical, laboratory and histological characteristics between groups were compared by use of the Mann-Whitney test (continuous variables) or χ²-test (categorical variables). Correlation analysis was performed by the Spearman test as appropriate. For all tests, results with P values less than 0.05 were considered statistically significant.

3. Results

Among the 64 children studied, 16 (25%) had hepatic steatosis on liver biopsy specimen. In particular, steatosis was mild in 10 (62.5%) patients and moderate in the remaining 6 (37.5%) children. None of the patients had signs of severe steatosis. Steatosis was macrovesicular and located in the perportal area rather than in the centrilobular area. Histopathological features of non-alcoholic steatohepatitis such as perisinusoidal and perivenular fibrosis and ballooned hepatocytes were absent.

All patients were symptom-free and none of them had a decompensated liver disease. Clinical, laboratory and histological features at liver biopsy were compared in children with and without steatosis and no difference was found between the two groups (Table 1).

The most represented HCV genotype was 1b in both groups (24 children without and 8 with steatosis). Five (10.4%) children without steatosis and 3 (18.7%) with steatosis were overweight, while 5 (10.4%) patients
without steatosis and 3 (18.7%) with steatosis were obese (P = N.S.).

Although no significant difference in the necroinflammatory and fibrosis scores was found between children with and without steatosis, it should be noted that 3 (18.7%) out of 16 patients with hepatic steatosis had a fibrosis score ≥2 with respect to 1 out of 48 patients without steatosis (2.1%, P < 0.05). It should also be noted that these 4 patients were the sole subjects having a more severe degree of fibrosis (fibrosis score 3 or 4), while in the remaining 60 children the fibrosis score ranged from 0 to 2.

Non-parametric correlation analysis showed that the severity of steatosis, expressed by steatosis score, significantly correlated with both ALT (r = 0.27, P < 0.05) and GGT (r = 0.25, P < 0.05). No relationship was found with relative BMI (r = 0.14, P = N.S.), HOMA (r = 0.11, P = N.S.) or duration of HCV infection.

However, it should be noted that only 3 children (2 without steatosis and 1 with steatosis) had GGT value over the normal range for age (1.7, 2.2 and 1.7 times the upper normal value, respectively).

Furthermore, 4 children (3 without and 1 with steatosis) had cholesterol serum level above the normal value (1.2, 1.05, 1.08 and 1.05 times the upper normal value, respectively), while no patient had hypertriglyceridemia.

Forty-seven children (34 patients without and 13 with steatosis) received a course of IFN therapy at standard dosage after liver biopsy. These patients were treated in the context of clinical trials, independently of the presence of hepatic steatosis.

A sustained response was observed in 18 (53%) out of 34 treated patients without hepatic steatosis and in 3 (25%) out of 13 treated children with steatosis (P = N.S.).

4. Discussion

The prevalence of histological evidence of hepatic steatosis found in our CHC children is similar to that reported in adults, accounting for about 25% [1]. Data on the prevalence of steatosis in children with CHC were lacking so far, since HCV infection is less common in children than in adults and liver biopsy is not routinely performed in chronically HCV-infected children. On the other hand, children with CHC probably represent a better model to evaluate the relationship between HCV infection and steatosis because in children cofactors of steatosis other than HCV are less commonly found. In this regard it seems particularly valuable that our patients did not have any underlying disease other
than CHC. The clinical significance of hepatic steatosis in patients with CHC is complex and has not been well clarified. Several studies have reported that steatosis is more severe in adult patients with HCV genotype 3 infection than in patients infected by other genotypes [3,4,6,16-19]. HCV genotype 3, in fact, appears to be a modulator of steatosis through a direct action on hepatic lipid homeostasis [4]. It is also well known that in HCV-infected adults with non-genotype 3 infection, overweight and obesity play an important role in the pathogenesis of hepatic steatosis, which is principally of metabolic origin [1,3,4]. At variance with these data, our results suggest a direct association between genotypes other than 3 and hepatic steatosis since children with steatosis infected with genotypes non-3 had similar relative BMI and percentages of overweight and obese subjects with respect to patients without steatosis. Furthermore, according to a previous report, our data show that the degree of insulin resistance, expressed by HOMA, was not associated with hepatic steatosis [20]. This observation is noteworthy in view of the debated role of HCV infection in the establishing of an insulin resistance state.

It remains unexplained the reason why in children and not in adults genotypes non-3 are associated with hepatic steatosis in absence of metabolic causes. It would have been very interesting to explore the behaviour of hepatic steatosis in genotypes non-3 infected children who achieved a sustained response to IFN therapy, but for ethical reasons in these subjects a follow-up biopsy was not performed.

However, owing to the low prevalence of genotype 3 in Italian children, in the present study only 3 patients were infected with this genotype, so the relationship between genotype 3 and steatosis was difficult to evaluate in this context. Therefore on the basis of the present data, we cannot exclude that genotype 3 is associated with steatosis also in children.

The lack of correlation between duration of HCV infection and presence of steatosis suggested a peculiar host susceptibility to develop steatosis which is not time-related (a genetically determined immune mechanism might be hypothesized).

Furthermore, no correlation was found between presence and severity of steatosis and viremia. At variance from Hwang et al., in our study no relationship was found between serum triglycerides levels and presence or severity of hepatic steatosis, while a correlation was found between serum GGT and degree of hepatic steatosis. It has been hypothesized that this finding may be the expression of an induction of microsomal enzyme system caused by steatosis [1].

As for the relationship between steatosis and severity of liver damage, it has been reported that adults with CHC and steatosis show a more severe liver disease in terms of necroinflammation, fibrosis and aminotransferases serum levels [1]. We found no relationship between steatosis and activity of necroinflammation in liver histology, and the median serum level of transaminases did not differ between children with and without steatosis. It is noteworthy that in the group of children with steatosis ALT serum levels significantly correlated with degree of steatosis. Furthermore, children with steatosis showed a significantly more severe fibrosis (fibrosis score >2). It remains to be shown to what extent steatosis itself, or steatosis acting synergistically with other co-factors, contributes to the severity of fibrosis.

The pathogenic role of genotypes non-3 in determining hepatic steatosis in children, hypothesized in the present study, is supported by in vitro studies conducted with HCV genotype 1-derived-constructs. These studies and those with transgenic mouse models have reported an overexpression of various HCV proteins, such as HCV core protein [21,22]. The effects of this overexpression result in induction of lipid accumulation and increase in lipid peroxidation [21,22]. These complex interactions could ultimately lead to, or at least participate in, steatosis. However, these findings have no proven steatogenic effect in vivo, so it is feasible that HCV core protein may not be the only viral protein involved in HCV-induced steatosis in vivo. The potential role of other viral factors has not yet been appropriately investigated.

Analyses of treatment outcomes indicated that, although response to IFN was not significantly related to presence of steatosis, children with steatosis were less likely to achieve sustained response to therapy. This result, in agreement with previous studies in adults with CHC [8,23], suggests that steatosis might induce a mechanism of resistance to IFN treatment.

In conclusion, histological evidence of steatosis is detectable in a quarter of children with CHC in absence of other causes. In children, differently from adults, genotypes other than 3 may be associated with hepatic steatosis independently from classical metabolic risk factors. Further studies are necessary to better understand the role and significance of steatosis in children with HCV infection.

References


Short communication

Hepatic steatosis is uncommon in children with chronic hepatitis B

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ABSTRACT

Background: Differently from chronic hepatitis C, factors associated with hepatic steatosis in children with chronic hepatitis B are not clearly elucidated.

Objective: Aim of this study was to investigate prevalence of steatosis at liver biopsy in HBV-infected children.

Study design: A retrospective study including 56 children with chronic hepatitis B undergoing liver biopsy at median age of 8.1 years. In all patients demographic, anthropometric, clinical and laboratory data were evaluated at the time of liver biopsy.

Results: Steatosis was present in 28 (4.8%) children. BMI was significantly higher in 2 patients with steatosis compared with those without steatosis. Demographic, biochemical and virological parameters did not differ between children with and those without steatosis.

Conclusions: Liver steatosis in HBV-infected children seems to be related to obesity and metabolic factors rather than to viral factors. Detection of steatosis in non-obese children with HBV infection requires a careful investigation to rule out other causes of fatty liver.

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1. Background

Recently, it has been reported that fatty liver is the most common form of paediatric liver disease.1 The list of potential causes of liver steatosis in children is very wide and principally includes nutritional disorders and metabolic liver diseases.2,3 As for the role of chronic viral hepatitis, the relationship between chronic hepatitis C virus infection and liver steatosis is well documented.4,5 In hepatitis C virus (HCV)-infected children the prevalence of histological evidence of hepatic steatosis accounts for 25–27%.6,4,5 Liver steatosis impacts on the degree of inflammation and fibrosis and may influence the long-term prognosis of chronic hepatitis C (CHC).5

Differently from CHC, factors associated with hepatic steatosis in chronic hepatitis B (CHB) are not clearly elucidated. The frequency of hepatic steatosis at liver biopsy in adults with CHB ranges from 18% to 76%7,8; it seems especially correlated with metabolic parameters (such as fasting glucose and overweight), while no correlation

Abbreviations: HCV, hepatitis C virus; CHC, chronic hepatitis C; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.

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has been found with HBV DNA titre and HBeAg status.8,9 Studies on prevalence of fatty liver in children with CHB are lacking. It is to note that the relationship between HBV infection and fatty liver might be better understood in children compared to adults, since pediatric patients have not so many co-factors responsible for steatosis such as alcohol intake, dyslipidemia, insulin-resistance, type 2 diabetes or metabolic syndrome.

2. Objectives

Aim of our study was to evaluate the prevalence of steatosis at liver biopsy in children with CHB and its relationship with anthropometric, biochemical and virological factors.

3. Study design

We retrospectively assessed 56 consecutive otherwise healthy children (38 males) with CHB who underwent liver biopsy for diagnostic purposes at the Pediatric Liver Unit of the University Federico II (Naples, Italy) during a 16-year period from 1989 to 2007.

Diagnosis of CHB was based on the presence of hepatitis B surface antigen (HBeAg) in serum for 6 months or longer. Patients with either concomitant chronic infections (hepatitis C virus, hepatitis delta virus, human immunodeficiency virus) or chronic systemic diseases (diabetes, malnutrition, dyslipidemia, thyroid diseases)
or exposure to hepatotoxic drugs or alcohol were excluded. Patients with history of antiviral treatment before liver biopsy were also excluded.

For each patient anthropometric data such as height, weight and body mass index (BMI), calculated as kg/m², were evaluated at the time of liver biopsy. Children were defined overweight in presence of BMI->90th percentile for age and obese in presence of BMI->95th percentile for age, according to CDC standards. The relative BMI was calculated and expressed as percentage of individual BMI/median BMI value for age and sex, according to age reference values °. Pubertal stage was evaluated according to Tanner criteria. ¹⁰

For each patient the following laboratory evaluation was performed at or near the time of liver biopsy: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, albumin, prothrombin time, international normalized ratio, complete blood cell count, fasting glucose, total cholesterol, triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL) serum levels, viral markers (HIV Ag/HIV Ab, HBeAg/HBe Ab, serum HBV DNA). Serum HBV DNA was quantitatively investigated, depending on the time, with a commercial hybridization method with a cut-off value of 5 pg/ml (Abbott Diagnostics, North Chicago, IL, USA) or with a commercial PCR assay (Amplifor HBV Monitor kit, Roche Diagnostic System, NJ, USA). Values obtained with different HBV DNA quantification assays were compared (1 μg HBV DNA = 285,000 copies).¹¹

Histological analysis of the liver sections was performed by an experienced pathologist (K.V.), blinded to the clinical data. Activity of necroinflammation and stage of fibrosis were evaluated according to Ishak scoring system (necroinflammation score: 0–18, fibrosis score: 0–6).¹² Hepatic steatosis was graded semi-quantitatively by determining the percentage of affected hepatocytes: grade 0 (<5% of hepatocytes involved), grade 1 (5–33% involved), grade 2 (34–66% involved), grade 3 (>66% involved hepatocytes); features of steatohepatitis were also searched.³ ¹³

The study was performed in accordance with the Declaration of Helsinki. Patients' parents or legal guardians provided written informed consent.

Statistical analysis was performed using a statistical program package (GraphPad Instat 2 Software). For all tests, results with P values less than 0.05 were considered statistically significant.

### 4. Results

Fifty-four patients had no steatosis at liver biopsy; steatosis was present only in 2 (4%) (2/50) children (grade 1 in both cases). In both cases, steatosis was macrovesicular and located in the periporal area. Histopathologic features of non-alcoholic steatohepatitis were not found.

Twelve (22.2%) of 54 patients without steatosis and both (100%) patients with steatosis were overweight (6 and 1, respectively) or obese (5 and 1, respectively) (P = 0.059). Relative BMI did not statistically differ between the two patients with steatosis (126.4 ± 1.2) and 12 overweight/obese children without steatosis (125.7 ± 12.3). Mean relative BMI was significantly higher in 2 patients with steatosis compared with the whole group without steatosis (P = 0.04).

No significant difference between patients with and without steatosis was found with regard to demographic parameters, biochemical, virological and histological features (Table 1). Since only 2 children had evidence of fatty liver, the present study was unable to identify potential risk factors for steatosis in this small subgroup of patients. Pre-pubertal (38 patients, 68.5%) and pubertal patients (18 patients, 32%) did not significantly differ as regard prevalence of steatosis and metabolic parameters.

### 5. Discussion

In the studied children with CHB the prevalence of hepatic steatosis was lower than that reported in paediatric patients with CHC and adults with CHB³⁴-⁶ and similar to that found in general paediatric population.¹ It is likely that higher percentage of steatosis in adult patients with CHB is related to presence of concomitant risk factors for fatty liver (alcohol consumption, type 2 diabetes, metabolic syndrome, etc)¹² This hypothesis is supported by the finding that in adults with CHB metabolic parameters (such as fasting glucose and overweight) were correlated with the presence of steatosis at liver histology, while no correlation was found with HBV DNA titre and HBeAg status.⁶⁷ In our population of HBV-infected otherwise healthy children, hepatic steatosis was only associated with a higher BMI, a parameter correlated with an increased risk of insulin-resistance and non-alcoholic fatty liver disease. No correlation was found between presence of steatosis and viral factors. As a consequence, differently from CHC, we expected to find no steatosis in a child
with chronic HBV infection and absence of metabolic risk factors for steatosis such as overweight or obesity, high levels of triglycerides, cholesterol or fasting glucose. If steatosis is documented in absence of these metabolic risk factors, other hepatic (e.g. metabolic liver diseases, autoimmune hepatitis, sclerosing cholangitis, chronic hepatitis C) or non-hepatic (e.g. inflammatory bowel disease, cystic fibrosis, celiac disease) causes of steatosis should be considered.

Although our results need to be confirmed in larger paediatric series, the present study shows that liver steatosis in children with CHB is a rare event which seems to be related to the host rather than the viral factors. These findings suggest that liver steatosis in non-obese children with CHB cannot be explained with HBV infection and should be carefully investigated to rule out the multiple causes of paediatric fatty liver.

**References**

CHAPTER 3

CHILDREN WITH HIV INFECTION
3.1 Perception of disease and adherence to antiretroviral therapy in HIV-infected children

3.11 Rationale

In the past two decades, the nature of pediatric HIV has radically shifted. Highly active antiretroviral therapy (HAART) involving multiple drug combinations have proven effective in controlling clinical disease progression and reducing mortality among both adults and children (1,2). As a result, while rates of new infections among infants have declined dramatically, perinatally infected children and youth are surviving longer (3,4).

While the advent of HAART has improved survival among children and adolescents with HIV, the regimens are demanding and require strict adherence in order to avoid medication resistance and achieve lasting clinical benefits (5-7). Adherence failure may result in early antiretroviral therapy (ART) failure, development of resistance to ART and subsequently reduction of treatment options. Owing to highly dynamic viral replication in HIV infected patients, it is now clear that even limited omissions of doses may lead to selecting resistant strains and ultimately to treatment failure (7,8). Most studies indicate that adherence to ART is suboptimal among children and adolescents. A recent review of 13 studies revealed a pediatric adherence ranging from 50% to 75% (6). An array of heterogeneous conditions affect treatment adherence in AIDS. Studies of caregivers of children with HIV have shown that compromised adherence is associated with the complexity of the regimen (9-11). A small number of studies have examined psychological influences on pediatric adherence, suggesting an important link with individual and family characteristics and situations (12-14).

Adherence among children and adolescents with HIV is affected by the quality of parent-child communication, level of caregiver stress and quality of life, caregiver cognitive functioning, children’s exposure to stressful life events and experiencing depression (12,13,15). In a previous cross-sectional assessment of adherence and its major determinants in 7 major Italian references centers for pediatric HIV-infection, we found that 21 (16%) of 129 children with HIV infection failed to adhere to treatment (14). In addition, we found that psychosocial rather than demographic or clinical features were major determinants of adherence. Indeed, the relationship between the caregiver and the child was the strongest determinant of adherence with foster parents being more adherent than relatives and biological parents.
Perception of disease and of the benefits of ART may favor acceptance of changes in therapy schedule, and eventually promote child adherence (16). The importance of promoting and maintaining optimal adherence in pediatric patients is recognized in several authoritative documents and guidelines, and careful monitoring is recommended (17,18). In Italy, children with HIV infection are cared for according to the recommendations of the Center for Disease Control (17) and the Italian Register for HIV infection. The latter coordinates the medical care of children in Italy and was established at the onset of the HIV epidemic (19-21). All HIV-positive children have free access to medical care, including antiretroviral and non-specific drugs, visits and procedures.

Adherence is a dynamic phenomenon and determinants of adherence change over time. However, few studies have examined the dynamics of adherence to ART. Drug switching due to non-adherence, failure of therapy, viral resistance, toxicity or even to improvements in HAART can influence the quality of life, the psychological dimension and the behavior of HIV-infected patients (22). Little is known about the effects of switching therapy on HIV-infected children and their caregivers and on adherence to ART. On June 6th 2007, the European Medicines Agency (EMEA) announced the recall by the manufacturer of the protease inhibitor nelfinavir (Viracept, Roche) from the market because of possible contamination of tablets with the genotoxic substance ethyl mesylate. As a consequence, all patients who were receiving nelfinavir had to be switched to a different drug regimen.

The first aim of this research was to investigate the effect of switching therapies on the perception of the benefits of ART and adherence to treatment in HIV-infected children and their caregivers.

The second aim of this research was to investigate in a multicenter study the evolution of adherence to ART 12 months after an initial (baseline) evaluation in a population of HIV-infected children.

3.12 Experimental procedures

Children and adolescents with HIV infection who switched ART
To investigate the effect of switching therapies on the perception of the benefits of ART and adherence, HIV-infected children and their caregivers attending our Pediatric AIDS Reference Center were considered for enrolment. Caregivers of enrolled children completed a questionnaire during a face-to-face interview. The questionnaire contained information on
demographic data, awareness of HIV status, perception of treatment efficacy, reaction to the “change of therapy” event, reasons for taking, missing or discontinuing drugs, and factors potentially influencing adherence. The questionnaire was administered by a psychologist trained in HIV infection at the time of or within one month after nelfinavir recall or treatment shift for other reasons. In the same period, caregivers of children who did not change therapy were administered the same questionnaire, except for the questions related to the change of antiretroviral drugs. Awareness of HIV status was established based on the child’s knowledge about HIV infection. Adherence was evaluated at enrolment and subsequently during the 4th month after shifting, as part of the routine follow-up for HIV-infected children (scheduled every 2 months).

**Children and adolescents with HIV infection included in the multicenter study**

To investigate the evolving pattern of adherence an observational, cross-sectional multicenter study was performed through a structured interview to the caregivers of HIV-infected children. The study was carried out in the same 7 major Italian centers for pediatric HIV-infection as the previous study (14). The same investigator (V.G.) visited each center for one to two days and interviewed caregivers of children with HIV symptomatic infection who were scheduled to visit the center for routine follow-up visits. A face-to-face structured interview based on a standardized scripted dialogue was administered to persons in charge of drug administration (caregivers). Data on socio-demographic and clinical features of enrolled children, their awareness of HIV status, prescribed antiretroviral therapy and adherence to drug regimen were collected. Each interview lasted for approximately 20 minutes.

**Definition of caregiver**

“Caregiver” was the person who routinely administered antiretroviral drugs to enrolled children and accompanied the child to the hospital for routine follow-up on the day of the interview. Caregivers were classified as biological parents, foster parents or second-degree relatives.

**Adherence assessment**

To estimate adherence quantitatively, each caregiver was asked how many doses of the total prescribed antiretroviral therapy had been omitted in the last 4 days. The 4-day instrument is a reliable surrogate marker for monthly adherence and was used in the previous study (9,23). A dose was defined as the intake of any pill, syrup or suspension of any antiretroviral drug in one day. Since adherence rates equal to or less than 95% of prescribed doses have been
associated with suboptimal virologic outcome, children were defined as non-adherent if they had taken less than 95% of all prescribed doses of antiretroviral therapy in the last 4 days. Threshold levels were prospectively labeled as follows: adherence (≥95% of prescribed doses regularly taken), low adherence (<95%, but >80% of doses taken) and poor adherence (<80% of doses taken). Compliance with administration time was intended as the percentage of doses taken within 2 hours of a target time.

**Clinical and laboratory parameters**
Clinical and immuno-virological parameters (count of CD4-positive lymphocytes and plasma HIV RNA levels) were evaluated at the time of the administration of both questionnaires. At the time of the multicenter study, the lower detection limit of the viral load assay was 200 copies of HIV RNA/ml.

**Statistical analysis**
Statistical analysis was performed using SPSS version 14.0 (SPSS, Chicago, IL, USA). The significance of the differences between the groups was assessed by the chi square test and Fisher’s exact test for dichotomous variables. In case of more variables, we applied the chi square test for independence. Continuous variables were compared with the Student t test and the Mann-Whitney test. When the study groups were more than 2, the ANOVA test was used for continuous variables. A two-sided test was used to indicate statistical significance at a p value of <0.05.

**3.13 Results**

**Effect of switching therapy on ART perception and adherence**
Thirty-eight (13 males; mean age 12.1±6.7 years) out of 45 HIV-infected children attending our center were enrolled in the study. One child was excluded because of a recent diagnosis of HIV infection. The remaining 6 children were not included because they did not attend our center during the study period (4 patients) or refused to participate (2 cases).
Based on treatment history, enrolled children were divided into 3 groups: patients who were shifted because of nelfinavir recall (group A; 8 children, 3 males; mean age 11.2 years); patients who were shifted in the month before nelfinavir recall or in the following 5 months for other reasons (group B; 12 children, 6 males; mean age 12.6 years), i.e. emergence of viral resistance (7 patients), toxicity (3 patients) and non-adherence (2 patients); and patients who
were not shifted (group C; 18 children, 4 males; mean age 10.4 years). Demographic and HIV RNA related parameters did not differ significantly between the three groups except that the primary caregiver was more frequently a biological parent in group A than in group B (p=0.02) and C (p>0.05). Overall, 12 (32%) children (mean age 14.7±2.1 years) were aware of their HIV status and managed their own therapy. In the remaining 26 (68%) children (mean age 9.3±3.4 years; p<0.0001), medications were administered by the primary caregiver.

Treatment-related feelings are reported in table 1. Most caregivers were satisfied with the health status of their children and all caregivers considered HAART necessary and effective, without significant differences between the groups. It is noteworthy that 18 (47%) caregivers were receiving HAART. There were a few worries with antiretroviral drugs, which were effectively dealt with by patients and their families. However, more caregivers of group B did not trust therapy (i.e., they did not believe in the efficacy of treatment) compared to groups A and C (p=0.03) (table 1).
Table 1. Perception of antiretroviral therapy and adherence to treatment in 38 HIV-infected children

<table>
<thead>
<tr>
<th></th>
<th>Group A (N=8)</th>
<th>Group B (N=12)</th>
<th>Group C (N=18)</th>
<th>Total (N=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>How do you consider health status of your child?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Good</td>
<td>6 (75%)</td>
<td>10 (83%)</td>
<td>16 (89%)</td>
<td>32 (84%)</td>
</tr>
<tr>
<td>- Poor</td>
<td>2 (25%)</td>
<td>2 (17%)</td>
<td>2 (11%)</td>
<td>6 (16%)</td>
</tr>
<tr>
<td><strong>Do you consider HAART necessary?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>8 (100%)</td>
<td>12 (100%)</td>
<td>18 (100%)</td>
<td>38 (100%)</td>
</tr>
<tr>
<td>- No</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Do you trust therapy?§</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>8 (100%)</td>
<td>9 (75%)</td>
<td>18 (100%)</td>
<td>35 (92%)</td>
</tr>
<tr>
<td>- No</td>
<td>0</td>
<td>3 (25%)</td>
<td>0</td>
<td>3 (8%)</td>
</tr>
<tr>
<td><strong>Was it a problem to take the therapy?§</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Never a problem</td>
<td>2 (25%)</td>
<td>4 (33%)</td>
<td>7 (39%)</td>
<td>13 (34%)</td>
</tr>
<tr>
<td>- Hardly ever a problem</td>
<td>5 (62%)</td>
<td>5 (42%)</td>
<td>8 (44%)</td>
<td>18 (47%)</td>
</tr>
<tr>
<td>- Frequent problem</td>
<td>1 (13%)</td>
<td>3 (25%)</td>
<td>3 (17%)</td>
<td>7 (19%)</td>
</tr>
<tr>
<td><strong>If it was a frequent problem, what was the reason?§</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Formulation of drugs</td>
<td>1 (100%)</td>
<td>1 (33%)</td>
<td>3 (100%)</td>
<td>5 (72%)</td>
</tr>
<tr>
<td>- Schedule of therapy</td>
<td>0</td>
<td>1 (33%)</td>
<td>0</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>- Social context</td>
<td>0</td>
<td>1 (34%)</td>
<td>0</td>
<td>1 (14%)</td>
</tr>
<tr>
<td><strong>Did your child comply with the drug timing?§</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Sometimes</td>
<td>3 (37%)</td>
<td>5 (42%)</td>
<td>2 (12%)</td>
<td>10 (26%)</td>
</tr>
<tr>
<td>- Often</td>
<td>2 (25%)</td>
<td>3 (25%)</td>
<td>8 (44%)</td>
<td>13 (35%)</td>
</tr>
<tr>
<td>- Always</td>
<td>3 (38%)</td>
<td>4 (33%)</td>
<td>8 (4%)</td>
<td>15 (39%)</td>
</tr>
<tr>
<td><strong>N. of patients adherent to therapy (%) §</strong></td>
<td>6 (75%)</td>
<td>8 (67%)</td>
<td>14 (78%)</td>
<td>28 (74%)</td>
</tr>
</tbody>
</table>

§ For patients who were shifted (groups A and B), these questions were related to the therapy before the shift. For patients who did not change therapy, the questions were related to the current therapy.

* More caregivers of group B did not trust therapy compared to group A and C (p=0.03).
Before switching, 28 (74%) patients met the criteria of adherence to therapy (≥95% of drug doses taken in 4 days), without significant differences between the groups (figure 1). Differently, adherence to the timing of medications was poor in a substantial number of cases but without differences between the groups. Patients adherent and not adherent to treatment did not differ by age (mean age 11.4±4.1 vs 10.5±4.1, respectively; p>0.05), sex (39% of males vs 20%, respectively; p>0.05) or race (Caucasian origin in 71% of cases vs 80%, respectively). Specific family factors were significantly associated with adherence rates. All children cared for by foster parents adhered to therapy compared to 62% of children cared for by biological parents (p=0.03) and 71% of children cared for by second-degree relatives (p>0.05). Increased adherence rates were associated with a higher education in caregivers. Fifty percent of caregivers of adherent patients had a schooling above 8 years vs 90% of caregivers of non adherent patients (p=0.05).

Reactions to drug shifting in 20 HIV-infected children who shifted therapy are reported in table 2. Shift of therapy, either because of nelfinavir withdrawal or for other reasons, generated anxiety and concern in most caregivers, without differences between the groups. Two (25%) caregivers of group A were very worried about nelfinavir toxicity. However, all caregivers were satisfied with the information received and reported they did not need more information. All but one caregiver (in group A) felt confident about the efficacy of the new treatment. The duration of treatment before switching did not differ between group A (median duration 60.5 months, range 7-96) and B (16.5 months, range 1-87; p>0.05) or between patients adherent and patients not adherent (median duration 45 months, range 1-96, vs 9.5 months, range 5-77; p>0.05). Adherence rates did not change significantly after switching therapy in groups A and B (87% and 58% of patients adherent after switching therapy in groups A and B, respectively; p>0.05) (figure 1). Finally, adherence rates were higher in the 12 patients who self-managed their therapy than in the 26 patients who received drugs from a caregiver, although the difference was not statistically significant (percentage of adherent patients 83% vs 64%, respectively: p>0.05).
At baseline After drug switching
Group A (N=8) Group B (N=12) Group C (N=18)

Figure 1. Percentage of patients adherent to therapy at baseline (for all groups) and after drug switching (for groups A and B)

Adherence: ≥95% of prescribed doses regularly taken.
Table 2. Reaction to drug shifting, perception of antiviral treatment in 20 HIV-infected children who shifted therapy

<table>
<thead>
<tr>
<th></th>
<th>Group A (N=8)</th>
<th>Group B (N=12)</th>
<th>Total (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>What was your feeling when your child needed to change therapy?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Reassurance</td>
<td>0</td>
<td>2 (17%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>- Indifference</td>
<td>1 (12%)</td>
<td>0</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>- Concern</td>
<td>6 (75%)</td>
<td>9 (75%)</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>- Anxiety</td>
<td>1 (13%)</td>
<td>1 (8%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td><strong>Why did you feel worried?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Side effects</td>
<td>2 (29%)</td>
<td>5 (50%)</td>
<td>7 (42%)</td>
</tr>
<tr>
<td>- Drug toxicity</td>
<td>2 (29%)</td>
<td>0</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>- Inefficacy of the new therapy</td>
<td>3 (42%)</td>
<td>5 (50%)</td>
<td>8 (47%)</td>
</tr>
<tr>
<td><strong>Do you trust new therapy?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>7 (87.5%)</td>
<td>12 (100%)</td>
<td>19 (95%)</td>
</tr>
<tr>
<td>- No</td>
<td>1 (12.5%)</td>
<td>0</td>
<td>1 (5%)</td>
</tr>
<tr>
<td><strong>If no, why?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Side effects</td>
<td>1 (100%)</td>
<td>-</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>- Long-term efficacy</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

§ Adherence to the new treatment was evaluated during the 4th month after shifting.

None of the analyzed variables differed statistically between the two groups.
Evolving pattern of adherence

In the first study 129 HIV-infected children (median age 96 months, range 4-204) were enrolled in 7 major Italian reference centres for pediatric HIV infection (14). One hundred and eight patients (84%) were adherent to therapy. Adherence with administration times was very poor and only 14 (11%) caregivers reported that they were complied with. An inverse relationship was detected between adherence and awareness of HIV infection. When adherence rates were analysed according to caregivers, it was observed that adherence was higher in children receiving drugs from foster parents than in those receiving treatment from biological parents and second degree relatives (14).

Of the 129 children enrolled in the first study, 112 (87%) patients (57 males; median age 108 months, range 12-216; mean duration of therapy at baseline 74 months ± 37) were available to be checked for adherence after a mean of 12 months. The other 17 children were not included because they did not attend the follow-up visit or their caregivers refused to take part in the second survey.

The overall adherence rate was 83% (figure 2). This included 88 (79%) children who took all doses and 5 who omitted one dose only in the last 4 days but were considered adherent according to the cut-off threshold (adherence rate >95%). Nineteen (17%) children did not adhere to treatment, and two of these children did not take any doses at all.

The changes in adherence pattern were as follows: of the 19 non-adherent children, 10 (53% of non-adherent) were non-adherent at baseline and were therefore labelled as “persistently non-adherent”. Conversely, 9 children (47% of 19 non-adherent patients) were adherent at baseline and were defined “new non-adherent”. Exactly the same number of children shifted from having been non-adherent at baseline to adherent and were defined “new adherent”. A comparison with baseline data revealed that non-adherent children had a significant risk of remaining non-adherent after 12 months (RR=5.44, O.R. 10.37, C.I. 1.56 to 11.55).

Similar to our previous findings, compliance with administration times was very poor with only 16 (14%) caregivers reporting that they did administer doses in due time. The vast majority (70 of 112 caregivers, 63%) reported that adherence with prescribed times was limited, and 26 (23%) of caregivers reported no adherence at all with administration times.
Figure 2. Rates of adherence in 112 HIV-infected children according to key informants
When we examined determinants of adherence, we found no significant difference between adherent and non-adherent patients with respect to gender, clinical class, severe or less than severe immune impairment as well as number of patients with undetectable viral load (figure 3). However, among patients with detectable HIV RNA, median viral load was significantly higher in non-adherent (HIV RNA median value 2870 copies/ml, range 250-100000) than in adherent children (HIV RNA median value 510 copies/ml, range 220-12200) (p=0.03). Twenty-seven children fully adherent either at baseline and after 12 months had a viral load below 200 copies/ml.

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**Figure 3. Rate of adherence in HIV-infected children with detectable or undetectable HIV RNA**

The lower detection limit of the viral load assay at the time of the study was 200 copies of HIV RNA/ml.
When we examined the correlation between adherence and ART regimens (number of doses), we found that children receiving more than six doses per day were more adherent than those receiving fewer than six doses (table 3). The cut-off of six doses per day was based on the median number of administrations per day in the overall population. Although the pattern of adherence varied widely in relation to individual drugs, no significant difference was found between adherent and non-adherent patients in relation to treatment type, with the exception of 6 patients taking lopinavir/ritonavir, who were all non-adherent (p<0.05 versus all the other individual drug). This result can be explained by the low palatability of lopinavir/ritonavir oral suspension.

Finally, adherence was strongly related to the caregiver, being significantly higher in children receiving treatment from foster parents than from biological parents (table 4). A similar pattern emerged from the baseline analysis in the same 112 caregivers, although the difference was not significant (table 4). Between the first and the second study, 18 children (11 males; median age 14.3 years, range 13-16.5) had been instructed to self-administer antiretroviral drugs. Five of these 18 children (28%) were non-adherent. The difference was statistically significant when these children were analyzed as a single group and compared with children receiving treatment from foster parents (table 4).
Table 3. Adherence according to number of doses of antiretroviral therapy per day

<table>
<thead>
<tr>
<th>No. of doses/day</th>
<th>At baseline</th>
<th></th>
<th></th>
<th>After 12 months</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adherent</td>
<td>Non adherent</td>
<td>p</td>
<td>Adherent</td>
<td>Non adherent</td>
</tr>
<tr>
<td>&lt;6</td>
<td>44 (75%)</td>
<td>15 (25%)</td>
<td>0.01</td>
<td>32 (73%)</td>
<td>12 (27%)</td>
</tr>
<tr>
<td>≥6</td>
<td>49 (92%)</td>
<td>4 (8%)</td>
<td></td>
<td>61 (90%)</td>
<td>7 (10%)</td>
</tr>
</tbody>
</table>

Data are shown as n (%)  

Table 4. Adherence according to the type of caregiver

<table>
<thead>
<tr>
<th>Type of caregiver</th>
<th>At baseline</th>
<th></th>
<th></th>
<th>After 12 months</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adherent</td>
<td>Non adherent</td>
<td>p</td>
<td>Adherent</td>
<td>Non adherent</td>
</tr>
<tr>
<td>Biological parents</td>
<td>61 (78%)</td>
<td>17 (22%)</td>
<td>0.05</td>
<td>49 (79%)</td>
<td>13 (21%)</td>
</tr>
<tr>
<td>Foster parents</td>
<td>32 (93%)</td>
<td>2 (7%)</td>
<td></td>
<td>31 (97%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Self-administered</td>
<td>0</td>
<td>0</td>
<td></td>
<td>13 (72%)</td>
<td>5 (28%)</td>
</tr>
</tbody>
</table>

Data are shown as n (%)
3.14 Discussion

Maintenance children’s medication routine in a variety of day-to-day situations is a challenge. Adherence support mechanisms need to go beyond reminder system and devote specific attention to fitting the medications into daily schedules that can include changes in routines, going away from home and being in a variety of social situations. The extent to which medication regimens can accommodate rather than interfere with social activities may be an important means of reinforcing and improving adherence, particularly among adolescents. Adherence is affected by clinical, social and behavioural factors (13,24). Shifts in ART regimens due to treatment failure, emergence of viral resistance or drug toxicity may impair adherence. The consequences of switching therapy in HIV-infected children and adolescents are poorly investigated for several reasons: the low number of HIV-infected children; the role of caregivers in drug administration; and less frequent need to change therapy compared to adult patients. The perception that therapy is protective or reliable is associated with higher levels of adherence, whereas the perception of therapy as enslaving is associated with lower levels of adherence in HIV-infected adults (25). A drug recall due to a possible toxic effect would be expected to result in impaired adherence. The nelfinavir recall, a unique event in the history of HAART, gave us the chance to investigate if changes of antiretroviral drugs affect the perception of the benefits of therapy and adherence rates. In 12 months, a total of 21 (47%) of 45 HIV-infected children seen at our Reference Center needed to change HAART and 38% of them did so because of nelfinavir recall. The switch because of recall involved 18% of the entire population of our HIV-infected children. Switching was a matter of concern for most caregivers and induced worries and a negative general feeling, although it did not affect adherence rates or result in decreased trust by HIV-infected children or their caregivers. In our population, drug recall was not more problematic than switching for other reasons and it did not affect adherence rate, perhaps because patients and their caregivers had received clear information about the medication and about the reasons for switching.

Adherence to ART is a dynamic phenomenon that changes with time. Our multicenter study shows a fairly constant distribution of rate of adherence. Quantitatively, non adherence remained stable at around 20% because of an equal flux of patients from adherence to non adherence and vice versa. This suggests that adherence should be frequently monitored and be a component of follow-up of children with HIV infection. Our assessment shows that also the determinants of adherence change over time, albeit to a lesser extent than adherence, which again suggests more frequent monitoring of adherence.
In the present study, a high percentage of adherent patients still had a viral load >200 copies/ml and some non-adherent subjects had an undetectable viral load, which resulted in a fairly low agreement between HIV RNA suppression and adherence rate. This inconsistency may have several explanations. First, adherence is a complex phenomenon and maintaining the prescribed adherence to medications is a continuous challenge even in adherent families. The self-report of medications taking during the previous 4 days does not adequately capture periods of non-adherence that are intermittent or present over longer time periods or that occur during periods of increases stress in the family. This may represent a limitation of the study. However, although self-report instrument may tend to overestimate adherence, the reports of missed doses is a practical and reliable method if used at each routine visit. Second, in some fully adherent children, suboptimal suppression of viral replication may be due to drug resistance. Third, metabolic and pharmacokinetic pattern may change with age and other conditions that are specific of children (i.e. the respect to timing of dosage and food consumption). Finally, although there was no association between viral load and complete adherence to antiretroviral therapy when viral load was dichotomized (more or less than 200 copies per ml), there was a significant trend for decreased level of serum HIV RNA in adherent patients compared to non-adherent subjects.

A key aspect of adherence among HIV-infected children and youth is represented by caregiver/family factors (13,14). Infants and young children depend almost entirely on a caregiver to administer medications. Adherence to treatment is, therefore, strictly dependent on caregivers. Biological parents of children with HIV are often HIV-infected and non-adherent themselves, more debilitated and depressed than foster parents. This can lead to a decreased attention to the maintenance of adherence in their HIV-infected children. This research confirms that adherence is higher in children receiving drugs from foster parents than in children receiving medication from biological parents (14). Better adherence in children living with foster parents may be due to the cultural background of foster parents, that are selected before adoption, and to a greater sense of responsibility in non-biological parents compared to biological ones (9,26).

A new class of caregivers emerged in the multicenter study, namely children who self-manage therapy. This group was the least adherent. Interestingly, all 5 non-adherent patients were also non-adherent at the baseline study. This finding carries an alarming message, namely, children who are managed by non-adherent caregivers tend to be non-adherent when they manage their own therapy. The latter is a worrying observation in the perspective of the increasing number of children who will directly manage their therapy.
Finally the relationship between adherence and more complex regimens has important clinical implications. This data is consistent with other reports showing that sicker adults with chronic diseases taking several drugs appeared to be highly compliant with therapy (27-31). However, in these studies, the exact number of doses taken/day was not assessed. Although the HAART pill burden has decreased in recent years due to simplified regimens, contrary to what might be expected out results show that HIV-infected patients taking more doses are more compliant with therapy than patients taking less doses. Is it possible to speculate that patients with more complex regimens may be, or believe to be, less healthy. As a consequence, they may have an increased perception of disease severity and, therefore, are likely to be more adherent to prescribed medication.

In conclusions, adherence is a crucial variable of treatment of AIDS in children and deserves early and continuous monitoring and implementation. Adherence tends to change over time and, more importantly, non adherence in early stages of life predicts later non adherence, when control of therapy may become more difficult or impossible. The caregiver, the relationship between patient and physician, and the perception of drug efficacy are key aspects to obtain and maintain adherence.
3.2 References


29. Wagner GJ, Rabkin JG. Measuring medication adherence: are missed doses reported more accurately then perfect adherence? AIDS Care 2000;12:405-08.


3.3 Publications

   Effects of antiretroviral drug recall on perception of therapy benefits and on adherence to antiretroviral treatment in HIV-infected children.

2. **Giannattasio A**, Albano F, Giacomet V, Guarino A.
   The changing pattern of adherence to antiretroviral therapy assessed at two time points, 12 months apart, in a cohort of HIV-infected children.
   (in press in Expert Opinion on Pharmacotherapy)

   Is the interruption of antiretroviral treatment during pregnancy an additional major risk factor for mother-to-child transmission of HIV type 1?.
Effects of Antiretroviral Drug Recall on Perception of Therapy Benefits and on Adherence to Antiretroviral Treatment in HIV-Infected Children

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Department of Pediatrics, University "Federico II", Naples, Italy

Abstract: In June 2007, the European Medicines Agency announced the recall by Roche of neflafaxiv from European Union markets because of contamination of tablets with ethyl mesylate. Based on this event, we investigated the effect of switching therapy because of neflafaxiv recall or for other reasons on the perception of therapy benefits and adherence to treatment in HIV-infected children and their caregivers. Thirty-eight children (mean age 12.1±6.7 years) were enrolled. A 35-item questionnaire was administered to the caregivers of enrolled children. Adherence was evaluated through a 4-day recall adherence instrument. Enrolled children were divided into 3 groups: patients who were shifted because of neflafaxiv recall (group A, 8 patients); patients who were shifted for other reasons (group B, 12 patients); patients who were not shifted in the last 6 months (group C, 18 patients). All caregivers considered antiretroviral therapy necessary and effective for their children. However, drug shifting generated anxiety in most of them, irrespective of the reason for shifting. At baseline, 74% patients adhered to therapy. Adherence rate was related to the type of caregivers being higher in children cared for by biological parents or second-degree relatives. Adherence rates did not change significantly in groups A and B after switching. Drug-switching raises concern in caregivers of HIV-infected children and induces a negative feeling irrespective of the reason for switching. However, switching, including the shift due to neflafaxiv recall, did not affect adherence rates.

Keywords: HIV, Antiretroviral therapy, Medication adherence, Children.

INTRODUCTION

Children affected by HIV and their families have to deal with physical symptoms, fear of AIDS-related death, social problems and the need to adhere to complex antiretroviral therapy that includes multiple drugs that must be taken several times a day [1, 2]. Although highly active antiretroviral therapy (HAART) has substantially changed the natural history of HIV-1 infection, the therapeutic regimens require strict adherence [2, 3]. In fact, nonadherence is a major predictor of therapeutic failure [4-7]. A level of adherence less than 95% of doses is associated with suboptimal control of viral load in adult patients [5].

Maintaining adherence to antiretroviral therapy over time is difficult, particularly in growing children. Caregiver factors are significant predictors of child adherence to this treatment [8]. In a previous study, we found that the relationship between care provider and child was associated with treatment adherence, whereas there was no relationship between adherence and sociodemographic or clinical features of HIV-infected children [9]. Also antiretroviral drugs and schedules have an impact on adherence rates [9]. Higher adherence rates have been reported in children with advanced HIV, possibly because of increased perception of disease severity [10]. Finally, psychosocial variables, poor caregiver-child communication, low caregiver-perceived quality of life and high levels of caregiver-perceived stress have been associated with non-adherence [8].

Therefore, an array of heterogeneous conditions affect treatment adherence in AIDS. Drug switching due to nonadherence, failure of therapy, viral resistance, toxicity or even to improvements in HAART can influence the quality of life, the psychological dimension and the behavior of HIV-infected patients [11]. On the other hand, perception of disease and of the benefits of antiretroviral therapy may favor acceptance of changes in antiretroviral therapy, and eventually promote child adherence [12]. However, little is known about the effects of switching therapy on HIV-infected children and their caregivers.

On June 6th 2007, the European Medicines Agency (EMEA) announced the recall by the manufacturer of the protease inhibitor neflafaxiv (Viracept, Roche) from the market because of possible contamination of tablets with the genotoxic substance ethyl mesylate. As a consequence, all patients who were receiving neflafaxiv had to be switched to a different drug regimen. After this unpredictable event, we decided to investigate the effect of switching therapies because of neflafaxiv recall, or for other reasons, on the perception of the benefits of therapy and adherence to treatment in HIV-infected children and their caregivers.

PATIENTS AND METHODS

HIV-infected children and their caregivers attending our Pediatric AIDS Reference Center (University "Federico II", Naples, Italy) between June and December 2007 were considered for enrolment. This is a major Italian center for the care of children with AIDS. HIV-infected children are managed according to guidelines established by the Centers for Disease Control and Prevention and the Italian Register for HIV Infection [13, 14]. Thirty-eight (13 boys; mean age 12.1±6.7 years) out of 45 HIV-infected children attending
Effect of Drug Recall in HIV-Infected Children

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our center were enrolled in the study. One child was excluded because of a recent diagnosis of HIV infection. The remaining 6 children were not included because they did not attend our center during the study period (4 patients) or refused to participate (2 cases). Informed consent to participate in the study was obtained from caregivers of all enrolled children.

Caregivers of enrolled children completed a questionnaire during a face-to-face interview. “Caregiver” was the person who routinely administered antiretroviral drugs to enrolled children and accompanied the child to the hospital for routine follow-up on the day of the interview. Caregivers were classified as biological parents, foster parents or second-degree relatives. The questionnaire contained information on demographic data, awareness of HIV status, perception of treatment efficacy, reaction to the “change of therapy” event, reasons for taking, missing or discontinuing drugs, and factors potentially influencing adherence. The questionnaire was administered by a psychologist trained in HIV infection at the time of or within one month after nelfinavir recall or treatment shift for other reasons. In the same period, caregivers of children who did not change therapy were administered the same questionnaire except for the questions related to the change of antiretroviral drugs. Awareness of HIV status was established based on the child’s knowledge about HIV infection. Adherence was evaluated at enrolment and subsequently during the 4th month after shifting, as part of the routine follow-up for HIV-infected children (scheduled every 2 months). To assess adherence, we used a 4-day recall instrument that has been shown to be a reliable surrogate marker of adherence [15, 16]. Patients were considered adherent if they took ≥95% of the total doses of medications in the last 4 days [15]. This study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of our institution.

The significance of the differences between the groups was assessed by the chi square test and Fisher’s exact test for dichotomous variables. In case of more variables, we applied the chi square test for independence. Continuous variables were compared with the Student t test and the Mann-Whitney test. When the study groups were more than 2, the ANOVA test was used for continuous variables. A two-sided test was used to indicate statistical significance at a p value of <0.05.

RESULTS

Based on treatment history, enrolled children were divided into 3 groups: patients who were shifted because of nelfinavir recall (group A; 8 children, 3 males; mean age 11.2 years); patients who were shifted in the month before nelfinavir recall or in the following 5 months for other reasons (group B; 12 children, 6 males; mean age 12.6 years), i.e. emergence of viral resistance (7 patients), toxicity (3 patients) and non-adherence (2 patients); and patients who were not shifted (group C; 18 children, 4 males; mean age 10.4 years). Demographic parameters did not differ significantly between the three groups except that the primary caregiver was more frequently a biological parent in group A than in group B (p = 0.02) and C (p = 0.05) (Table 1). All children on nelfinavir were switched to another protease inhibitor (lopinavir/ritonavir in all cases).

Overall, 12 (32%) children (mean age 14.7 ± 2.1 years) were aware of their HIV status and managed their own therapy. In the remaining 26 (68%) children (mean age 9.3 ± 3.4 years; p = 0.001), medications were administered by the primary caregiver. HIV-related features at the time of enrolment did not differ between the groups (Table 1). However, the number of patients with HIV-RNA less than 40 copies/ml was higher in group C patients than in group B patients, although the difference was not statistically significant (p = 0.06).

Table 1. Demographic Characteristics and HIV-Related Features at Enrolment in 38 HIV-Infected Children

<table>
<thead>
<tr>
<th>Group A (N = 8)</th>
<th>Group B (N = 12)</th>
<th>Group C (N = 18)</th>
<th>Total (N = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/Females</td>
<td>3/5</td>
<td>6/6</td>
<td>4/14</td>
</tr>
<tr>
<td>Mean age in years ± SD</td>
<td>11.2 ± 3.1</td>
<td>12.6 ± 3.9</td>
<td>10.4 ± 4.4</td>
</tr>
<tr>
<td>Caregiver*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biological parents</td>
<td>7 (37%)</td>
<td>4 (33%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Relatives</td>
<td>0</td>
<td>3 (25%)</td>
<td>4 (22%)</td>
</tr>
<tr>
<td>Foster parents</td>
<td>1 (13%)</td>
<td>5 (42%)</td>
<td>4 (22%)</td>
</tr>
<tr>
<td>Caregiver school education $</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 8 years</td>
<td>7 (87.5%)</td>
<td>5 (45%)</td>
<td>11 (65%)</td>
</tr>
<tr>
<td>&gt; 8 years</td>
<td>1 (12.5%)</td>
<td>6 (55%)</td>
<td>6 (35%)</td>
</tr>
<tr>
<td>Mean value of CD4+ cells in mm$^3$ ± SD</td>
<td>827 ± 332</td>
<td>795 ± 335</td>
<td>940 ± 311</td>
</tr>
<tr>
<td>Mean value of HIV RNA in copies/ml in patients with detectable HIV RNA ± SD</td>
<td>16495 ± 37806</td>
<td>33672 ± 42353</td>
<td>4912 ± 7784</td>
</tr>
<tr>
<td>Number of patients with viral load less than 40 copies/ml (%)</td>
<td>5 (62%)</td>
<td>5 (42%)</td>
<td>14 (78%)</td>
</tr>
</tbody>
</table>

SD = standard deviation.

*p = 0.02 for group A versus group B.

$Primary care was more frequent in biological parents compared to group B (p = 0.02) and C (p = 0.05).

School education was not available for 2 caregivers.
Treatment-related feelings are reported in Table 2. Most caregivers were satisfied with the health status of their children and all caregivers considered HAART necessary and effective, without significant differences between the groups. It is noteworthy that 18 (47%) caregivers were receiving HAART. There were a few worries with antiretroviral drugs, which were efficiently dealt with by patients and their families. However, more caregivers of group B did not trust therapy (i.e., they did not believe in the efficacy of treatment) compared to groups A and C (p = 0.03) (Table 2).

Before switching, 28 (74%) patients met the criteria of adherence to therapy (>95% of drug doses taken in 4 days), without significant differences between the groups. Differently, adherence to the timing of medications was poor in a substantial number of cases but without differences between the groups (Table 2). Patients adherent and not adherent to treatment did not differ by age (mean age 11.4±4.1 vs. 10.5±0.1, respectively, p=0.05), sex (39% of males vs. 20%, respectively; p=0.05) or race (Caucasian origin in 71% of cases vs. 80%, respectively; p=0.05).

Specific family factors were significantly associated with adherence to treatment rates. All children cared for by foster parents adhered to therapy compared to 62% of children cared for by biological parents (p = 0.03) and 71% of children cared for by second-degree relatives (p=0.05). Increased adherence rates were associated with a higher education in caregivers. Fifty percent of caregivers of adherent patients had a schooling above 8 years vs 90% of caregivers of non-adherent patients (p = 0.05).

Reactions to drug shifting and adherence to antiretroviral drugs in 20 HIV-infected children who shifted therapy are reported in Table 3. Shift of therapy, either because of nelfinavir withdrawal or for other reasons, generated anxiety and concern in most caregivers, without differences between the groups. Two (25%) caregivers of group A were very worried about nelfinavir toxicity. However, all caregivers were satisfied with the information received and reported they did not need more information. All but one caregiver (in group A) felt confident about the efficacy of the new treatment.

### Table 2: Perception of Antiretroviral Therapy and Adherence to Treatment in 58 HIV-Infected Children

<table>
<thead>
<tr>
<th>Perception of Therapy and Adherence</th>
<th>Group A (N=19)</th>
<th>Group B (N=11)</th>
<th>Group C (N=18)</th>
<th>Total (N=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>How do you consider health status of your child?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Good</td>
<td>6 (32%)</td>
<td>10 (91%)</td>
<td>16 (90%)</td>
<td>32 (67%)</td>
</tr>
<tr>
<td>- Poor</td>
<td>2 (11%)</td>
<td>2 (18%)</td>
<td>2 (11%)</td>
<td>6 (16%)</td>
</tr>
<tr>
<td><strong>Do you consider HAART necessary and effective?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>8 (100%)</td>
<td>11 (100%)</td>
<td>10 (100%)</td>
<td>29 (100%)</td>
</tr>
<tr>
<td>- No</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Do you trust therapy?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>8 (100%)</td>
<td>9 (73%)</td>
<td>10 (100%)</td>
<td>27 (56%)</td>
</tr>
<tr>
<td>- No</td>
<td>0</td>
<td>3 (25%)</td>
<td>0</td>
<td>3 (6%)</td>
</tr>
<tr>
<td><strong>Why?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Side effects of drugs</td>
<td>-</td>
<td>1 (33%)</td>
<td>-</td>
<td>1 (33%)</td>
</tr>
<tr>
<td>- Long-term efficacy</td>
<td>-</td>
<td>2 (67%)</td>
<td>-</td>
<td>2 (67%)</td>
</tr>
<tr>
<td>- Other reasons</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td><strong>Was it a problem to take the therapy?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Never</td>
<td>2 (25%)</td>
<td>4 (33%)</td>
<td>7 (39%)</td>
<td>13 (27%)</td>
</tr>
<tr>
<td>- Hardly ever a problem</td>
<td>5 (42%)</td>
<td>5 (42%)</td>
<td>8 (44%)</td>
<td>18 (37%)</td>
</tr>
<tr>
<td>- Forget about it</td>
<td>1 (11%)</td>
<td>3 (25%)</td>
<td>3 (17%)</td>
<td>7 (14%)</td>
</tr>
<tr>
<td><strong>If it was a frequent problem, what was the reason?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- formulation of drugs (taste, pill size...)</td>
<td>1 (100%)</td>
<td>1 (25%)</td>
<td>3 (100%)</td>
<td>5 (71%)</td>
</tr>
<tr>
<td>- schedule of therapy (meals, sleep, school...)</td>
<td>0</td>
<td>1 (33%)</td>
<td>0</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>- Side effects</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>- Social context</td>
<td>0</td>
<td>1 (33%)</td>
<td>0</td>
<td>1 (14%)</td>
</tr>
<tr>
<td><strong>Did your child comply with the drug timing?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Never</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>- Sometimes</td>
<td>3 (37%)</td>
<td>5 (42%)</td>
<td>2 (12%)</td>
<td>10 (21%)</td>
</tr>
<tr>
<td>- Always</td>
<td>2 (22%)</td>
<td>3 (25%)</td>
<td>8 (44%)</td>
<td>13 (27%)</td>
</tr>
<tr>
<td>- Occasionally</td>
<td>3 (33%)</td>
<td>3 (25%)</td>
<td>8 (44%)</td>
<td>15 (31%)</td>
</tr>
<tr>
<td><strong>Number of patients adherent to therapy (%)</strong></td>
<td>6 (75%)</td>
<td>8 (73%)</td>
<td>14 (78%)</td>
<td>28 (74%)</td>
</tr>
</tbody>
</table>

For patients who were shifted (groups A and B), these questions were related to the therapy before the shift. For patients who did not change therapy, the questions were related to the current therapy.

* More caregivers of group B did not trust therapy compared to group A and C (p = 0.05).
Table 3. Reaction to Drug Shifting, Perception of Antiviral Treatment and Adherence to Treatment in 20 HIV-Infected Children who Shifted Therapy

<table>
<thead>
<tr>
<th>What was your feeling when your child needed to change therapy?</th>
<th>Group A (N=5)</th>
<th>Group B (N=15)</th>
<th>Total (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Reassurance</td>
<td>0</td>
<td>2 (13%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>- Indifference</td>
<td>1 (20%)</td>
<td>0</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>- Concern</td>
<td>4 (73%)</td>
<td>9 (60%)</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>- Anxiety</td>
<td>1 (13%)</td>
<td>1 (6%)</td>
<td>2 (10%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Why did you feel worried?</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Side effects</td>
<td>2 (29%)</td>
<td>5 (33%)</td>
<td>7 (42%)</td>
</tr>
<tr>
<td>- Drug toxicity</td>
<td>2 (29%)</td>
<td>0</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>- Inefficacy of the new therapy</td>
<td>3 (43%)</td>
<td>5 (33%)</td>
<td>8 (47%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Do you trust new therapy?</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Yes</td>
<td>7 (87.5%)</td>
<td>12 (100%)</td>
<td>19 (95%)</td>
</tr>
<tr>
<td>- No</td>
<td>1 (12.5%)</td>
<td>0</td>
<td>1 (5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>If so, why?</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Side effects</td>
<td>1 (100%)</td>
<td>-</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>- Long-term efficacy</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>- Other reasons</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Number of patients adherent in the new therapy (% of total)

| - New treatment prescribed by caregiver                        | 7 (87%)       | 7 (53%)        | 14 (70%)    |

Adherence to the new treatment was evaluated during the 12th month after shifting. None of the analyzed variables differed statistically between the two groups.

The duration of treatment before switching did not differ between group A (median duration 60.5 months, range 7-96) and B (16.5 months, range 1-87; p<0.05) or between patients adherent and patients not adherent (median duration 45 months, range 1-96 vs 9.5 months, range 5-77; p<0.05). Adherence rates did not change significantly after switching therapy in groups A and B (87% and 58% of patients adherent after switching therapy in groups A and B, respectively, p<0.05). Finally, adherence rates were higher in the 12 patients who self-managed their therapy than in the 28 patients who received drugs from a caregiver, although the difference was not statistically significant (percentage of adherent patients 83% vs 64%, respectively; p<0.05).

DISCUSSION

In HIV-infected patients, shifts in antiretroviral therapy regimens are frequently due to treatment failure, emergence of viral resistance or drug toxicity. In a recent study of factors associated with willingness to accept changes in antiretroviral medication, 75% of HIV-infected adults accepted the change of HAART recommended by their providers [17]. Patients’ willingness to accept HAART changes is influenced by their attitude and beliefs about treatment, and the effectiveness of patient-provider communication [17], whereas side effects, social isolation and complexity of the antiretroviral regimen are associated with decreased adherence [18]. The consequences of switching therapy are difficult to investigate in HIV-infected children and adolescents for several reasons: the low number of HIV-infected children, the role of caregivers in drug administration, and less frequent need to change therapy compared to adult patients. We used the self-management of an HIV-infected child, which was a unique event in the history of HAART, to investigate if changes of antiretroviral drugs affect the perception of the benefits of therapy and adherence rates.

In HIV-infected pediatric patients, caregiver/family factors are the factors most closely associated with non-adherence to antiretroviral regimens [8]. Children receiving therapy from foster parents were more adherent than those receiving drugs from biological parents or relatives [9]. Interestingly, adherence was related to the relationship between the care provider and the child, and to the child’s awareness of HIV infection [9]. This study confirms that adherence is higher in children receiving drugs from foster parents than in children receiving medication from biological parents [9]. Furthermore, patients who self-managed their therapy tended to be more adherent than patients managed by caregivers.

Adherence may be increased by the perception of the beneficial effects of HAART. The perception that therapy is protective or reliable is associated with higher levels of adherence, whereas the perception of therapy as enslaving is associated with lower levels of adherence in HIV-infected adults [9]. A drug recall due to a possible toxic effect would be expected to result in impaired adherence. In the present study, almost all caregivers reported trust in physicians as well as in HIV medications, and adherence rates were even higher than those previously reported [9, 15]. The higher percentage of caregivers who did not trust therapy in group B may be explained by the presence in this group of patients who had already undergone treatment shifts because of a history of treatment failure due to viral resistance, side effects or non-adherence.

Although this study enrolled a small number of children, it describes a unique occurrence in HIV treatment. In 12 months, a total of 21 (47%) of 45 HIV-infected children seen
at our Reference Center needed to change HAART and 38% (8/21) of them did so because of nelfinavir recall. The switch because of recall involved 18% of the entire population of our HIV-infected children. Switching was a matter of concern for most caregivers and induced worries and a negative general feeling, although it did not affect adherence rates or result in decreased trust by HIV-infected children or their caregivers. In our population, drug recall was not more problematic than switching for other reasons and it did not affect adherence rate, perhaps because patients and their caregivers had received clear information about the medication and about the reasons for switching.

In conclusion, trust, the relationship between patient and physician, and the perception of drug efficacy are central to adherence. Shifting therapy because of drug recall was troublesome and generated anxiety, although it did not decrease adherence in HIV-infected children.

ACKNOWLEDGEMENTS

We thank Vania Giacometti for her comments and help in reviewing the manuscript. We thank Jean Ann Gilder for editing the manuscript. The research was supported by a grant from Sky Italia s.r.l.

ABBREVIATIONS

HAART = Highly active antiretroviral therapy
EMEA = European Medicines Agency
SD = Standard Deviation

REFERENCES

The changing pattern of adherence to antiretroviral therapy assessed at two time points, 12 months apart, in a cohort of HIV-infected children

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University Federico II, Department of Pediatrics, Via S. Raimondo, 5, 80131 Naples, Italy

Objective: In a previous multicentre study including 129 HIV-infected children, non-adherence to antiretroviral therapy was detected in 16% of patients and was mainly related to psychosocial rather than clinical or demographic features. The aim of this study was to explore the evolving pattern of adherence and its determinants in the same population of children.

Methods: An observational, cross-sectional multicentre study was done through a structured interview to the caregivers of HIV-infected children. Adherence was quantitatively evaluated through a 4-day recall adherence instrument.

Results: One hundred and twelve children were included. Nineteen (17%) omitted more than 5% of doses in the preceding 4 days and were considered non-adherent. Of these, 10 had been found non-adherent in the previous study. In parallel, nine of the originally non-adherent children had become adherent. Adherence rates were higher in children receiving therapy from foster parents than in children receiving therapy from biological parents. Dose number significantly correlated with adherence.

Conclusions: Adherence to antiretroviral therapy is a dynamic phenomenon that changes with time. The evolving pattern shows a fairly constant distribution of adherence because a similar number of patients gain and lose adherence. Psychosocial rather than clinical features are major determinants of adherence.

Keywords: adherence, antiretroviral therapy, children, HIV infection


1. Introduction

Pediatric HIV infection has evolved from a rapidly progressive, fatal disease in the early years of the epidemic to a chronic infection with prolonged survival in patients receiving highly active antiretroviral therapy (HAART). However, effective control of HIV infection depends on adherence and regular assumption of prescribed medications. Nearly perfect adherence (95%) is required to achieve and sustain viral suppression, to slow disease progression and to obtain optimal long-term benefits. Adherence is therefore a primary focus of research in HIV disease and a target of interventions.

Most data on adherence in HIV disease concern adult patients and factors that contribute to non-adherence. Recent meta-analyses suggest that efforts to improve adherence to antiretroviral therapy among adults can be effective [6]. By contrast, little is known about the determinants of adherence in children affected by HIV. In a previous cross-sectional assessment of adherence and its major determinants in seven major Italian reference centres for paediatric HIV infection, we found that 21 (16%)
Changing pattern of adherence to antiretroviral therapy

Table 1. Rates of adherence in 112 HIV-infected children according to key informants.

<table>
<thead>
<tr>
<th>Dose taken (% of total prescribed)</th>
<th>Baseline n (%)</th>
<th>After 12 months n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>87 (78)</td>
<td>88 (79)</td>
</tr>
<tr>
<td>&gt; 95% to &lt; 100%</td>
<td>6 (5)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>90%</td>
<td>14 (12)</td>
<td>6 (7)</td>
</tr>
<tr>
<td>75%</td>
<td>1 (1)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>60%</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>50%</td>
<td>2 (2)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>0</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
</tbody>
</table>

To design a program to improve treatment adherence in children with HIV infection, we must understand the dynamics of adherence to antiretroviral therapy. Very few studies have examined the evolution of adherence over time [n]. The present survey allowed us to investigate the evolution of adherence to antiretroviral therapy 12 months after an initial (baseline) evaluation in a population of HIV-infected children.

2. Patients and methods

The study was carried out in the same seven major Italian centres for pediatric HIV-infection as in the previous study. The same investigator (VG) visited each centre for 1 - 2 days and interviewed caregivers of children with HIV-symptomatic infection who were scheduled to visit the centre for routine follow-up visits. A face-to-face structured interview based on a standardised script dialogue was given to persons in charge of drug administration (caregiver). Data on socio-demographic and clinical features of enrolled children, their awareness of HIV status, prescribed antiretroviral therapy, and adherence to drug regimen were collected. Each interview lasted for approximately 20 min. Each patient was identified with a unique code. The investigator was unaware of the history and disease state of enrolled children. The physician in charge of the HIV-infected children was made aware of the result of interview only after the data analysis.

Clinical and immunovirological parameters (count of CD4-positive lymphocytes and plasma HIV RNA levels) were evaluated at the time of the administration of the questionnaire. At the time of the study, the lower detection limit of the viral load assay was 200 copies of HIV RNA/ml.

To estimate adherence quantitatively, each caregiver was asked how many doses of the total prescribed antiretroviral therapy had been omitted in the previous 4 days. The 4-day interval was a reliable surrogate marker for monthly adherence and was used in the previous study [10,12]. A dose was defined as the intake of any pill, syrup or suspension of any antiretroviral drug in one day. Since adherence rates of ≤ 95% of prescribed doses have been associated with suboptimal virologic outcome, children were defined non-adherent if they had taken less than 95% of all prescribed doses of antiretroviral therapy in the previous 4 days. Threshold levels were prospectively labelled as follows: perfect adherence (≥ 95% of prescribed doses regularly taken), low adherence (< 95%, but > 80% of doses taken) and poor adherence (< 80% of doses taken). Compliance with administration time was intended as the percentage of doses taken within 2 h of a target time.

Statistical analysis was performed using SPSS version 14.0 (SPSS, Chicago, IL, USA). The significance of the differences between features of adherent and non-adherent children and their caregivers was assessed by the χ² test and Fisher's exact test. Continuous variables were compared by the student's t-test or non-parametric Mann-Whitney U test. A two-tailed test was used to indicate statistical significance at a p-value of < 0.05.

3. Results

Of the 129 children enrolled in the first study (baseline), 112 (87%) patients (57 males; median age 108 months, range 12 - 216; mean duration of therapy at baseline 74 months ± 37) were available to be checked for adherence after a mean of 12 months. The other 17 children were not included because they did not attend the follow-up visit or their caregivers refused to take part in the second survey.

The overall adherence rate was 83% (Table 1). This included 88 (79%) children who took all doses and five who omitted one dose only in the previous 4 days but were considered adherent according to the cut-off threshold (adherence rate > 95%). Nineteen (17%) children did not adhere to treatment, and two of those children did not take any doses at all.

The changes in adherence patterns were as follows in the 19 non-adherent children, 10 (53%) of non-adherent were non-adherent at baseline and were therefore labelled "persistently non-adherent". Conversely, nine children (47%) of 19 non-adherent patterns) were adherent at baseline and were defined "new non-adherent". Exactly the same number of children shifted from having been non-adherent at baseline to adherent and were defined "new adherent". A comparison of baseline data revealed that non-adherent children had a significant risk of remaining non-adherent after 12 months (RR = 5.44, OR = 10.37, CI 1.56 - 11.55).
Table 2. HIV-related features of 112 enrolled children.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Baseline</th>
<th>After 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Adherent</td>
</tr>
<tr>
<td>Clinical class</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>A</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>B</td>
<td>38</td>
<td>31</td>
</tr>
<tr>
<td>C</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>CD4 T cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 15%</td>
<td>21 (18.7)</td>
<td>17 (81.0)</td>
</tr>
<tr>
<td>&gt; 15%</td>
<td>91 (81.3)</td>
<td>76 (83.5)</td>
</tr>
<tr>
<td>Viral load:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 200 copies/ml</td>
<td>32 (29.6)</td>
<td>27 (84.4)</td>
</tr>
<tr>
<td>&gt; 200 copies/ml</td>
<td>80 (71.4)</td>
<td>65 (82.5)</td>
</tr>
</tbody>
</table>

Data are shown as n (%).

Table 3. Adherence according to type of therapy and number of doses per day.

<table>
<thead>
<tr>
<th>Antiretroviral regimen</th>
<th>Baseline</th>
<th>After 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Adherent</td>
</tr>
<tr>
<td>HAART</td>
<td>87 (75.0)</td>
<td>76 (87.3)</td>
</tr>
<tr>
<td>No HAART</td>
<td>25 (22.0)</td>
<td>17 (68.0)</td>
</tr>
<tr>
<td>No. of doses/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 6</td>
<td>59 (50.7)</td>
<td>46 (78.1)</td>
</tr>
<tr>
<td>≥ 6</td>
<td>53 (47.8)</td>
<td>42 (82.3)</td>
</tr>
</tbody>
</table>

Data are shown as n (%).

Similar to our previous findings, compliance with administration times was very poor with only 16 (14%) caregivers reporting that they did administer doses in due time. The vast majority (95% of 112 caregivers, 63%) reported that adherence with prescribed times was limited, and 26 (23%) of caregivers reported no adherence at all with administration times. When we examined determinants of adherence, we found no significant difference between adherent and non-adherent patients with respect to gender, clinical class, or less than severe immune impairment and number of patients with undetectable viral load (Table 2). However, among patients with detectible HIV RNA, median viral load was significantly higher in non-adherent (HIV RNA median value 2870 copies/ml, range 250–300 000) than in adherent children (HIV RNA median value 510 copies/ml, range 220–12 390; p = 0.03). Twenty-seven children fully adhered either at baseline or after 12 months had a viral load below 200 copies/ml. Ninety-two children were receiving HAART whereas the remaining 20 were on only inhibitors of viral reverse transcriptase (NRTI; Table 3). Among the latter group, all patients were on two-drug regimens with a combination of two NRTIs. Adherence rate tended to be higher in children on HAART versus children on only NRTI (Table 3). Interestingly, there was a significant correlation between number of doses and adherence. The mean number of doses per day was 5.6 ± 1.30 and the median was six doses a day. Children receiving more than six doses per day (n = 68) were more adherent than those receiving fewer than six doses. The cut-off of six doses per day was based on the median number of administrations per day in the overall population. Of 61 adherent patients taking more than six doses per day, 34 (56%) received medications from biological parents, 19 (31%) from foster parents and eight (13%) self-administered drugs. Out of seven non-adherent patients, four (57%) received therapy from biological parents, one (14%) from foster parents and two (29%) self-administered antiretroviral medications (p > 0.05). Although the pattern of adherence varied widely in relation to individual drugs, no significant difference was found between adherent and non-adherent patients in relation to treatment type, with the exception of...
Changing pattern of adherence to antiretroviral therapy

![Graph showing adherence rates for different antiretroviral drugs](image)

**Figure 1. Adherence of HIV-infected children to individual antiretroviral drugs.**

Data for individual antiretroviral drugs taken by more than five patients are shown. A percentage of non-adherence more than 5% is reported. Adherence rates were 99% in the use of stavudine (n = 10), lopinavir (n = 8) and indinavir (n = 10) (data not reported in figure).

ZDV: Zidovudine; 3TC: Lamivudine; ZDV/3TC/Lamivudine/Abacavir combination; ABC: Abacavir; ddI: Didanosine; ddI/Abacavir; LPV/r: Lopinavir/ritonavir; NFV: Nelfinavir; ZDV: Zidovudine.

Six patients taking lopinavir/ritonavir, who were all non-adherent (p < 0.05 vs all other the individual drugs) (see Figure 1). This result can be explained by the low palatability of lopinavir/ritonavir oral suspension.

Finally, adherence was strongly related to the caregivers, and it was significantly higher in children receiving treatment from foster parents than from biological parents (Table 4). A similar pattern emerged from the baseline data in the 112 caregivers, although the difference was not significant (Table 4). Between the first and the second study, 38 children (11 males, median age 14.3 years, range 13 – 16.5) had been instructed to self-administer antiretroviral drugs. Among these, 11 (61%) lived with biological parents and seven (39%) lived with foster parents. Five of these 18 children (28%) were non-adherent, four (80%) of them lived with biological parents and one (20%) with foster parents. The difference was statistically significant when these children were analysed as a single group and compared with children receiving treatment from foster parents (Table 4).

### 4. Discussion

Adherence is affected by clinical, social, and behavioural factors [23,34]. Our results show that the pattern of adherence is unstable, thus supporting the concept that adherence is a time-related phenomenon in children with HIV infection. Quantitatively, non-adherence remained stable at around 20% because of an equal rate of patients from adherence to non-adherence and vice versa. This suggests that adherence should be frequently monitored and be a component of follow-up of children with HIV infection. Our assessment shows that the determinants of adherence change over time, albeit to a lesser extent than adherence, which again suggests more frequent monitoring of adherence.

In the present study, a high percentage of adherent patients still had a viral load > 200 copies/ml and some non-adherent subjects had an undetectable viral load, which resulted in a fairly low agreement between HIV RNA suppression and adherence rate. This inconsistency may have several explanations: First, adherence is a complex phenomenon and maintaining the prescribed adherence to medications is a continuous challenge even in adherent families. The self-report of medications taken during the previous 4 days does not adequately capture periods of non-adherence that are intermittent or present over longer time periods or that occur during periods of increased stress in the family. This may represent a limitation of the study. However, although a self-report instrument may tend to overestimate adherence, the reports of missed doses is a practical and reliable method if used at each routine visit. Second, some fully adherent children, suboptimal suppression of viral replication may be due to drug resistance. Third, metabolic and pharmacokinetic pattern may change with age and other conditions that are specific of children (i.e., the respect to timing of dosage and food consumption). Finally, although there was no association between viral load and complete adherence to antiretroviral therapy when viral load was dichotomized (more or less than 200 copies/ml), there was a significant trend for decreased level of viral load in adherent patients compared with non-adherent subjects.

In this study, caregivers report that psychosocial factors of caregivers and children play a major role in adherence and that foster parents are more reliable caregivers than biological parents. Family/caregiver related factors are crucial to paediatric adherence because infants and young children depend almost entirely on a caregiver to administer medications. Adherence to treatment, therefore, strictly dependent on caregivers. Biological parents of children with HIV are often HIV-infected and non-adherent themselves, and more debilitated and depressed than foster parents. This can lead to decreased attention given to the maintenance of adherence in their HIV-infected children. Furthermore, better adherence in children living with foster parents may be due to the cultural background of foster parents, who are selected before adoption, and to a greater sense of responsibility in non-biological parents compared with biological ones [31,35].

A new class of caregivers emerged in the second study, namely children who self-manage therapy. This group was the least adherent. Interestingly, all five non-adherent patients were also non-adherent at the baseline study. This finding carries an alarming message, namely, children who are managed by non-adherent caregivers tend to be non-adherent when they manage their own therapy. The latter is a worrying observation in the perspective of the increasing number of children who will directly manage their therapy.
Table 4. Relation between adherence and caregivers.

<table>
<thead>
<tr>
<th>Type of caregiver</th>
<th>Baseline</th>
<th>After 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Adherent</td>
</tr>
<tr>
<td>Biological parents</td>
<td>78 (78.2)</td>
<td>61 (79.5)</td>
</tr>
<tr>
<td>Foster parents</td>
<td>34</td>
<td>32 (92.9)</td>
</tr>
<tr>
<td>Self-administered</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are shown as n(%).

Finally, the relationship between adherence and more complex regimens has important clinical implications. These data are consistent with other reports showing that children with chronic diseases taking several drugs seem to be highly compliant with therapy. However, in these populations, the exact number of doses taken each day was not assessed. Although the HAART pill burden has decreased in recent years owing to simplified regimens, contrary to what might be expected our results show that HIV-infected patients taking more drugs are more compliant with therapy than patients taking fewer doses. It is possible to speculate that patients with more complex regimens may be, or believe to be, less healthy. As a consequence, they may have an increased perception of disease severity and, therefore, are likely to be more adherent to prescribed medication.

5. Conclusion

Adherence is a crucial variable of treatment of AIDS in children and deserves early and continuous monitoring and implementation because it tends to change over time and, more importantly, because non-adherence in early stages of life predicts later non-adherence, when control of therapy may become more difficult or impossible.

Declaration of interest

The research was supported by a grant from the television company Sky Italia s.r.l, who provided an unrestricted grant that contributed, in part, to the travelling expenses of the investigator.

Bibliography

Each of special note have been highlighted at either at moment (†) or of considerable interest (+) to readers.

1. Lawrence J. Adherence to antiretroviral therapy. AIDS Patient Care STDS 2001:15:107-8
5. In this multicenter study social factors were detected as main factor affecting non-adherence to antiretroviral therapy.
8. This is a recent meta-analysis including a large number of HIV-infected adults (59 randomized controlled trials with a total of 1839 participants). The results of the meta-analysis indicate that HIV-1 adherence behavioral interventions can be efficacious in improving adherence rates in HIV-infected adults.
Changing pattern of adherence to antiretroviral therapy

- In this study, including 125 HIV-infected children, self-reported adherence to antiretroviral therapy collected in a standardized manner was described as a useful measure of medication taking. Furthermore, an association between full adherence to treatment and maintenance of viral suppression was reported.


- In this study, a clear discrepancy between caregivers' and physicians' estimates of adherence to antiretroviral therapy was observed, the former being overestimated.


- This study showed that caregivers identified factors, such as caregiver stress, patient-child communication and lower caregiver quality of life, as the most strongly associated with non-adherence. These results underline that special efforts addressing psychosocial and family factors are required to improve child adherence to HAART.


- The study investigated potential indicators of compliance to medication in 6054 adults with chronic diseases. The authors found that compliance was significantly related to the number of drug regimens, being higher in patients taking several drugs.


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Is the Interruption of Antiretroviral Treatment During Pregnancy an Additional Major Risk Factor for Mother-to-Child Transmission of HIV Type 1?

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Background. There is currently an expert's agreement discouraging interruption of antiretroviral treatment (ART) during the first trimester of pregnancy in women infected with human immunodeficiency virus type 1 (HIV-1). However, this recommendation is poorly supported by data. We evaluated the effects of discontinuing ART during pregnancy on the rate of mother-to-child transmission.

Methods. Logistic regression models were performed in a prospective cohort of 975 women who were perinatally exposed to HIV-1 to evaluate adjusted odds ratios for confounding factors on mother-to-child transmission, including maternal interruption of ART.

Results. Among 975 pregnant women infected with HIV-1, ART was interrupted in 81 (8.4%) in the first trimester and in 11 (1.2%) in the third trimester. In the first trimester, the median time at suspension of ART was 6 weeks (interquartile range [IQR], 3-13 weeks); and the time without treatment was 8 weeks (IQR, 7-11 weeks). In the third trimester, the median time at suspension of ART was 32 weeks (IQR, 23-36 weeks); and the time without treatment was 6 weeks (IQR, 2-9 weeks). The plasma viral load was similar in women who had treatment interrupted in the first trimester and in those who did not have treatment interrupted. Overall, the rate of mother-to-child transmission in the whole cohort was 1.9% (95% confidence interval [CI], 0.7%-2.4%), whereas it was 6.9% (95% CI, 1.9%-13.2%) when ART was interrupted in the first trimester and 18.2% (95% CI, 4.5%-72.7%) when ART was interrupted in the third trimester. In the multiple logistic regression models, only interruption of ART during either the first or the third trimester was associated with a higher rate of mother-to-child transmission.

Conclusions. Discontinuing ART during pregnancy increases the rate of mother-to-child transmission of HIV-1, either when ART is stopped in the first trimester and subsequently resumed or when it is interrupted in the third trimester. This finding supports recommendations to continue ART in pregnant women who are already receiving treatment for their health.

After the results of the Pediatric AIDS Clinical Trials Group Protocol 076 Study Group [1], several prospective studies have confirmed the effectiveness of antiretroviral treatment (ART) during pregnancy in decreasing the rate of mother-to-child transmission.

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of HIV-1 (2–5). In developed countries, the synergic effect of ART in the mother and newborn, cesarean delivery, and avoidance of breastfeeding have resulted in a rate of MTCT of <2% (2–5). In the HAART era, the rate of MTCT is ~1%, probably because of the strong effect of HAART on the maternal viral load (6). Therefore, there is a consensus on the benefits of HAART for the mother’s health and for prevention of MTCT provided that drugs with a potential toxicity for the offspring are avoided (7). Data from prospective registries (8) support lack of teratogenicity with exposure to antiretroviral drugs during pregnancy, but with the increasing use of new molecules, monitoring of birth defects is continually needed. An increasing number of treated women are now identified as pregnant, and presently there is an expert’s opinion discouraging a temporary discontinuation of ART during the first trimester (7). However, this opinion is only partially supported by evidence. To date, insufficient data support an increase in the rate of MTCT as a consequence of ART discontinuation during pregnancy. We performed an analysis involving a large cohort of children prospectively enrolled in the Italian Register for HIV Infection in Children, to evaluate the effect of discontinuation of ART during pregnancy on the rate of MTCT.

METHODS

Data collection. The Italian Register for HIV Infection in Children is a nationwide multicenter study of children perinatally exposed to HIV-1 that was inaugurated in 1985. Data come from a network of 106 participating pediatric centers located throughout Italy, representing the overall population of exposed infants in Italy (9). Informed consent at the local pediatric center and National Privacy Agency permission are obtained. Both at risk children identified at birth and infected children identified after birth are enrolled. In this study, only exposed children identified at birth who were born to treated mothers and who had known infection status were considered (9). Data with regard to mother-infant pairs were collected as described elsewhere (8, 9). Detailed data on the type of regimen(s), gestational age at beginning and end of ART (for each single regimen if switched occurred during pregnancy), and ART administered intrapartum and/or to the newborn were included. Mucosal data were obtained at each pediatric center from an infectious diseases report and/or a gynecologist’s report, and they were recorded at the infant’s first examination, at birth. From 2001, information on the maternal plasma viral RNA load and CD4+ cell counts at the time of delivery and information on the last antiretroviral regimen (the type and the date of initiation) prior to pregnancy were collected. Because this information was uniformly available only since 2001, only children born in 2001 or later were evaluated in the present study. Viral loads and CD4+ cell counts were evaluated as described elsewhere (3, 9). Both baseline and follow-up information on the infant’s infection status, HIV-1 antibodies, and viral markers (HIV-1 DNA or RNA detection) were collected (3).

Case definitions. Infections in children (3, 9, 10), elective cesarean delivery (11), and duration of ART during pregnancy (9) were defined as reported. To evaluate the effect of type of ART on the rate of MTCT, we defined monotherapy as zidovudine (according to the Pediatric AIDS Clinical Trials Group Protocol 076 Study Group protocol), double therapy as any regimen including 1 nucleoside reverse transcriptase inhibitors (NRTI), and HAART as any combination regimen with ≥3 antiretroviral drugs (no mother received a triple NRTI regimen). Among HAART regimens, a nevirapine-based regimen was defined as any triple regimen including 2 NRTIs plus nevirapine, and a protease inhibitor (PI)-based regimen was defined as any triple (or more) regimen including NRTIs plus a PI. When ≥1 regimen was received during pregnancy, the more complex (i.e., HAART vs. double) was considered. Maternal interruptions of ART were defined as the discontinuation of any ART for >15 days. Shorter periods of interruption, in fact, could not be reported by the mothers. Moreover, it is usually believed that plasma viral RNA load significantly decreases after 2 weeks of treatment, and perinatal guidelines recommend monitoring maternal viral load 2–6 weeks after initiating or changing ART (7). The gestational age at ART initiation was defined as the week at the beginning of the first ART regimen for women who did not receive therapy during the periconceptional period.

Statistical analysis. Analyses were performed on data reported through December 2005 for children followed up since birth who were born from 1 January 2002 through 31 December 2004. Children born from 1 January 2003 through 30 June 2005 were excluded from analysis because of the large percentage of exposed children with an undetermined HIV-1 status.

Data were expressed as median and interquartile range (IQR) or as mean and 95% CI. Differences in proportions were evaluated by the y2 test. A nonparametric 3-sample test was performed to test the hypothesis of median equality. The analysis of variance was used to test for differences in maternal viral load at delivery. To evaluate the association of ART interruption with the rate of MTCT, logistic regression models were performed to estimate adjusted ORs for factors potentially influencing the rate of MTCT: infant’s sex, gestational age >37 weeks, >37 weeks, or unknown), plasma viral RNA load at delivery (<2,000, 2,000–4,999, 5,000–6,999, 7,000–8,999; or unknown), maternal viral RNA load at delivery (≥4,000, 2,000–4,999, >5,000 copies/ml; or unknown), mode of delivery (vaginal delivery, elective cesarean delivery, cesarean delivery other than elective, or unknown), maternal ART in pregnancy (zidovudine monotherapy, double therapy, HAART, or unknown), maternal interruption of ART (yes or no), interruption ART (yes, no, or unknown), and perinatal ART (yes, no, or unknown). Because
no child was breast-fed, the mode of feeding was not included in the regression analysis. To better evaluate interaction between quantitative variables potentially associated with the rate of MTCT, an additional logistic regression model with log10 plasma viral RNA load and log10 CD4+ cell count as continuous variables was performed for a subgroup of mother-child pairs with both variables known.

RESULTS

Mother-infant pairs. Overall, 927 of 1016 mother-infant pairs receiving ART entered the study; 79 (7.6%) of the exposed infants were excluded because they were lost to follow-up before initiation of antiretroviral therapy. The characteristics of the 927 mothers and children are shown in Table 1. Intervention and exclusive prophylaxis consisted of zidovudine in all but 13 cases in which nevirapine was given. Maternal plasma viral load was known in 630 (67.2%) of the 927 mother-child pairs. Of the 630 women with known viral load, the majority (486 [76.9%]) showed an undetectable plasma viral load (tested by an assay with a cutoff value of 50 copies/ml; n = 486) and 164 (25.8%) showed ≥ 50 copies/ml. Characteristics of mother-child pairs were compared according to known or unknown maternal plasma viral load. The frequency of prepartum ART administration was higher when maternal viral load was known (486 [74.1%] of 630 versus 164 [25.9%] of 630 women; χ2 = 13.91; P = .0001) and frequency of prepartum HAART administration was higher when maternal viral load was known (486 [74.1%] of 630 versus 164 [25.9%] of 630 women; χ2 = 13.91; P = .0001), but there was no difference observed in the frequency of mode of delivery or caesarean delivery (546 [84.1%] of 630 versus 150 [93.4%] of 307 women; χ2 = 0.023; P = .882), the use of neonatal ART (614 [95.1%] of 650 infants versus 301 [98.0%] of 307 infants; χ2 = 0.03; P = .862), and the infant's infectious status (8 [2.8%] of 608 cases versus 4 [1.3%] of 307 cases; χ2 = 0.03; P = 0.862). The maternal viral load decreased as the complexity of the ART regimen increased. The mean plasma viral load was 2.6 log10 copies/ml (95% CI 2.2–2.9 log10 copies/ml) and 1.9 log10 copies/ml (95% CI 1.3–2.5 log10 copies/ml) in women receiving monotherapy, double therapy, or HAART, respectively (analysis of variance: F = 17.12; P < .001). The median gestational age at the beginning of treatment was 18 weeks (IQR, 13–23 weeks) in the first trimester, 10 (IQR, 6–15 weeks) in the second trimester, and 13 weeks (IQR, 7–22 weeks) in the third trimester. The proportion of women with detectable viral load did not differ according to trimester at start of ART when monotherapy was given (n = 50; 78.0%) during the second trimester, and 50.0% during the third trimester. χ2 = 2.27; P = .13. In addition, the pro-

Table 1. Characteristics of 927 mother-infant pairs in our study of mother-to-child transmission of HIV-1 infection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) of mother-infant pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal ART</td>
<td></td>
</tr>
<tr>
<td>Monotherapy</td>
<td>96 (10.4)</td>
</tr>
<tr>
<td>Double therapy</td>
<td>107 (11.4)</td>
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<tr>
<td>HAART</td>
<td>654 (69.8)</td>
</tr>
<tr>
<td>Type of ART</td>
<td></td>
</tr>
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<td>Before antiretroviral ART (n = 91)</td>
<td>77 (85.5)</td>
</tr>
<tr>
<td>Other regimens for HIV ART</td>
<td>14 (15.5)</td>
</tr>
<tr>
<td>Type of ART</td>
<td></td>
</tr>
<tr>
<td>Before antiretroviral ART (n = 91)</td>
<td>77 (85.5)</td>
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<td>Other regimens for HIV ART</td>
<td>14 (15.5)</td>
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<td>Type of ART</td>
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<tr>
<td>Before antiretroviral ART (n = 91)</td>
<td>77 (85.5)</td>
</tr>
<tr>
<td>Other regimens for HIV ART</td>
<td>14 (15.5)</td>
</tr>
</tbody>
</table>

NOTE: HAART was defined as ≥2 antiretroviral drugs, ART: antiretroviral therapy.
Table 2. Characteristics of the 12 mother-child pairs in which the infants were infected.

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Maternal ART regimen</th>
<th>At beginning of pregnancy</th>
<th>After interruption</th>
<th>Infant ART regimen</th>
<th>Maternal ART</th>
<th>CTC cell count at delivery, cells/μL</th>
<th>CD4+ cell count at delivery, cells/μL</th>
<th>Gestational age, weeks</th>
<th>Intermittent ART</th>
<th>Interim ART</th>
<th>IRCT (months)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>A2T</td>
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<td>A2T</td>
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<td>Yes</td>
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<td>No</td>
<td>Yes</td>
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<tr>
<td>2</td>
<td>A2T</td>
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<tr>
<td>3</td>
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<tr>
<td>4</td>
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<tr>
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<td>Yes</td>
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<td>12</td>
</tr>
</tbody>
</table>

**NOTE.** All deliveries were elective Cesarean deliveries. ART, antiretroviral; IDU, intravenous drug user; IDU, intravenous drug user; LAM, lamivudine; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PIs, protease inhibitors. Mean values are presented for continuous variables. *P* values are presented for categorical variables. Significant *P* values are in bold.

A portion of women with detectable viral load did not differ according to the trimester during which ART was initiated when double therapy was given (n = 136; 81.8%) during the first trimester, 37.7% during the second trimester, and 47.1% during the third trimester (χ² = 0.56; P = .576). However, the proportion of women with detectable viral load was significantly higher when HAART (given to 45 women) was started during the third trimester (45.9%), compared with during the second (17.4%) or first trimester (17.8%); χ² = 23.81; *P* < .0001. Women treated with HAART who had a known plasma viral load had similar mean plasma viral loads when compared with those who were treated with a PI-based regimen (1.05 log₁₀ copies/mL; 95% CI, 1.77–2.07 log₁₀ copies/mL) were compared with those who were treated with a nevirapine-based regimen (1.85 log₁₀ copies/mL; 95% CI, 1.65–1.92 log₁₀ copies/mL; Student’s test, 1.29; *P* = .197). Discontinuation and subsequent restart of ART was reported in 81 (53.9%) of the 151 mother-child pairs in the first trimester of pregnancy (median time at suspension, 6 weeks [IQR, 5–6 weeks] median time without treatment, 8 weeks [IQR, 7–11 weeks]) and in 11 (1.2%) of the 90 mother-child pairs in the second trimester (median time at suspension, 7 weeks [IQR, 6–8 weeks] median time without treatment, 6 weeks [IQR, 2–9 weeks]). Only 1 mother, who gave birth to an uninfected child, had treatment interrupted during the first and the third trimesters. ART was more frequently interrupted at the beginning of pregnancy in women treated with HAART (74 [11.4%] of 651 women) than in women treated with double therapy (31 [1.2%] of 257 women) or with monotherapy (14 [4.0%] of 350 women; χ² test, 23.50; *P* < .0001).
To evaluate the role of different risk factors for MTCT, univariate and multiple logistic regression models were performed. Maternal monotherapy or double therapy, interruption of ART during either the first or the third trimester, delivery by a mode other than elective cesarean delivery, and a viral load at delivery >4.78 log_{10} copies/mL were associated with an increased rate of MTCT (table 3). Plasma viral load >4.78 log_{10} copies/mL was associated with a 30-fold increased risk of transmission.

Other factors (maternal CD4 cell count at delivery, trimester at the start of ART, child’s sex, and intrapartum and neonatal ART) were not significantly associated with the rate of MTCT.

When we included in an additional model the 79 exposed infants lost to follow-up, with the assumption that all these children were uninfected, results were unchanged (adjusted OR for treatment interruption in the first trimester, 11.4; in the third trimester, 14.1). The probability of infection in children

<table>
<thead>
<tr>
<th>Table 3. Univariate and logistic regression analysis of risk factors for mother-to-child transmission of HIV-1 infection.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor</strong></td>
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<tr>
<td>-----------</td>
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<tr>
<td><strong>Type of maternal ART</strong></td>
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<tr>
<td>Double</td>
</tr>
<tr>
<td>HAART</td>
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<tr>
<td>First</td>
</tr>
<tr>
<td>Second</td>
</tr>
<tr>
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<tr>
<td>No</td>
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<tr>
<td>During the second trimester</td>
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</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Viral load at delivery, log_{10} copies/mL</strong></td>
</tr>
<tr>
<td>&lt;2.5</td>
</tr>
<tr>
<td>2.5-4.5</td>
</tr>
<tr>
<td>4.5-7</td>
</tr>
<tr>
<td>&gt;7</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td><strong>CD4 cell count at delivery, cells/μL</strong></td>
</tr>
<tr>
<td>&lt;200</td>
</tr>
<tr>
<td>200-499</td>
</tr>
<tr>
<td>&gt;500</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Sex of child</strong></td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td><strong>Intrapartum ART</strong></td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td><strong>Neonatal ART</strong></td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
</tbody>
</table>

**NOTE.** HAART was defined as ≥3 antiretroviral drugs. ART, antiretroviral treatment; NA, not applicable.
in this group was very low, because missing infected children are reported by periodic cross-matchings with the Italian National AIDS Registry, to which all AIDS cases must be reported [11].

To check the interference of missing values for maternal plasma viral load, 3 logistic regression models were performed. Interruption of ART in the first trimester was associated with an adjusted OR of 10.33 (95% CI, 2.02–52.91; P = .005), and interruption of ART in the third trimester was associated with an adjusted OR of 4.66 (95% CI, 1.48–14.12; P = .007) in the whole cohort, with the inclusion of maternal viral load as a categorical variable. When only cases with known maternal viral load were included, the adjusted OR for interruption of ART in the first trimester was 11.25 (95% CI, 1.58–83.08; P = .006) and in the third trimester was 24.33 (95% CI, 0.77–765.92; P = .007). When maternal viral load was excluded, the adjusted OR for interruption of ART in the first trimester was 8.32 (95% CI, 1.88–36.00; P = .005) and in the third trimester was 40.32 (95% CI, 3.24–387.64; P = .001) in the whole cohort.

Finally, a logistic regression model on a subgroup (n = 338) of mother-child pairs with both known plasma viral load and known CD4+ cell count was performed, including all factors as continuous variables. Treatment interruption in the first trimester was associated with an adjusted OR of 6.70 (95% CI, 1.48–27.18), and treatment interruption in the third trimester was associated with an adjusted OR of 20.48 (95% CI, 0.98–941.66). Plasma viral load was associated with an adjusted OR of 2.90 (95% CI, 1.27–6.91), and CD4+ cell count was associated with an adjusted OR of 1.87 (95% CI, 0.97–3.53). Thus, treatment interruption in the first trimester was associated with a 10-fold increased risk of MTCT, whereas a plasma viral load increase of 1 log10, captured doubled the risk of MTCT.

**DISCUSSION**

To our knowledge, this is the first study to reveal that discontinuing ART during pregnancy increases the rate of MTCT of HIV-1, either when ART is stopped in the first trimester and subsequently restarted, or when it is interrupted in the third trimester. The last finding was partly expected, because discontinuation of treatment could lead to a systemic rebound [12, 18] and because a higher viral load near the time of delivery increases the risk of MTCT, as we and others have clearly shown [14, 15]. The incremental risk of MTCT associated with treatment interruption is high among all treated women, even among HAART-treated women, because the virologic rebound is stronger if the virologic potency of therapy is higher. Therefore, our findings confirm the recommendation that pregnant women infected with HIV-1 who receive ART should continue treatment after the first trimester, particularly at the end of pregnancy [7]. More interestingly, we found that interruption of ART early in pregnancy increased the rate of MTCT both in the whole cohort and among children born to HAART-treated women. To our knowledge, no other large study has investigated this issue. Of note, in our cohort, temporary ART discontinuation early in pregnancy is more frequent in women receiving HAART, probably because of the lack of drug toxicity in the embryos. However, temporary discontinuation led to a 10-fold increase in the rate of MTCT, overcoming all other risk factors, except for the independent factor of high maternal plasma viral RNA load at delivery. When viral load increased by 1 log10, captured, the risk of MTCT was, indeed, more than doubled. A small proportion of HAART-treated women may have high plasma viral loads because of poor compliance or mutations associated with viral resistance [16]. Nevertheless, we found no difference in pre-delivery plasma viral load between mothers who temporarily discontinued therapy and mothers who did not. It might be speculated that a transient viral load increase, occurring close to the time of ART interruption, favors HIV-1 transmission.

We also investigated whether a lower antiretroviral potency of the regimen administered after suspension could lead to higher risk of MTCT, but the proportions of nevirapine-based or PI-based regimens were similar before and after interruption. However, it is possible that virological failure occurred more frequently because of nevirapine resistance mutations [17] in those women who discontinued and restarted the same nevirapine-based regimen. Viral load at delivery was similar in the women treated with a PI-based regimen and in those treated with a nonnucleoside reverse transcriptase inhibitor-based regimen in both the whole cohort and in those who had therapy interrupted. In addition, we found a trend toward higher viral loads in women treated with a PI-based regimen than in those treated with an nucleoside reverse transcriptase inhibitor-based regimen, as recently reported by the EuroSIDA Collaborative Study [18]. It is possible that women with lower baseline viral loads (before or at the beginning of pregnancy) are more often treated with nucleoside reverse transcriptase inhibitor-based regimens, or the pharmacokinetic peculiarities of ART in pregnancy may be involved [18].

Because our data are from a large pediatric cohort, a limitation of our analyses is that some detailed information on the mothers was lacking. First of all, maternal viral load at delivery was unknown for ~30% of mother-child pairs. However, when mothers-child pairs were compared according to whether maternal viral load was known or unknown, exactly the same MTCT rate was found. As an additional check, 3 different logistic regression models were performed, but the main role of ART interruption in pregnancy was constantly confirmed. Another critical point is the unavailability in our database of information on the reasons for ART discontinuation during...
pregnancy. Therapy could be discontinued for several reasons: the development of virological failure or resistance mutations, the occurrence of adverse effects, or the desire to avoid fetal exposure during the first trimester. On the other hand, ART interruption was not homogeneously distributed during the whole pregnancy, peaking at 6 and 32 weeks. We think that avoidance of fetal exposure during the first trimester and maintenance of treatment are the most probable reasons for ART discontinuation, and if so, it would be unlikely that women who had ART suspended were at a higher risk of MTCT because of virological failure or resistance mutations. Unfortunately, we had no data on maternal viral load during gestation. Moreover, we could not assess whether infected children born to mothers who had ART interrupted acquired the infection in utero or perinatally [19], because we did not systematically measure HIV-1 RNA or DNA load during the last 24–48 h of life.

We identified all interactions between variables that we thought were biologically convincing. Other important factors might be compliance to ART and genotype resistance in the mothers, but these data were lacking in our database. Finally, the very low number of infected children in the whole cohort and, consequently, the wide 95% CIs found, are additional caveats. Additional studies, prospectively performed using large cohorts of mother-child pairs starting from the beginning of gestation, may confirm our findings. It may be surprising that we found no relationship between interrumpption and maternal therapy and the rate of MTCT, but the number of mother-child pairs who were not receiving interrruption and maternal prophylaxis were too small to draw any conclusion. Moreover, we found no differences according to trimester at start of ART, even if a higher proportion of HAART-treated women had a detectable viral load at delivery when treatment was used during the third trimester, compared with the first or second trimester. This finding supports recently revised guidelines [7] that recommend that women who do not require ART for their own health, ART for MCJT prophylaxis may be started during the second trimester, preferably with a 3-drug regimen.

The main finding of our study is that no other factors but the discontinuation of ART and high maternal viral load at delivery are associated with an increased risk of MTCT. Thus, although concerns about potential toxicity in the fetus need to be completely clarified, counseling on the temporary discontinuation of ART in the first trimester should consider both the need for maternal health [7] and the increased risk of HIV-1 transmission to the offspring. We believe that our findings may be useful for physicians who care for women infected with HIV-1 and for specialists who determine guidelines.

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References

3.4 Psychosocial issues in children growing with HIV infection

3.41 Rationale

Children and adolescents with HIV infection have to face greater challenges to their psychosocial well-being than their counterparts not affected by HIV (1-4). Children living with HIV must deal with lack of adult influence, illness or death of a parent, poverty, disclosure of their own or their parents’ HIV status to members of their community (5,6). As a consequence, being a child or an adolescent with HIV implies major problems in terms of quality of life, school attendance and social interactions with peers. Thus, notwithstanding the spectacular improvement in the virological, immunological and metabolic control of the disease due to highly active antiretroviral therapy (HAART), a number of psychosocial problems may affect the health status of HIV-infected patients (5). In a recent Cochrane review, no rigorous studies of interventions for improving the psychosocial well-being of children affected by HIV and AIDS were identified (7). Current psychosocial practice is based on anecdotal knowledge, descriptive studies and situational analyses.

The identification of needs is mandatory to improve the psychosocial well-being of this vulnerable population. Assessment of psychosocial problems in HIV is hampered by the lack of standard instruments to evaluate these issues in growing children and adolescents (8). The International Classification of Functioning, Disability and Health (ICF) of the World Health Organization adapted for children and young people (ICF-CY) is an age-specific descriptor system devised to evaluate the health status and disabilities of a person or a group of persons and ideally completes the information to outweigh the global impact of a given disease (8-11). It encompasses information on health using a bio-psycho-social approach that takes into account physical health as well as the social context and other factors that may favor, or impair, school, work, and social achievements. Application of the ICF to pediatric HIV infection may provide a framework for standardizing data collection and for evaluating health care interventions.

The aim of this study was to investigate the feasibility of applying the ICF to determine the health status and needs of a population of children and adolescents with HIV infection seen at a reference center for pediatric AIDS in Europe.
3.42 Experimental procedures

**Patients with HIV infection**

Children in follow-up at two HIV reference centers located in Southern and Northern Italy, namely in Naples and Brescia, were enrolled. In both institutions, the children are managed according to the recommendations of the Center for Disease Control (12) and the Italian Register for HIV infection (13-15). The latter coordinates the medical care of children in Italy and was established at the onset of the HIV epidemic (13-15).

**ICF tool**

ICF comprises a standard checklist validated at international level. The specific version for children and adolescents, designated ICF-CY, was devised in 2006 by the World Health Organization (www.who.int/classifications/icf/en/). The checklist may be completed by health care personnel or by other caregivers such as teachers, social workers and parents.

The ICF encompasses 4 major areas of health and functioning of a person:

1) impairments of body structures;
2) impairments of body functions;
3) environmental factors;
4) activity limitations and restrictions to social life.

Impairments are defined as problems in body function or structure. Information about impairment of body structures and functions was obtained from medical records and physical examination of patients. The level of impairment detected in these two areas are scored from 0 (no problem) to 4 (complete impairment).

The section of the ICF-CY regarding environmental factors includes a number of domains related to the physical, social, and attitudinal environment in which the child lives. The environmental factors section encompasses products and technology, natural environmental features and human-made changes to the environment, supports and relationships, attitudes, services, systems and policies that may positively or negatively affect the life of a patient. Environmental factors are labeled as facilitators or barriers to the development of an individual and are scored from 0 (no facilitator or no barrier) to 4 (complete facilitator or complete barrier).

Activity limitations are difficulties a person may have in carrying out daily activities. Participation restrictions are problems a person may experience when involved in life or social situations. Specifically, this section encompasses 9 domains: learning and applying knowledge, general tasks and demands, communication, mobility, self care, domestic life, interpersonal interactions and relationships, major life areas, and finally community.
social, and civic life. This section is scored from 0 (no limitation or restriction) to 4 (complete limitation or restriction). For all sections, the item was classified “code 8” (“not specified”) in case of insufficient information to specify the severity of the impairment, whereas the item was scored “code 9” (“not applicable”) if it is inappropriate to apply any other code.

**Data collection**

Information related to sections 1 and 2 were collected by the physicians in charge of children (A.G.). Data regarding sections 3 and 4 were collected by a specifically trained psychologist (G.G.) by means of interviews conducted in a protected setting. The key responder was the person responsible for the care of the child and who accompanied the child to the hospital for routine follow-up. Key responders were biological or foster parents or second-degree relatives. Each interview lasted approximately one hour. Key responders were informed about the objectives of the study and gave their consent to participate.

**Statistical analysis**

Data were analyzed with the SPSS package version 12. Considering the high number of issues in each section of the questionnaire, in this report we focus on the more frequent critical issues. Erratic issues, detected in less than 3% of the patients (1 child), are not reported herein, but the corresponding author will provide information on all items of the ICF checklist upon request.

**3.43 Results**

Forty-one families of HIV-infected children were enrolled, 25 from Naples and 16 from Brescia.

**Table 1** shows the main sociodemographic and HIV-related features of the enrolled children. There was a high prevalence of children of low socioeconomic status, and most of them were orphans. Most patients were adolescents. Overall, the major clinical, immunological and virological parameters of HIV infection were well controlled; viral load was low and immune derangement was limited.
Table 1. Main sociodemographic and HIV-related features of 41 enrolled patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients enrolled, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal education (years)*:</td>
<td></td>
</tr>
<tr>
<td>≤8</td>
<td>20 (80)</td>
</tr>
<tr>
<td>&gt;8</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Single parent</td>
<td>12 (29)</td>
</tr>
<tr>
<td>One or both parents unemployed</td>
<td>18 (44)</td>
</tr>
<tr>
<td>Natural father not alive</td>
<td>9 (22)</td>
</tr>
<tr>
<td>Natural mother not alive</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Foster families</td>
<td>9 (22)</td>
</tr>
<tr>
<td>Children aware of their HIV diagnosis</td>
<td>13 (32)</td>
</tr>
<tr>
<td>Age range (years)</td>
<td></td>
</tr>
<tr>
<td>6-9</td>
<td>9 (22)</td>
</tr>
<tr>
<td>10-13</td>
<td>10 (24)</td>
</tr>
<tr>
<td>&gt;14</td>
<td>22 (54)</td>
</tr>
<tr>
<td>Patient whose viral load was &lt;40 copies/ml</td>
<td>30 (73)</td>
</tr>
<tr>
<td>Median viral load among patients with HIV RNA &gt;40 copies/ml (range)</td>
<td>2935 (480-65400)</td>
</tr>
<tr>
<td>Median CD4 (cell/mm) (range)</td>
<td>923 (259-2245)</td>
</tr>
</tbody>
</table>

* Information not available for some families.
Table 2 summarizes the main findings regarding impairments of body structures. The main problems reported were related to the immune system and skin, mainly due to lipodystrophy. The latter is considered a “skin” problem in the questionnaire. As shown in table 3, the main impairments of body function concerned vision and the gastrointestinal tract.

Table 2. Major impairments regarding body structures of the ICF checklist in 41 HIV-infected children and adolescents

<table>
<thead>
<tr>
<th>Impairments</th>
<th>Patients with impairments, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorax*</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>Immune system**</td>
<td>13 (32)</td>
</tr>
<tr>
<td>Endocrine system***</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Skin problems/fat distribution §</td>
<td>11 (27)</td>
</tr>
</tbody>
</table>

* Lung tuberculosis  
** Score 2 and 3 according to CDC criteria  
*** Growth hormone deficiency  
§ Includes lipodystrophy

Table 3. Relevant impairments regarding body functions of the ICF checklist in 41 HIV-infected children and adolescents

<table>
<thead>
<tr>
<th>Impairments</th>
<th>Patients with impairments, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye function*</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Gastrointestinal function**</td>
<td>6 (15)</td>
</tr>
</tbody>
</table>

* Myopia (5 patients), strabismus (1 patient), palpebral ptosis (1 patient)  
** Chronic hepatitis B (1 patient), chronic hepatitis C (3 patients), liver steatosis (1 patient), sclerosing cholangitis (1 patient)
Table 4 reports the main environmental factors that were perceived to affect the quality of life of subjects enrolled in the study. Many environmental factors of the ICF checklist were considered by the key informants as barriers, whereas none was considered a facilitator. In addition, the barriers were scored “severe” or “complete” by more than 50% of the key informants interviewed. A major issue was limited availability of food and drugs. When the reasons for the limited availability of foods and drugs were investigated, the most frequent answers were poverty and unemployment. The majority (83%) of key informants considered care providers, and in particular social workers, as “barriers” because they received inadequate support from these institutions (public health services, school…). Moreover, when key informants were asked why they had classified other family members, friends and health and education care providers as “barriers”, the most frequent answer was that they had not told these people about child’s HIV status due to the fear of social stigma.

Table 4. Environmental factors of the ICF checklist scored as barriers in 41 HIV-infected children and adolescents

<table>
<thead>
<tr>
<th>Barriers</th>
<th>Families reporting the barriers, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insufficient availability of:</strong></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Drugs</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Daily consumption products</td>
<td>8 (19)</td>
</tr>
<tr>
<td>Transportation facilities</td>
<td>5 (12)</td>
</tr>
<tr>
<td><strong>Inadequate support by:</strong></td>
<td></td>
</tr>
<tr>
<td>Other family members</td>
<td>11 (27)</td>
</tr>
<tr>
<td>Relatives and friends</td>
<td>22 (54)</td>
</tr>
<tr>
<td>Care providers (health, education or social)</td>
<td>34 (83)</td>
</tr>
<tr>
<td><strong>Inadequate approach by:</strong></td>
<td></td>
</tr>
<tr>
<td>Other family members</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>Relatives and friends</td>
<td>8 (19.5)</td>
</tr>
<tr>
<td>Care providers (health, education or social)</td>
<td>6 (15)</td>
</tr>
</tbody>
</table>
Table 5 shows the frequency of activity limitations and participation restrictions in the children enrolled in this study. Few parents reported specific limitations and restrictions, and the degree of severe or complete limitations and restrictions was approximately 50%.

Table 5. Reported activity limitations and participation restriction in 41 HIV-infected children and adolescents

<table>
<thead>
<tr>
<th>Domains of activity limitations and participation restrictions</th>
<th>Families reporting limitations and restrictions, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relationship with relatives and friends</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Learning in informal setting</td>
<td>5 (12)</td>
</tr>
<tr>
<td>Learning at school</td>
<td>7 (17)</td>
</tr>
</tbody>
</table>
3.44 Discussion

This is the first attempt to apply the ICF to children and adolescents with AIDS in order to obtain a functional profile of their psychosocial problems. ICF is an adjunct to the WHO International Classification of Disease (ICD-X) and serves to obtain information on both physical health status and social well-being by investigating environmental and social factors that may influence the activities and the quality of life of an individual. Thus far, ICF has been applied in several chronic childhood conditions that are mainly characterized by physical disabilities (16-23). Application of this tool to children with HIV infection, a disease with a heavy burden of psychosocial issues, may also yield information useful for planning health care interventions.

Impairments of body structures and functions were neither frequent nor severe in our patients and were well controlled by antiretroviral therapy. This is expected in a child in follow-up in a reference centre for AIDS in Europe, where a high standard of pharmacological care is guaranteed. It was easy to collect such information with the ICF, which may thus be a standard means for monitoring both the evolution of the disease and the impact of interventions. In contrast with the limited frequency and intensity of clinical problems, many barriers were reported in the psychosocial sections. Indeed, most key informants labeled the environmental factors in the checklist as barriers rather than facilitators to the participation in social activities of their children. Perceived insufficient availability of food, drugs, other products for personal use, as well as of transportation facilities, were considered a major critical issue. This was probably due to the high prevalence of poverty and unemployment in the families of HIV-infected children and the frequent absence of biological parents.

A second critical issue reported by key informants was the lack of support provided by other family members and by care providers from the social, education and health sectors. The ICF checklist does not investigate the reasons for this lack of support, but several parents reported that they had not disclosed the health status of their children to family members or to other care providers. It is thus conceivable that, in several cases, support had not been provided because people who were in the position to provide (e.g. school teachers or primary care pediatricians) were unaware of the clinical status of the child. The reasons for not disclosing HIV status are probably the stigma and the fear of social exclusion associated with AIDS. Most caregivers did not seem confident that the social, education, and health institutions could take initiatives to effectively fulfill the needs of their child. However, almost all parents reported that their child had good social relationships and attended school regularly. Finally, it is noteworthy that most adolescents enrolled in the study had not received any information
about their disease. Often the key informants themselves denied their consent to disclosure of HIV diagnosis.

In conclusion, the ICF provides a common language for assessing the functional status of children and adolescents that can enhance communication between health care workers in charge of children with HIV. The scenario that emerges from this study is that the quality of life of HIV-infected children and adolescents is associated with poor psychosocial conditions rather than with the impairment of physical health. ICF is an easy to apply instrument to investigate the clinical and psychosocial status of children and adolescents with HIV infection and to plan interventions aimed at achieving an at least reasonable level of social participation by HIV-infected children and their families. The improvement of the psychosocial condition of HIV-infected patients should proceed in parallel with the spectacular progresses that have been made in the clinical, immunologic and virologic control of the HIV infection.
3.5 References

3.6 Publications

Psychosocial issues in children and adolescents growing with HIV infection evaluated with an age-specific descriptor system
Antonietta Giannattasio, MD\(^1\), Annunziata Officioso, psychologist\(^1\), Grazia Isabella Continisio, psychologist\(^1\), Giovanna Griso, psychologist\(^1\), Cinzia Storace, social worker\(^1\), Simonetta Coppini, psychologist\(^2\), Daniela Longhi, psychologist\(^2\), Carmela Mango, nurse\(^1\), Alfredo Guarino, MD\(^1\), Raffaele Badolato, MD\(^2\), Alfredo Pisacane, MD\(^1\).
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(submitted)
CHAPTER 4

STRATEGIES TO IMPROVE VACCINATION RATES IN CHILDREN WITH CHRONIC MEDICAL CONDITIONS
4.1 Pneumococcal and influenza vaccination rates in children with chronic medical conditions

4.11 Rationale

Among vaccine preventable diseases, two are of outstanding importance and provide an interesting area of evaluation: pneumococcus and influenza infections. The first is a relatively common and potentially severe infection for which vaccines are widely available, effective and relatively widespread. Influenza has peculiar features related to variable degree and the need of annual vaccination.

Subjects with chronic medical conditions are at increased risk for severe complications related to vaccine-preventable infections and should be extensively immunized for both infections (1,15). Influenza is associated with increased morbidity, hospitalisation and mortality in children with chronic medical conditions compared to healthy children (3,20,22). Similarly, the incidence of invasive Streptococcus pneumoniae infection in children with sickle cell disease, asplenia or human immunodeficiency virus (HIV) infection are 20- to 100-fold higher than those of healthy children during the first 5 years of life (5,18,24).

Chronic diseases at risk of complications and hospitalisation due to influenza and/or pneumococcal infections include chronic cardiopulmonary disorders (such as cystic fibrosis and chronic cardiac diseases), chronic metabolic diseases (including diabetes mellitus), renal dysfunction, hemoglobinopathies and immunosuppression (including HIV infection) (1,15).

Criteria for influenza immunization in USA by Advisory Committee for Immunization Practice (ACIP) now include all children aged 6 months to 18 years (14). In Italy, influenza vaccination is not routinely offered to healthy children, whereas it is recommended and offered without costs to children with chronic conditions. The same is true for pneumococcal vaccination (25).

Despite long-standing recommendations to provide pneumococcal and annual influenza vaccinations to children with chronic medical conditions, immunization rates in these vulnerable populations are poor (16). A cluster survey performed by means of interviews carried out at the subjects’ home, showed less than 10% coverage for influenza and pneumococcal vaccinations in most primary care settings in Italy (17). A more recent a study evaluating influenza vaccination in Italian children with chronic disease at high risk of influenza complications confirmed a low rate of vaccination (11).
Several factors hamper implementation of these vaccinations, including problems in identifying at risk children and limited awareness of specific recommendations (4,7). Vaccination of at-risk children is largely a matter of organisation. In Italy, as well as in many other western Countries, children with chronic conditions are generally seen at specific Reference Centres, often located in Universities or major Children’s Hospitals. However, children with chronic diseases are seen also by primary care pediatricians (or family pediatricians), like all children in the Italian Health Service. Moreover, vaccination centres provide vaccinations to both healthy and at-risk children. Therefore, there are multiple services through which a child with a chronic disease can receive immunization.

The aims of this research were to investigate the rates of pneumococcal and influenza vaccinations in selected groups of high risk children and to comparatively define the correlates of missed vaccination in different diseases. Influenza and pneumococcal vaccinations were selected because of the different features of infections and models of immunization.

4.12 Experimental procedures

Study setting and populations enrolled
The study was conducted between January and June 2008. Children aged 2-18 years with the following conditions were included: HIV infection (HIV), cystic fibrosis (CF), type I diabetes mellitus (DM) and children who received liver transplantation (LTx). Children <2 years and those diagnosed as having any of the selected chronic conditions since less than 1 year were excluded, in order to evaluate the rates for influenza vaccination in two subsequent seasons. Physicians involved in the management of children with chronic diseases, including pediatricians of the Reference Centres and primary care pediatricians, were enrolled and received a specific questionnaire. Parents or legal guardians of children received a specific questionnaire providing that informed consent had been given.

Data sources
Patients with chronic medical conditions were identified through the Regional Reference Centres for each disease. The Reference Centres for HIV infection, for CF and for LTx (University of Naples “Federico II”) and all three Reference Centres for DM located in Campania Region (University of Naples “Federico II”, Second University of Naples and “San Sebastiano” Hospital of Caserta) took part into the study.
**Key instrument**

A questionnaire was administered face-to-face to caregivers of subjects aged between 2 and 18 years with high risk medical conditions during their routine clinical evaluation at the Reference Centre. Parental report of vaccination status has been previously validated and has a reasonable sensitivity, specificity and reliability (26,28). In addition to the basic questions of having received vaccinations against pneumococcus and influenza, the questionnaire included a demographic section with age, sex and age at diagnosis. Twelve items on *Streptococcus pneumoniae* infection and 13 on influenza were included to investigate caregivers' awareness about infection, their children's vaccination status and factors that might have affected vaccination status. The latter consisted of the following items: (1) who recommended immunization; (2) who administered the vaccines and (3) the reasons for receiving or missing vaccination. Children were considered immunized against pneumococcus if they have received heptavalent pneumococcal conjugate vaccine or pneumococcal polysaccharide vaccine, according to their age. Inactivated influenza vaccine is the only vaccine available in Italy.

In addition, a specific questionnaire on pneumococcal and influenza diseases was delivered to a representative sample of 113 primary care pediatricians, corresponding to approximately 15% of the total number of primary care pediatricians of Campania Region (southern Italy). Items included the specific risks of pneumococcal and influenza infections in children with chronic medical conditions, knowledge of current national recommendations on vaccination and the number of patients with high risk conditions routinely seen at their practice. Finally, physicians of Reference Centres were interviewed in order to investigate whether they routinely recommend pneumococcal and influenza vaccinations to at-risk children.

**Statistical analysis**

Data are expressed as medians and ranges or means and standard deviations (SD). The chi square and the *t* tests for independent samples were used to compare categorical and continuous variables, respectively. The Mann-Whitney test was used to compare non parametric data. A *p* value <0.05 was considered statistically significant.
4.13 Results

*Pneumococcal and influenza vaccination rates*

The questionnaire was administered to 343 caregivers of high risk children (174 males; median age 13 years, range 2-18) including 40 patients with HIV, 39 with CF, 59 with LTx and 205 with DM.

The demographic features were similar in all 4 groups (table 1).

**Table 1. Demographic features of 343 at-risk children**

<table>
<thead>
<tr>
<th>Patients</th>
<th>HIV</th>
<th>CF</th>
<th>LTx</th>
<th>DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>40</td>
<td>39</td>
<td>59</td>
<td>205</td>
</tr>
<tr>
<td>Males</td>
<td>17 (43%)</td>
<td>18 (46%)</td>
<td>34 (58%)</td>
<td>105 (51%)</td>
</tr>
<tr>
<td>Median age in years (range)</td>
<td>11 (2-18)</td>
<td>11 (2-18)</td>
<td>9.5 (2-18)</td>
<td>13 (2-18)</td>
</tr>
<tr>
<td>Median age at diagnosis in years (range)</td>
<td>3 (1-12)</td>
<td>0.3 (0.1-15)</td>
<td>0.1 (0.1-13)</td>
<td>8 (2-16)</td>
</tr>
</tbody>
</table>

*HIV=* human immunodeficiency virus infection  
*CF=* cystic fibrosis  
*DM=* type I diabetes mellitus  
*LTx=* liver transplantation
Vaccination rates against pneumococcus were low and largely below 50% in all high risk categories, the lowest being in subjects with DM (figure 1). Vaccinated and not vaccinated children did not differ by sex (17/29 males versus 155/314, respectively; p>0.05) or mean duration of chronic disease (6.3±4.41 versus 7±5 years; p>0.05). However, children who received vaccine were younger than those who did not (mean age 8.2±4.3 versus 12.5±4 years, respectively; p<0.0001).

Figure 1. Pneumococcal vaccination rates in 343 high risk children
Inset: percentage of patients with DM vaccinated for pneumococcus seen at each of the three Reference Centres.
DM vs HIV: p=0.0007; DM vs CF: p=0.02; DM vs LTx: p=0.002.
In 2006-2007, a total of 208 (61%) of the 343 children with chronic disease conditions were vaccinated for influenza and 135 (39%) were not. A very similar pattern was observed in the same population in 2007-2008 (figure 2). Similarly to findings for pneumococcus, vaccination rate for influenza was significantly lower in patients with DM compared with HIV (p=0.01), CF (p<0.0001) and LTx (p=0.0006). Vaccination rates in children seen in the 3 Reference Centres for DM were broad ranging from 21% to 61% (figure 2). Vaccinated children were younger than not vaccinated (mean age 11±4.2 versus 12±4.2 years, respectively; p=0.03), but the mean duration of the chronic conditions was significantly higher in vaccinated subjects (7.8±4.9 years) than in non vaccinated subjects (6.5±5 years; p=0.001). This apparently surprising result is explained by the lower age at diagnosis of children with HIV, CF and LTx compared to DM.
Figure 2. Influenza vaccination rates in 343 high risk children

Influenza vaccination rates in two subsequent winters. Inset: percentage of patients with DM vaccinated for influenza seen at each of the three Reference Centres.

*p<0.05.
Role of physicians working in different settings in recommending vaccinations

To investigate the role in promoting immunization by the physicians working in specific Reference Centres, key responders were asked to report who recommended vaccinations. As shown in figure 3, physicians of the Reference Centres for HIV and LTx actively recommended immunization against pneumococcus, while this was not so in the 3 centres for DM. A similar number of physicians of the Reference Centres and primary care pediatricians recommended pneumococcal vaccination for CF patients. Again, the Reference Centre had a major role in recommending influenza vaccination to children with HIV, CF and LTx, whereas primary care pediatricians had a main role in case of children with DM (figure 4). This data may be due to the different management of these chronic conditions: children with HIV and LTx were rarely seen by primary care pediatricians, while for children with DM the pattern was more scattered and depended on the individual Reference Centre.
Figure 3. Reasons for receiving pneumococcal vaccination in 343 high-risk children

Role of the Reference Centre in recommending vaccination in HIV vs CF and DM: p<0.05.
Role of the Reference Centre in recommending vaccination in LTx vs CF and DM: p<0.05.
“Others” includes vaccination because of previous serious influenza illness, recommendation by relatives or friends, information garnered from the mass media or not specified reason.
Figure 4. Reasons for receiving influenza vaccination in 343 high risk children

** DM versus HIV, CF and LTx: p<0.0001.

“Others” includes vaccination because of previous serious influenza illness, recommendation by relatives or friends, information garnered from the mass media or not specified reason.
Reasons for missing vaccinations

There was a wide spectrum of reasons why patients were not vaccinated for pneumococcus and influenza, ranging from lack of information to parental “ideological” attitudes and concern. Lack of information prevailed in all groups, particularly as regards pneumococcal vaccination (figure 5, A and B).
Figure 5. Reasons for not having pneumococcal or influenza vaccinations in 343 high risk children

A. Reasons for not having pneumococcal vaccination.
B. Reasons for not having influenza vaccination.

“Others” includes child barriers (“child was sick”), parents barriers (“no time”, “forgot”), system barriers (“no vaccine available”), vaccination considered unnecessary by physician or not specified reason.
Key informants were specifically asked whether they would consider pneumococcus and influenza infections as potentially serious diseases in their children. As a control question, we also asked whether they would consider these infections a severe problem in healthy children. Overall, a small number of parents (79/343, 23%) were aware of pneumococcus infection and this was perceived as a potentially severe infection by only 15% of the key informants of at-risk children. Influenza was perceived as a serious infection by 53 (15%) caregivers, but as many as 243 (71%) considered it a severe disease in children with chronic medical conditions. A greater proportion of caregivers of CF (36 of 39, 92%) and LTx patients (50 of 59, 85%) were concerned about influenza infection than caregivers of HIV (25 of 40, 62%; p=0.002 and p=0.01, respectively) and DM children (134 of 205, 65%; p=0.0005 and p=0.003, respectively).

Awareness of the severity of pneumococcus and influenza infections, as reported by key informants, was then correlated with vaccination rates. Significantly more children of key informants who were aware that pneumococcus infection could be severe, were vaccinated than children of key informants who did not consider pneumococcus infection as severe (12% versus 32%; p<0.0001). No relationship between infection awareness and vaccination rate was found for influenza.

**Reported knowledge of health care providers about national vaccine recommendations**

Physicians working in 5 of 6 the Reference Centres reported that they actively recommended immunization against pneumococcal and influenza to high risk children as part of their routine approach to children with chronic conditions. Physicians working within the same Reference Centre had a similar vaccination policy. In one of the 3 Reference Centres for DM, pediatricians advised influenza vaccination only to patients with poor metabolic control, whereas vaccination was considered unnecessary and not recommended in other children with DM. Interestingly, only 20 (25%) of the 80 children with DM followed at that Centre had been immunised, thus supporting the role of Reference Centre in promoting or not immunization.

A total of 113 primary care pediatricians were interviewed. They reported that the proportion of at-risk children in the age range of 0-14 years followed at their practices was approximately 10% of all children in their practice. The estimate included children with any chronic condition that would require pneumococcal and/or influenza vaccinations.

Most interviewed pediatricians were aware of the relevance of pneumococcal (102/113, 90%) and influenza (109/113, 96%) infections in children with chronic medical conditions and reported they recommend these vaccinations to at-risk children. When asked to indicate
which high risk conditions required pneumococcal and influenza vaccinations according to the National Vaccine Programme, the majority of them correctly identified the main categories.

4.14 Discussion

Vaccination against influenza of healthy children aged 6 months-18 years is recommended in the USA (14) but not in Italy (25). However, vaccination of children with chronic medical conditions against a wide spectrum of infectious diseases has been included in the Essential Levels of Care and is provided free of charge (25).

Although it is well known that pneumococcal infection can be more severe than influenza and that pneumococcal vaccine is protective for a long time, the vaccination rate against pneumococcus was low in all studied groups and far lower than influenza. This rate was consistent with the findings of a previous Italian study (17). Data from the United States showed that coverage rate for pneumococcal polysaccharide vaccine in 2003 was only 37% among persons 18-64 year of age at increased risk for pneumococcal diseases (6). As for pediatric age, recent data reported an estimated coverage rate for pneumococcal conjugate vaccines of 31, 38 and 49% among children born in 2000, 2001 and 2002, respectively (21). These low rates of pneumococcal vaccination may be due to lack of knowledge by care providers or to low priority by public health authorities.

Furthermore, “once in the life” administration instead of annual administration (as required by influenza vaccine) could reduce the opportunities that physicians have to check vaccination status. In our study vaccinated children were younger than non vaccinated. This result may be due to the Italian vaccination program that promotes pneumococcal vaccination in children aged less that 5 years, while no clear indications are provided for children aged 5 to 18 years (25).

Approximately 60% of children with the selected chronic conditions were vaccinated against influenza. This rate was stable over two subsequent seasons. Recently, in a large retrospective observational study, a poor influenza vaccination coverage has been reported in adolescents with high risk conditions, with 85% of subjects who were not vaccinated in 2002 (19). Immunization rate against influenza found in our study was higher than in other subjects with other chronic conditions (2,8,23,27) and than that reported in a previous study in Italian at-risk children (17).

A suboptimal vaccination rate in at-risk children may be in part related to a lack of information, fear of side effects of vaccination in children who are already ill, to
organisational problems and difficulties to identify at-risk subjects. Furthermore, from our study it appears that another potential reason of the low coverage rates could be the lack of awareness of the severity of pneumococcal and influenza infections in at-risk children, as reported by key informants of patients with HIV and DM. Immunization rates against pneumococcus and influenza were closely related to the underlying condition, with the lowest rate for both vaccinations occurring in patients with DM. This may be due to the lack of awareness of the risks related with pneumococcus and influenza infections by physicians in charge of children with DM. There was a clear relationship between immunization rates and the policy of the Reference Centres as appeared by the comparative evaluation of rates in the 3 Reference Centres for DM. According to the responses by parents and physicians, Reference Centres rather than primary care pediatricians were more instrumental in increasing the rate of influenza vaccination in patients with HIV, CF and LTx. In contrast, primary care pediatricians had a major role in children with DM. The low vaccination coverage in at-risk children largely depends on having three categories of physicians, namely disease specialists, primary care physicians and vaccination service physicians, equally charged with vaccination, with no clear distinction between them. It was previously found that one of the most important factors positively associated with vaccination was recommendation by a health care provider (9,11,13). However, it has been reported that health care workers’ knowledge about recommendations on vaccinations is limited (11,12), and, in a previous study on the determinants of influenza vaccination, the majority of parents of infants stated they received information through mass media rather than from physicians (10). In summary, 3 major findings emerge from this study. Firstly, although both pneumococcal and influenza vaccines in Italy are provided free of charge, the immunization rate for pneumococcal infection is very low, whereas the immunization rate for influenza is relatively high, with a pattern consistent over time. Secondly, for most chronic conditions, vaccination is performed upon the advice of a Reference Centre. Third, the main reason of unvaccination was the lack of knowledge by parents of the benefits of pneumococcal and influenza vaccinations in children with chronic underlying diseases. In conclusion, immunization should be considered part of the global care of children with chronic medical conditions. Immunization includes three different actions: to recommend vaccination, to administer vaccine, to check vaccination status. For diseases principally managed by Reference Centres (such as HIV, CF and LTx), physicians of the Reference
Centres should also take the responsibility to administer vaccines. For diseases with a less defined management, more detailed information on vaccination should be provided to families by primary care pediatricians. To achieve optimal immunization rates in at-risk groups a complex array of responsibilities and functions is required. A special effort is required to implement pneumococcal immunization among Italian high risk children.
4.2 References


4.3 Publications

   Preparing for an influenza pandemic in Italy: resources and procedures in paediatric hospital units.

2. Pandolfi E, Marino MG, Gesualdo F, Romano M, **Giannattasio A**.
   Vaccinare i bambini con patologia cronica? E’ meno difficile di quello che sembra!

   V+: strategie per il miglioramento della copertura vaccinale nei bambini con patologia cronica.

4. **Giannattasio A**, Lo Vecchio A, Guarino A.
   Vaccination in at-risk children: too much may be harmful.
   (Rapid response) BMJ 30 September 2009
   [http://www.bmj.com/cgi/eletters/339/aug17_2/b3363](http://www.bmj.com/cgi/eletters/339/aug17_2/b3363)

   Pathways and determinants of vaccination rates against influenza and pneumococcus in children with type 1 diabetes
   * Department of Paediatrics, University Federico II, Naples, Italy
   § Department of Paediatrics, Second University of Naples, Italy.
   §§ Department of Paediatrics, “Sant’Anna e San Sebastiano” Hospital, Caserta, Italy.
   *(submitted)*
4.4 Influenza and pneumococcal vaccinations in HIV-infected children: assessment of methodological quality of current recommendations

4.41 Rationale

Pediatric human immunodeficiency virus (HIV) infection has evolved from a rapidly progressive, fatal disease to a chronic infection with prolonged survival thanks to the advent of highly active antiretroviral therapy (HAART). However, HIV-infected subjects are at increased risk of severe complications related to vaccine-preventable infections (1). Prevention is therefore an important area of HIV care. Among the factors that must be considered when contemplating vaccination of HIV-infected patients are the safety, immunogenicity and efficacy of a given vaccine. Selected vaccines may be administered to all HIV-infected children. Individuals with HIV infection, like healthy children, should receive DTP, Hib, HBV vaccines (1). Other vaccines are more appropriate in selected patients with HIV infection. For example, live virus vaccines, such as measles and varicella, should be administered only to HIV-infected patients that are not severely immunosuppressed (CD4+ cells greater than 15%) (2).

Specific features distinguish influence from other vaccine-preventable diseases: it is a frequent (almost inevitable) disease and it is an annual risk so that vaccination must be repeated every year. HIV-infected naïve patients have an approximately 10-fold higher influenza-attributable mortality rate than the general population (3). Even in patients on HAART, hospitalization rates during the influenza season are higher than in the general population and similar to that of at-risk groups of patients (4). The increased risk is not limited to influenza. There is evidence that the incidence of invasive *Streptococcus pneumoniae* infection in children with HIV infection is 20- to 100-fold higher than in healthy children during the first 5 years of life (5,6).

The increased influenza- and pneumococcal-related diseases in HIV-infected individuals prompted the recommendation that HIV-infected patients be considered a high-risk group and a specific target for annual influenza and pneumococcal vaccinations (7-9). The past 10 years have seen a plethora of guidelines, consensus statements and systematic reviews on preventive aspects of HIV-infected children. However, the increase in the number of guidelines and recommendations has been accompanied by growing concern about their quality (10-16).

The aim of this study was to identify and assess the quality of guidelines and of consensus statements concerning influenza and pneumococcal vaccinations in HIV-infected children. To
indirectly evaluate the reliability and quality of the studies on which the recommendations were based, we also analyzed systematic reviews for rigor and production methodology. Rather than focusing on their content, our primary objective was to review the methods applied in writing the recommendations and to identify the fields of major weaknesses.

4.42 Experimental procedures

Search strategy
The following definitions were applied in this study. A guideline is a systematically developed statement to assist practitioners make decisions about appropriate care for specific diseases based on evidence (17,18). Consensus statements are documents representing the opinion of a panel of experts to help clinicians the most agreed-upon approach to clinical practice. A systematic review is a methodologically rigorous process with which to analyze and summarize scientific evidence (19). The development of a systematic review is based on a “step by step” process that includes formulation of review questions, definition of inclusion/exclusion criteria, detailed search strategy and a critical appraisal of the studies selected through specific quality scales and checklists (19).

We screened PubMed and Embase for guidelines, consensus statements and systematic reviews on influenza and pneumococcal vaccinations in HIV-infected patients. The following search terms were used: influenza vaccines, pneumococcal vaccines and HIV infection. The keywords used for the search strategy in Medline were: (“HIV infections”[Mesh] AND “Influenza Vaccines” [Mesh] AND “Guidelines” OR “Systematic review” [Mesh]); (“HIV infections”[Mesh] AND “Pneumococcal Vaccines” [Mesh] AND “Guidelines” OR “Systematic review” [Mesh]). For papers identified within PubMed, we applied the definition of guideline and systematic review based on the Mesh term that identified them in the database. To be more specific, if a paper was identified in PubMed with guidelines in Mesh, it was labeled as such. Inclusion criteria were: studies in English; published in the last ten years.

We also explored relevant websites of agencies involved with HIV infection, vaccinations or guidelines such as AIDSinfo (http://www.aidsinfo.nih.gov/); American Academy of Pediatrics (AAP, www.aap.org); Centers for Disease Control and Prevention (CDC, www.cdc.gov); National Guideline Clearinghouse (NGC, www.guidelines.gov); Cochrane Database of Systematic Review (www.cochrane.org); Scottish Intercollegiate Guidelines (SIGN, www.sign.ac.uk); Canadian Medical Association (CMA, www.cma.ca). Where one review was an update of a previous review, only the most recent publication was considered. To identify
additional relevant studies, we also searched the reference lists of published guidelines, protocols and systematic reviews.

**Selection criteria**

Articles were selected in a two-step procedure. First, two investigators (AG and ALV) independently screened all titles and abstracts concerning influenza and pneumococcal vaccinations in HIV-infected patients potentially relevant for the present study. Studies meeting inclusion criteria were fully read. Three issues on influenza and pneumococcal vaccinations were considered: recommendations for vaccination, and the efficacy and safety of vaccines.

**Instruments to assess quality**

We evaluated the scientific quality the selected guidelines using the Appraisal of Guidelines for Research and Evaluation (AGREE) instrument, which is an internationally validated instrument for assessment of the quality of the reporting and of some aspects of recommendations (20). AGREE provides an assessment of the predictive validity of a guideline. It provides a framework with 6 domains and a total of 23 items for the assessment of several components that are integral to guideline development: “Scope and Purpose” (3 items), “Stakeholder Involvement” (4 items), “Rigor of Development” (7 items), “Clarity and Presentation” (4 items), “Applicability” (3 items), “Editorial Independence” (2 items). Each item is scored with a 4-point scale ranging from ‘strongly disagree’ (1) to ‘strongly agree’ (4). The score for each domain is obtained by summing all the scores of the individual item in a domain and is expressed as percentage of the maximum possible score for that domain. Although a threshold to label a domain “good” or “bad” has not been established, a domain scoring less than 50% is considered not appropriate. The final step of AGREE is a judgment of a guideline application as “strongly recommended”, “recommended (with provisos)”, “would not recommend” or “unsure”.

Four independent investigators (AG, ALV, AF and VG) extracted data and evaluated the scientific quality of guidelines. Reviewers also determined whether the guideline was evidence-based or consensus-based. The AGREE instrument was also used to evaluate the quality of consensus statements, as previously reported (13). Consensus statements may have a less rigorous development process than guidelines; consequently, a lower score should be expected. Agreement between reviewers on the scores given to each item of the AGREE instrument was also evaluated.
The quality of systematic reviews was assessed with methodological checklists produced by the Scottish Intercollegiate Guidelines Network for the evaluation of effectiveness of health assistance (21). This instrument includes the following methodological items: “the study addresses an appropriate and clearly focused question”; “a description of the methodology used is included”; “the literature search is sufficiently rigorous to identify all the relevant studies”; “study quality is assessed and taken into account”; “there are enough similarities between the studies selected to make combining them reasonable”; “what types of study are included in the review?”. A six-point scale (from “well covered” to “adequately addressed”, “poorly addressed”, “not addressed”, “not reported” and “not applicable”) was used to evaluate the validity of each item. A final question (“how was the scientific quality rate of the overview?”), based on the previous items, and expressed by a score ranking from – to ++ (indicating superior quality) was used to assess the overall scientific quality of the systematic review.

**Statistical analysis**

Dichotomous variables were analyzed by the chi square test and Fisher’s exact test. Continuous variables were compared with the Student t test and the Mann-Whitney test. A two-sided test was used to indicate statistical significance at a p value of <0.05. To measure the agreement between reviewers, we used the Free-marginal multirater kappa (multirater $k_{free}$). We choose this statistical instrument because it was appropriate for the typical agreement study (22). Fleiss’ multirater kappa, generally used to assess agreement between more than two raters, is influenced by prevalence and bias, which can lead to the paradox of high agreement but low kappa. The response categories were dichotomized into strongly agree/agree versus disagree/strongly disagree because this analysis of agreement was considered sufficient at this level to estimate consistency of rating between evaluators. The measure reflects the degree of agreement in classification over that expected by chance and ranges between 0 and 1. The $k$ statistic was then applied to each of the 23 items of the AGREE instrument.

**4.43 Results**

The search in Pubmed and Embase yielded 22 citations that met the inclusion criteria (12 citations on influenza and 10 citations on pneumococcus vaccinations). Eight additional citations were identified in the websites. After screening titles and abstracts and removing duplicates, 19 (63%) of the 30 citations were considered relevant. Based on full text reading,
18 met the selection criteria (1,2,8,9,23-36). One article was excluded because it did not contain recommendations (37). Nine of the 18 selected articles were guidelines or consensus statements, and 9 were systematic reviews.

*Quality assessment of guidelines and consensus statements*

Five guidelines and 4 consensus statements were analysed and the AGREE scores are reported in table 1.
Table 1. Quality assessment of recommendations of influenza and pneumococcal vaccinations in HIV-infected children

<table>
<thead>
<tr>
<th>Study [type of article]*</th>
<th>Domain scores (%)</th>
<th>Mean score (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scope/purpose</td>
<td>Stakeholder involvement</td>
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<tr>
<td>Kaplan JE [G] (2)</td>
<td>83.3</td>
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<td>Fiore AE [G] (8)</td>
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<td>NY 2006 [G] (36)</td>
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</tr>
<tr>
<td>Mean score</td>
<td>59.6</td>
<td>31.2</td>
</tr>
</tbody>
</table>

G=guideline
CS=consensus statement
The overall mean score ranged from 27.2% to 54.4%. One guideline scored ≥50% of the AGREE criteria thereby meeting the standard of good overall quality (2). Interestingly, consensus statements had a total score comparable to that of guidelines (mean scores 54% vs 53%, respectively; p=0.7).

The domain “Scope and Purpose”, which evaluates the aims of a guideline, the specific clinical questions and the target patient population, had the highest mean score. Seven (78%) of 9 analyzed papers performed well in this domain scoring ≥50%. Similarly, the “Clarity and Presentation” domain, which deals with the language and format of the guideline, had a good total score, and the score was ≥50% in 5 (55.6%) cases. Lower scores were obtained for the “Stakeholder Involvement” domain, which evaluates the degree of a guidelines to represent the views of its target users, for “Applicability”, pertaining to the organizational, behavioral and cost implications of applying the guideline, and for “Editorial Independence”, which addresses the independence of the recommendations and disclosure of possible conflict of interest. The score for these domains was lower than 50% in all papers but one. The “Rigor of Development” domain had a low mean score with only two (22.2%) guidelines scoring more than 50%. This domain deals with the process used to search for evidence and criteria for selecting them, the methods to formulate the recommendations, the link between the recommendations and the supporting evidence, consideration of health benefits, side effects and risks, external revisions of the guideline and procedures to update it. Low scores were largely due to poor reporting. Specifically, two (22.2%) guidelines described methods for searching and selecting the evidence, only one (11.1%) described the methods used to formulate the recommendation and another (11.1%) was externally reviewed. The strongest item of this domain was that on health benefits, side effects and risks when formulating the recommendation, which was well described in 7 (77.7%) cases.

Of the 9 guidelines and consensus statements evaluated, 3 (33.3%) were evidence-based. The AGREE score tended to be higher for evidence-based guidelines than for non evidence-based recommendations (mean score 44.2% vs 35.1%, respectively; p=0.1), but the difference was significant only for the “Clarity and Presentation” domain (mean score 77.1% for evidence-based recommendations vs 50.4% for non evidence-based recommendations; p=0.009).

After completing the AGREE instrument, the appraisers came to a consensus with respect to an overall recommendation for each guideline. A guideline was recommended (“strongly recommended” or “recommended with provisos”) if it had a fairly good quality with the AGREE and was considered useful to health-care providers. Overall, the reviewers recommended 5 (2,8,31,32,34) of the 9 guidelines (55.6%) for use in local practice.
Agreement among reviewers

The agreement among reviewers for the 23 items of the AGREE Instrument is reported in table 2. Agreement was higher than 60% for most items. Only 5 (22%) items had an agreement score less than 50%. Agreement was not fair (<40%) or poor (<20%) for any of the 23 items. The percentage of agreement among reviewers was lowest for the “Applicability” domain (for all the items of this domain, agreement was less than 60%); agreement was highest for the “Clarity and Presentation” domain (>60% for all items).

Table 2. Agreement among reviewers for AGREE Instrument

<table>
<thead>
<tr>
<th>Agreement, %</th>
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<td>61-80</td>
<td>12 (52%)</td>
</tr>
<tr>
<td>&gt;81</td>
<td>0</td>
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</table>
Quality assessment of systematic reviews

The quality score of the systematic reviews analyzed is reported in table 3. The question addressed by the study was sufficiently clear in 6 (66.7%) of 9 reviews. The methodological quality areas had the lowest scores. In four reviews search strategies were not reported. Overall, only 3 (33%) reviews fulfilled all or most quality criteria, and 2 (22%) fulfilled some criteria.
<table>
<thead>
<tr>
<th>Author</th>
<th>Scope and purpose</th>
<th>Search methods stated</th>
<th>Search comprehensive</th>
<th>Study quality assessment</th>
<th>Combine findings</th>
<th>Overall scientific quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overton ET (1)</td>
<td>C</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Anema A (23)</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>++</td>
</tr>
<tr>
<td>Zanetti AR (24)</td>
<td>C</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Neuzil KM (25)</td>
<td>B</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Brydak LB (26)</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>NA</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>Destefano F (27)</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>++</td>
</tr>
<tr>
<td>Bliss SJ (28)</td>
<td>B</td>
<td>A</td>
<td>C</td>
<td>B</td>
<td>A</td>
<td>++</td>
</tr>
<tr>
<td>Spencer DC (29)</td>
<td>C</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Pai VB (30)</td>
<td>B</td>
<td>B</td>
<td>C</td>
<td>B</td>
<td>NA</td>
<td>+</td>
</tr>
</tbody>
</table>

A=well covered; B=adequately addressed; C=poorly addressed, NA=not addressed.
Analysis of the content of recommendations

All guidelines, consensus statements and systematic reviews recommended influenza and pneumococcal vaccinations for HIV-infected children. Two systematic reviews on pneumococcus (25,27) did not give any indication for vaccination because this was not a specific aim of the studies. The only contraindication to influenza vaccination in HIV-infected subjects was age below 6 months (as for healthy children); in addition, the live intranasal influenza virus vaccine was contraindicated (1,8). There were minor differences between papers on the optimal type of pneumococcal vaccine in relation to patient’s age. Some authors recommended eptavalent conjugate vaccine in children aged \( \leq 5 \) years and 23-valent pneumococcal polysaccharide vaccine in adolescents and adults, whereas others recommended eptavalent pneumococcal conjugate vaccine in children below the age of 2 years (29). Most guidelines and systematic reviews did not include indications for these specific pneumococcal vaccines. Antibody response against vaccines can be lower in the presence of severe immunosuppression (CD4+ cell count less than 200 cells/ml) (1,8,25,31). However, in the absence of contraindications and of specific side effects, all guidelines recommend inactivated influenza and pneumococcal vaccinations in all HIV-infected children because of the expected benefits.

4.44 Discussion

We have investigated the methodological quality of guidelines and systematic reviews on influenza and pneumococcal vaccinations in HIV-infected children using the AGREE instrument, a widely accepted instrument designed for this purpose (20, 38-40). Application of AGREE has frequently revealed serious methodological flaws in guidelines that are used in clinical practice (12-14,39). Recently, the quality of 215 evidence-based pediatric guidelines was assessed with AGREE (12). The poorest performance was for the domains “Applicability” and “Editorial Independence”. However, overall, pediatrics guidelines scored better than guidelines in other fields. For example, no guideline of 11 examined on oral mucositis scored \( >50\% \) in all domains, which equates with a low overall quality (14). In addition, the quality of lung cancer guidelines was found to be poor, with only 37% of guidelines recommended for use in clinical practice (38).

Our analysis shows that the quality of most recommendations on influenza and pneumococcal vaccinations in HIV-infected children is poor. None of the guideline scored \( >50\% \) on all domains and “Applicability” and “Editorial Independence” were again the weakest fields. Also
the “Rigor of Development” domain, which is considered the best indicator of scientific quality, scored >50% only in two cases, mainly because most guidelines performed poorly on external revision and procedures for updating the guideline. It should be pointed out that when no specific information is provided (e.g. methods used in the development process or conflicts of interest), the resulting score will be low. Therefore, low scores can be explained by bad reporting rather than by an inappropriate development.

To indirectly support that the mean quality score of consensus statements was similar to that of guidelines. This result may be due to the poor quality of guidelines rather than to the high quality of consensus statements.

Similarly to guidelines, the most common weakness of systematic reviews was the unclear, not reported or inadequate search strategy. However, most articles classified as reviews were not systematic reviews but narrative reviews, which generally do not include a thorough and systematic search of papers. Few guidelines graded their recommendations, whereas the majority did not and the former obtained higher scores. Guidelines and consensus statements recommended by reviewers for use in local practice obtained an AGREE score higher than guidelines that were not recommended. For all recommended guidelines except one, the “Rigor of Development” domain scored higher than for not recommended guidelines.

The AGREE instrument is designed only to evaluate the development process of a guideline, and not to assess the clinical significant of recommendations. Consequently, a high AGREE score does not imply that the recommendation is correct or appropriate (41). However, no substantial differences in the evaluation of each appraiser were found; this indicates that the judgment of quality was consistent. Despite the methodological limitations, all papers recommended influenza and pneumococcal vaccinations for children with HIV infection.

In conclusion, when drawing-up guidelines for vaccination in HIV-infected patients, much more attention should focus on the domains of “Stakeholder Involvement”, “Applicability”, “Editorial Independence” and “Rigor of Development”. Our analysis of the weaknesses and strengths of guidelines and systematic reviews should alert physicians to the importance of the methodological process in developing or implementing a guideline or a systematic review. Concomitantly, users should critically evaluate the process leading to a recommendation before applying it in clinical practice.
4.5 References


4.5 Publications

CHAPTER 5

CONCLUDING REMARKS
5.1 Conclusions

Children with chronic diseases have often a long life. They grow, and become adolescents and young adults thanks to improvements of quality of care and progresses in medical treatments. A careful understanding of the natural history of HBV and HCV infections in children is important in making decisions regarding treatment. Our research shows that chronic hepatitis B and C acquired in childhood have a mild and often benign course. Histological analysis shows a low prevalence of cirrhosis and a slow progression of fibrosis. Furthermore, a very low frequency of liver steatosis was found in chronically HBV-infected children, while a higher prevalence of steatosis was found in children with chronic hepatitis C. Conventional interferon alpha, lamivudine and adefovir are approved to treat chronic hepatitis B in children. The rapid emergence of resistant HBV associated with long-term lamivudine therapy, as well as poor tolerability associated with conventional interferon alpha, are factors that should be considered before initiating antiviral therapy. To date, it seems reasonable not to treat all children, but only those with more-severe liver disease and/or with positive predictive factors of response. Treatment of children with chronic HCV infection has provided promising results.

Highly active antiretroviral therapy (HAART) has proven effective in controlling clinical disease progression and reducing mortality of both adults and children with HIV infection. Although HAART has substantially changed the natural history of HIV-1 infection, the complex therapeutic regimens require strict adherence. Children growing with HIV face new problems related to long-term side effects of therapy, adherence to treatment schedules, disclosure of their own or their parents’ HIV status to other members of community, quality of life, and psychosocial problems. The results obtained in this thesis shows that adherence is a crucial variable of treatment of AIDS in children and needs early and continuous monitoring and implementation. Adherence tends to change over time and, more importantly, children non-adherence in early stages of life predicts later non-adherence, when control of therapy may become more difficult or impossible. The caregiver, the relationship between patient and physician, and the perception of drug efficacy are key aspects to obtain and maintain adherence. The improvement of the psychosocial conditions of HIV-infected patients should proceed in parallel with the spectacular progresses that have been made in the clinical, immunologic and virologic control of the HIV infection. The identification of needs by means the International Classification of Functioning, Disability and Health (ICF), a standardized
instrument, may yield essential information for planning health care interventions. ICF provides a common tool for assessing the functional status of children and adolescents that can increase effective communication between different health care professionals in charge of children with HIV.

Public health policies are fundamental to optimize the distribution of resources. Immunization should be considered part of the global care of children with chronic medical conditions. Strategies are needed at national and regional levels to indicate who should be offered vaccinations, how to prioritise target populations, and what are the pathways to ensure rapid vaccination in large cohort of subjects. To ensure an effective vaccination policy in at risk children, three order of actions should be applied: clear information to families on where to go to receive vaccination; coordination of services and physicians involved in the care of children to avoid redundancy of vaccination offer; control of immunization performed routinely as well as in exceptional circumstances. A suboptimal vaccination rate in at risk children may be in part related to a lack of information, fear of side effects of vaccination in children who are already ill, to organisational problems and difficulties to identify and call at risk subjects. The results presented in this thesis show that the lack of awareness of the severity of pneumococcal and influenza infections in at risk children is another reason for the low coverage rates.

Immunization includes three different actions: to recommend vaccination, to administer vaccine, to check vaccination status. To achieve optimal immunization rates in at risk groups a complex array of responsibilities and functions is required. Strategies should differ according to the different models of chronic conditions. For diseases principally managed by Reference Centres (e.g. HIV infection, chronic liver diseases), physicians of the Reference Centres should be in charge for immunization. For diseases with a less defined management (e.g. neurological disorders), detailed information on vaccination and check of vaccination status should be guaranteed by primary care pediatricians.

Another tool to improve quality of care is the use of practice guidelines. Clinical practice guidelines are systematically developed statements to assist practitioners in making decisions about appropriate health care in specific clinical circumstances. Their purpose is to make explicit recommendations with a definite intent to influence what clinicians do. The primary goal of practice guidelines in pediatrics is to improve the health of infants and children by ensuring that they receive up-to-date, evidence-based care. Several studies have shown that adherence to evidence-based guidelines leads to improvement in the quality of care provided.
However, for many health conditions, there is a gap between what medical science has shown to be effective practice and what is actually done. The number of guidelines is rapidly mounting, also in pediatrics. However, the plethora of guidelines has been accompanied by growing concern about differences among guideline recommendations and about the quality of guidelines. A well performed guideline should be scientifically valid, usable, reliable, and should improve the outcome of patients. But, it is rarely known how a guideline performs in clinical practice. Evaluation of guidelines should include both the methods used to develop recommendations and the applicability of the recommendations (benefits, adverse effects and costs). The analysis of guidelines for vaccination in HIV-infected patients shows weaknesses in several domains. Our analysis should alert physicians to the importance of the methodological process in developing or implementing a guideline. Concomitantly, users should critically evaluate the process leading to a recommendation before applying it in clinical practice.

In conclusion, the research presented in this thesis highlights new problems that children with chronic diseases have to face, shows some weaknesses of current public health models and provide information to improve quality of care of at risk children. Overall, it indicates that the rapidly changing pattern of infections on one side, and management on the other should promote changes in organization to maximize efficiently and ultimately improve the quality of care of high risk children and their families.
CHAPTER 6

CURRICULUM VITAE AND GRANTS
6.1 Curriculum Vitae

Is HCV infection associated with liver steatosis also in children?  
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   Vaccinare i bambini con patologia cronica? E’ meno difficile di quello che sembra!

29. Marino MG, Pandolci E, Carlkoni E, Ciofi degli Atti M, Tozzi AE e il gruppo di lavorto
   V+.
V+: strategie per il miglioramento della copertura vaccinale nei bambini con patologia cronica.


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6.2 Grants

1. Nuovi modelli assistenziali per l’ottimizzazione della copertura vaccinale in bambini a rischio in Regione Campania.
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2. Strategie per il miglioramento della copertura vaccinale nei bambini con patologia cronica.
   Progetto finanziato dal CCM-Ministero della Salute, numero 188BGCTRGU; accordo OPBG/CCM del 18.09.07.

3. I bisogni come risorsa nel processo di cura: ricerca intervento sui bisogni dei bambini e degli adolescenti sieropositivi e delle loro famiglie.
   Progetto finanziato da Sky s.r.l Italia.

4. Prevention of pulmonary exacerbations in children with Cystic Fibrosis through the modification of intestinal microflora.
   Progetto finanziato da Fondazione Italiana per la Ricerca sulla Fibrosi Cistica; project number FFC 20/2006.

5. Modulation of intestinal and extraintestinal inflammation in infants with Cystic Fibrosis by early modification of intestinal microflora.
   Progetto finanziato da Fondazione Italiana per la Ricerca sulla Fibrosi Cistica; project number FFC 23/2009.