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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

1. Williams R. Global challenges in liver disease. Hepatology 2006;44:521-26.
2. Romanò L, Azara A, Chiaramonte M, et al. Low prevalence of anti-HCV antibodies among Italian children. Infection 1994;22:350-51.
3. Bortolotti F, Iorio R, Resti M, et al. Epidemiological profile of 806 Italian children with hepatitis C virus infection over a 15-year period. J Hepatol 2007;46:783-90.
4. Prati D. Transmission of hepatitis C virus by blood transfusions and other medical procedures: a global review. J Hepatol 2006;45:607-16.
5. Bortolotti F, Resti M, Giacchino R, et al. Changing epidemiologic pattern of chronic hepatitis C virus infection in Italian children. J Pediatr 1998;133:378-80.
6. Schwimmer JB, Balistreri WF. Transmission, natural history and treatment of hepatitis C virus infection in the pediatric population. Semin Liver Dis 2000;20:37-46.
7. Resti M, Azzari C, Mannelli F, et al. Mother to child transmission of the hepatitis C virus: prospective study of risk factors and timing of infection in children born to women seronegative for HIV1. BMJ 1998;317:437-41.
8. Conte D, Fraquelli M, Prati D, et al. Prevalence and clinical course of chronic hepatitis C virus (HCV) infection and rate of HCV vertical transmission in a cohort of 15,250 pregnant women. Hepatology 2000;31:751-55.
9. Vogt M, Lang T, Frosner G, et al. Prevalence and clinical outcome of HCV infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med 1999;341:866-70.
10. Minola E, Prati D, Suter F, et al. Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C. Blood 2002;99:4591.
11. Jonas MM. Children with hepatitis C. Hepatology 2002;36:S173-78.
12. Casiraghi MA, Paschale MD, Romano L, et al. Long-term outcome (35 years) of hepatitis C after acquisition of infection through minitransfusions of blood given at birth. Hepatology 2004;39:90-96.
13. Tovo PA, Pembrey LJ, Newell ML, and The European Pediatric Hepatitis C Virus Infection Network. Persistence rate and progression of vertically acquired hepatitis C infection. J Infect Dis 2000;181:419-24.
14. Mast EE, Hwang L, Seto DSY, et al. Risk factors for perinatal transmission of hepatitis C virus (HCV) and the natural history of HCV infection acquired in infancy. J Infect Dis 2005;192:1880-89.

15. De Rossi A. Virus–host interactions in pediatric HIV-1 infection. Curr Opin HIV AIDS 2007;2:399-04.
16. Centers for Disease Control and Prevention. HIV/AIDS surveillance report, 2004. Atlanta, GA: US Department of Health and Human Services, CDC; 2004. Available at: http://www.cdc.gov/hiv/stats/2004surveillancereport.pdf.
17. European Collaborative Study. Mother-to-child transmission of HIV infection in the era of highly active antiretroviral therapy. Clin Infect Dis 2005;40:458-65.
18. Murphy EL, Collier AC, Kalish LA, et al. Highly active antiretroviral therapy decreases mortality and morbidity in patients with advanced HIV disease. Ann Intern Med 2001;135:17-26.
19. Smit C, Geskus R, Walker S, et al. Effective therapy has altered the spectrum of cause-specific mortality following HIV seroconversion. AIDS 2006;20:741-49.
20. de Martino M, Tovo PA, Balducci M, et al. Reduction in mortality with availability of antiretroviral therapy for children with perinatal HIV-1 infection. Italian Register for HIV Infection in Children and the Italian National AIDS Registry. JAMA 2000;284:190-97.
21. Gortmaker SL, Hughes M, Cervia J, et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 2001;345:1522-28.
22. Gona P, Van Dyke RB, Williams PL, et al. Incidence of opportunistic and other infections in HIV-infected children in the HAART era. JAMA 2006;296:292-00.
23. Selik RM, Lindegren ML. Changes in deaths reported with human immunodeficiency virus infection among United States children less than thirteen years old, 1987 through 1999. Pediatr Infect Dis J 2003;22:635-41.
24. Gibb DM, Duong T, Tookey PA, et al. Decline in mortality, AIDS, and hospital admissions in perinatally HIV-1 infected children in the United Kingdom and Ireland. BMJ 2003;327:1019.
25. McConnell MS, Byers RH, Frederick T, Peters VB, Dominguez KL, Sukalac T, Greenberg AE, Hsu HW, Rakusan TA, Ortiz IR, Melville SK, Fowler MG. Trends in antiretroviral therapy use and survival rates for a large cohort of HIV-infected children and adolescents in the United States, 1989-2001. J Acquir Immune Defic Syndr 2005;38:488-94.
26. Judd A, Doerholt K, Tookey PA, et al. Morbidity, mortality, and response to treatment by children in the United Kingdom and Ireland with perinatally acquired HIV infection during 1996-2006: planning for teenage and adult care. Clin Infect Dis 2007;45:918-24.

27. Nagler SF, Adnopoz J, Forsyth BWC. Uncertainty, stigma and secrecy: psychological aspects of AIDS for children and adolescents. In: Geballe S, Gruendel J, Andiman W., editors. Forgotten children of the AIDS epidemic. New Haven:Yale University Press; 1995, pp 71-82.
28. Mellins CA, Ehrhardt AA. Families affected by pediatric acquired immunodeficiency syndrome: sources of stress and coping. J Dev Behav Pediatr 1994;15:S54-60.
29. Diekema DS. American Academy of Pediatrics Committee on Bioethics. Responding to parental refusals of immunization of children. Pediatrics 2005;115:1428-31.
30. Kroger AT, Atkinson WL, Marcuse EK, et al. General recommendations on immunization: Recommendations of the ACIP. MMWR Recomm Rep 2006;55:1-48.
31. Briss PA, Rodewald LE, Hinman AR, et al. Reviews of evidence regarding interventions to improve vaccination coverage in children, adolescents, and adults. The Task Force on Community Preventive Services. Am J Prev Med 2000;18:97-140.
32. Centers for DiseaseControl and Prevention. Achievements in public health, 1900-1999. Impact of vaccines universally recommended for children-- United States, 1990-1998. MMWR. Morbidity and Mortality Weekly Report 1999;48:243-48.
33. Filia A, Brenna A, Panà A, et al. Health burden and economic impact of measles-related hospitalizations in Italy in 2002-2003. BMC Public Health 2007;7:169.
34. Neuzil KM, Wright PF, Mitchel EF Jr, et al. The burden of influenza illness in children with asthma and other chronic medical conditions. J Pediatr 2000;137:856-64.
35. O'Brien MA, Uyeki TM, Shay DK, et al. Incidence of outpatient visits and hospitalisations related to influenza in infants and young children. Pediatrics 2004;113:585-93.
36. Bhat N, Wright JG, Broder KR, et al. Influenza-associated deaths among children in the United States, 2003-2004. N Engl J Med 2005;353:2559-67.
37. Levine OS, Farley M, Harrison LH, et al. Risk factors for invasive pneumococcal disease in children: a population-based case-control study in North America. Pediatrics 1999;103:E28.
38. Piano Sanitario Nazionale 2005-2008. Available at: http://www.ministerosalute.it/resources/static/psn/documenti/psn_2005-2008.PDF
39. Fredrickson K, McLaren RP, Enger KS, et al. Influenza vaccination coverage among children aged 6-23 months - six immunisation information system sentinel sites, United States, 2005-06 influenza season. MMWR Morb Mortal Wkly Rep 2006;55:1329-30.
40. Bonanni P. Vaccination and risk groups: how can we really protect the weakest? Hum Vaccin 2007;3:217-19.

41. Daley MF, Barrow J, Pearson K, et al. Identification and recall of children with chronic medical conditions for influenza vaccination. Pediatrics 2004;113:e26-33.

CHAPTER 2

CHRONIC VIRAL HEPATITIS IN CHILDHOOD

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 x^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/m^2 3 times per week for 12 months), 9 children had received IFN recombinant alpha-2a (5 MU/m^2 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/m^2 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**.

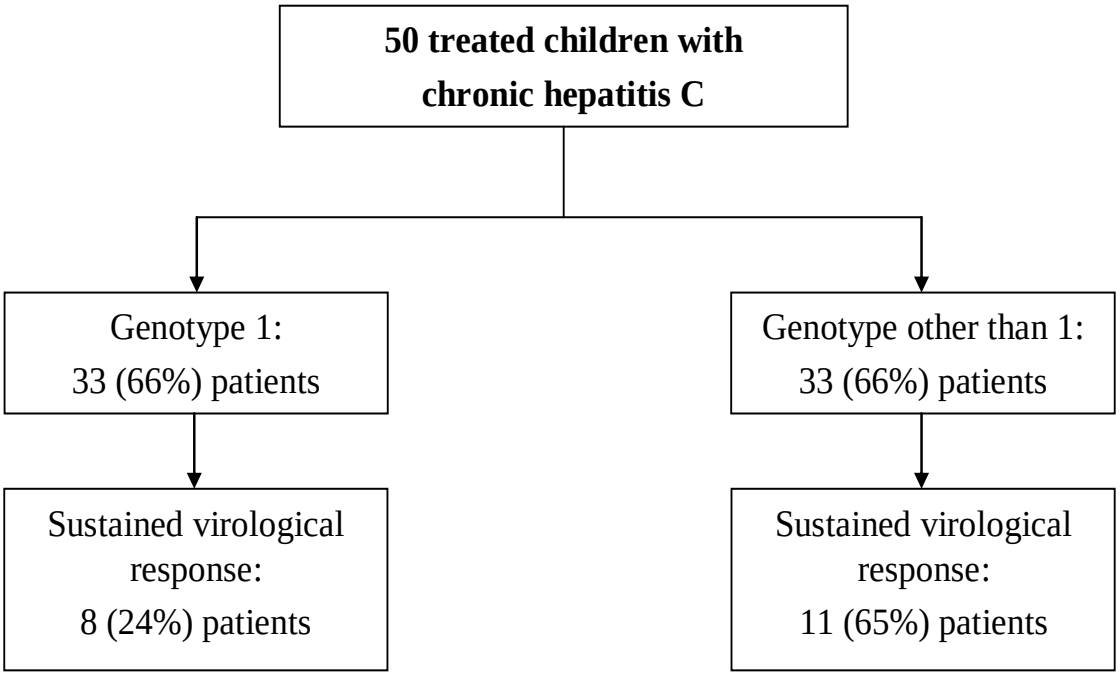

Figure 1. Flow diagram of the outcome in 50 treated children with chronic hepatitis C

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**.

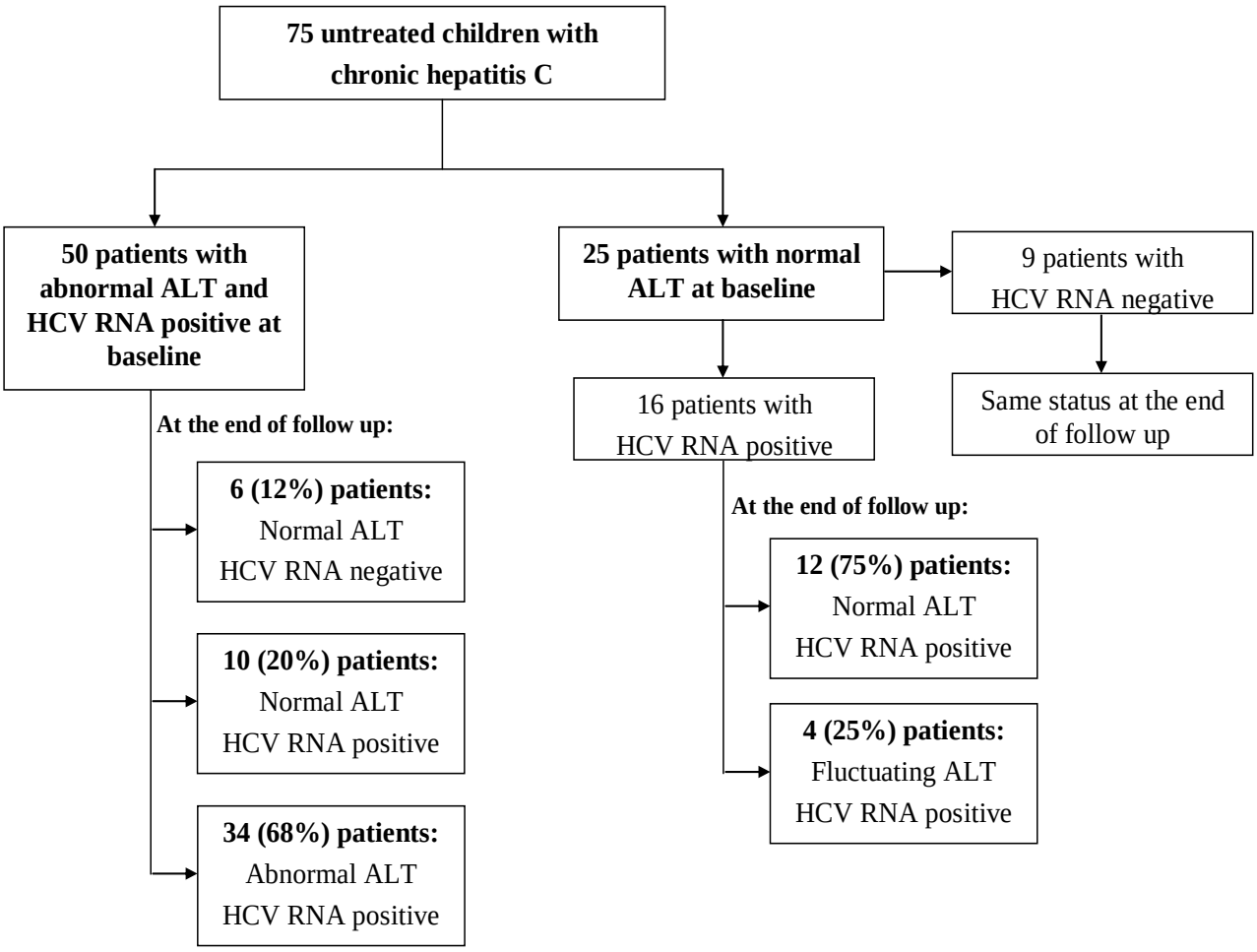

Figure 2. Flow diagram of the outcome in 75 untreated children with chronic hepatitis C

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.

2.2 References

1. Hsu EK and Murary KF. Hepatitis B ans C in children. Nature Clinical Practice 2008;5:311-320.
2. Fattovich G. Natural history of hepatitis B. J Hepatol 2003; 39:S50–8.
3. Lok AS and McMahon BJ. Chronic hepatitis B: update 2009. Hepatology 2009;50:661-2.
4. Hsu YS, Chien RN, Yeh CT, et al. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology 2002; 35:1522–7.
5. Boxall EH, Sira J, Standish RA, et al. Natural history of hepatitis B in perinatally infected carriers. Arch Dis Child Fetal Neonatal Ed 2004; 89:F456–F460.
6. Bortolotti F, Jara P, Crivellaro C, et al. Outcome of chronic hepatitis B in Caucasian children during a 20-year observation period. J Hepatol 1998;29:184-90.
7. Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med 1997;336:1855-59.
8. Czauderna P, Mackinlay G, Perilongo G, et al. Hepatocellular carcinoma in children: results of the first prospective study of the International Society of Pediatric Oncology Group. J Clin Oncol 2002;20:2798-2804.
9. Ni YH, Chang MH, Wang KJ, et al. Clinical relevance of hepatitis B virus genotype in children with chronic infection and hepatocellular carcinoma. Gastroenterology 2004;127:1733–8.
10. Barbera C, Bortolotti F, Crivellaro C, et al. Recombinant interferonalpha2a hastens the rate of HBeAg clearance in children with chronic hepatitis B. Hepatology 1994;20:287-90.
11. Bortolotti F, Jara P, Barbera C, et al. Long term effect of alpha interferon in children with chronic hepatitis B. Gut 2000; 46:715-8.
12. Jonas MM, Mizerski J, Badia IB, et al. Clinical trial of lamivudine in children with chronic hepatitis B. N Engl J Med 2002;346:1706-13.
13. Resti M, Azzari C, Manelli F, et al. Mother to child transmission of hepatitis C virus: prospective study of risk factors and timing of infection in children born to women seronegative for HIV-1. BMJ 1998;317:437-41.
14. Thomas SL, Newell ML, Peckham CS, et al. A review of hepatitis C virus (HCV) vertical transmission: risks of transmission to infants born to mothers with and without

HCV viraemia or human immunodeficiency virus infection. Int J Epidemiol 1998;27:108-17.

15. Minola E, Prati D, Suter F, et al. Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C. Blood 2002;99:4591.
16. Casiraghi MA, Paschale MD, Romano L, et al. Long-term outcome (35 years) of hepatitis C after acquisition of infection through minitransfusions of blood given at birth. Hepatology 2004;39:90-96.
17. Tovo PA, Pembrey LJ, Newell ML, and The European Pediatric Hepatitis C Virus Infection Network. Persistence rate and progression of vertically acquired hepatitis C infection. J Infect Dis 2000;181:419-24.
18. Feld JJ and Liang TJ. Hepatitis C-identifying patients with progressive liver injury. Hepatology 2006;43:S194-S206.
19. Jara P, Resti M, Hierro L, et al. Chronic hepatitis C virus infection in childhood: clinical patterns and evolution in 224 white children. Clin Infect Dis 2003;36:275-80.
20. Guido M, Rugge M, Jara P, et al. Chronic hepatitis C in children: the pathological and clinical spectrum. Gastroenterology 1998;115:1525–29.
21. Vogt M, Lang T, Frosner G, et al. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med 1999;341:866-70.
22. Locasciulli A, Testa M, Pontisso P, et al. Prevalence and natural history of hepatitis C infection in patients cured of childhood leukemia. Blood 1997;90:4628-33.
23. Kage M, Fujisawa T, Shiraki K, et al. Pathology of chronic hepatitis C in children: child liver study group of Japan. Hepatology 1997;26:771-75.
24. Badizadegan K, Jonas MM, Ott MJ, et al. Histopathology of the liver in children with chronic hepatitis C viral infection. Hepatology 1998;28:1416-23.
25. Birnbaum AH, Shneider BL, Moy L. Hepatitis C in children. N Engl J Med 2000;342:290-1.
26. Zancan L, Strafella MS, Brugiolo A, et al. Chronic hepatitis C virus infection in childhood and early cirrhosis: it is possible? J Pediatr Gastroenterol Nutr 2000;30:350-1.
27. Rumbo C, Fawaz RL, Emre SH, et al. Hepatitis C in children: a quaternary referral center perspective. J Pediatr Gastroenterol Nutr 2006;43:209-16.
28. Barshes NR, Udell IW, Lee TC, et al. The natural history of hepatitis C virus in pediatric liver transplant recipients. Liver Transpl 2006;12:1119-23.

29. Jacobson KR, Murray K, Zellos A, et al. An analysis of published trials of interferon monotherapy in children with chronic hepatitis C. J Pediatr Gastroenterol Nutr 2002;34:52-8.
30. Strader DB, Wright T, Thomas DL, et al. Diagnosis, management, and treatment of hepatitis C. Hepatology 2004;39:1147-71.
31. Gonzalez-Peralta RP, Kelly DA, Haber B, et al. Interferon alfa-2b in combination with ribavirin for the treatment of chronic hepatitis C in children: efficacy, safety, and pharmacokinetics. Hepatology 2005;42:1010-18.
32. Lackner H, Moser A, Deutsch J, et al. Interferon-alpha and ribavirin in treating children and young adults with chronic hepatitis C after malignancy. Pediatrics 2000;106:E53.
33. Suoglu OD, Elkabes B, Sokucu S, et al. Does interferon and ribavirin combination therapy increase the rate of treatment response in children with hepatitis C? J Pediatr Gastroenterol Nutr 2002;34:199-206.
34. Wirth S, Lang T, Gehring S, et al. Recombinant alfa-interferon plus ribavirin therapy in children and adolescents with chronic hepatitis C. Hepatology 2002;36:1280-84.
35. Figlerowicz M, Sluzewski W, Kowala-Piaskowska A, et al. Interferon alpha and ribavirin in the treatment of children with chronic hepatitis C. Eur J Pediatr 2004;163:265-67.
36. Iorio R, Pensati P, Botta S, et al. Side effects of alpha-interferon therapy and impact on health-related quality of life in children with chronic viral hepatitis. Pediatr Infect Dis J 1997;16:984-90.
37. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-39.
38. Detmer J, Lagier R, Flynn J, et al. Accurate quantification of HCVRNA from all HCV genotypes using branched DNA (b-DNA) technology. J Clin Microbiol 1996;34:901-07.
39. Simmonds P, McOmish F, Yap PL, et al. Sequence variability in the 5 non-coding region of hepatitis C virus: identification of a new virus type and restrictions on sequence diversity. J Gen Virol 1993;74:661-68.
40. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-99.
41. Iorio R, Giannattasio A, Cirillo F, et al. Long-term outcome in children with chronic hepatitis B: a 24-year observation period. Clin Infect Dis 2007;45:943-9.
42. Chang MH, Chen PJ, Chen JY, et al. Hepatitis B virus integration in hepatitis B virus-related hepatocellular carcinoma in childhood. Hepatology 1991;13:316-20.
43. Guido M, Bortolotti F, Leandro G, et al. Fibrosis in chronic hepatitis C acquired in infancy: is it only a matter of time? Am J Gastroenterol 2003;98:660-63.

44. Lin SM, Sheen IS, Chien RN, et al. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971-75.
45. Lau DT, Everhart J, Kleiner DE, et al. Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. Gastroenterology 1997;113:1660-67.
46. Bortolotti F, Guido M, Bartolacci S, et al. Chronic hepatitis B in children after e antigen seroclearance: final report of a 29-year longitudinal study. Hepatology 2006;43:556–62.
47. Murata K, Sugimoto K, Shiraki K, et al. Relative predictive factors for hepatocellular carcinoma after HBeAg seroconversion in HBV infection. World J Gastroenterol 2005;11:6848-52.
48. Lo Nigro L, La Spina M, Mirabile E, et al. Use of PEG-interferon alfa-2a plus ribavirin as treatment for chronic HCV hepatitis in a child cured of ALL. Pediatr Blood Cancer 2004;43:185.

2.3 Publications

1. Iorio R, **Giannattasio A**, Cirillo F, D'Alessandro L, Vegnente A.
   Long-term outcome of children with chronic hepatitis B: a 24-year observation period.
   Clin Infect Dis 2007;45:943-49.

2. Iorio R, Verrico A, **Giannattasio A**.
   Is liver biopsy mandatory in children with chronic hepatitis C?
   World J Gastroenterol 2007;713:4025-26.

3. Bortolotti F, Jorio R, Resti M, Cammà C, Marcellini M, Giacchino R, Marazzi MG, Verucchi G, Zancan L, Barbera C, Maggiore G, Vajro P, **Giannattasio A**, Bartolacci S.
   Epidemiological profile of 806 Italian children with hepatitis C virus infection over a 15-year period.
   J Hepatol 2007;46:783-90.

4. Iorio R, , Cirillo F, Telizzi V, **Giannattasio A**.
   Children with chronic hepatitis C: what future?
   Hepatology 2008;48:691-92.

MAJOR ARTICLE

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 "Frederico 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 43 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]. Seroconversion 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

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have been reported in 23% of treated children, compared with 13% 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 favorable 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 seroconversion 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 Department of Pediatrics at University "Frederico II" (Naples, Italy) over a 24-year period were retrospectively evaluated and compared for the clinical, biochemical, and virological outcomes.

PATIENTS AND METHODS

Patients. All children with CHB (defined as the presence of hepatitis B surface antigen [HBsAg] in serum for ≥6 months) who were observed for >5 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. Exclusion criteria included presence of concomitant systemic diseases, 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 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), α-fetoprotein level, serum immunoglobulin level, and non–organ-specific autoantibody levels. Serum aspartate aminotransferase and alanine aminotransferase (ALT) levels were evaluated using standard methods (upper limit of normal, 50 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. Ultrasonographic 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 biochemical data. Specimens were scored with regard to hepatitis activity (grades, 0–18) and fibrotic changes (stages, 0–6), according 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 carrier state was defined as a detectable HBsAg level, an undetectable 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 HBsAg 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 (HBsAg, antibody to 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 (cutoff value, 5 pg/mL; Abbott Diagnostics) or 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 χ^2 test or Fisher's exact test. Comparison of continuous data was performed using the Mann-Whitney *U* test and the Kruskal-Wallis test. A *P* value <.05 was considered to be statistically significant.

RESULTS

One hundred eight consecutive patients (65 male patients; 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 CHB was evaluated for a median period of 12.1 years (range, 5–23 years).

During the observation period, 67 children remained untreated, 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 57 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 (33.3%; 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 micronodular 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, 5.4–20.9 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, 3–15.5 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 9 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<10^4 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 10^4 copies/mL to 10^5 copies/mL. HBV infection resolved in 4 patients (9.8%) who seroconverted to antibody to HBsAg. 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 1. Baseline characteristics of 108 children with chronic hepatitis B virus (HBV) infection.

Characteristic	IFN-treated children (n = 41)	Untreated children (n = 67)
Sex, M:F	26:15	39:28
Age, median years (range)	5.7 (1–14.7)	6.7 (1.1–13.2)
Route of infection[a]		
Vertical	14 (34.1)	32 (47.8)
Horizontal	15 (36.6)	13 (19.4)
Unknown	12 (29.3)	22 (32.8)
Age at diagnosis, median years (range)	3.9 (0.3–14)	4 (0.2–12)
ALT level		
Normal	5 (12.2)	18 (26.9)
Elevated	36 (87.8)	49 (73.1)
Median ALT level × ULN (range)	2 (1.4–7.9)	2.2 (1.1–26)
Detectable HBeAg and HBV DNA levels	41 (100)	62 (92.5)

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.

[a] Vertical transmission was defined as transmission of HBV infection from a mother with a detectable HBsAg level in the absence of other risk factors for HBV infection.

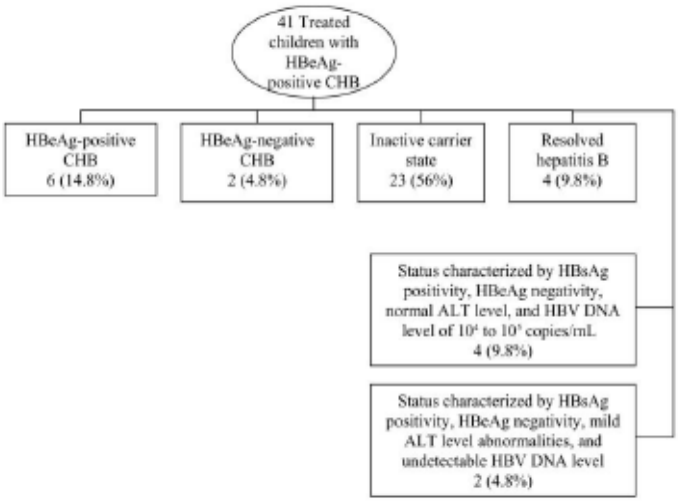

Figure 1. Flow diagram of the outcome in 41 treated children with chronic hepatitis B (CHB). Hepatitis B early antigen (HBeAg)–negative chronic hepatitis B (CHB) is defined as CHB in the absence of a detectable HBeAg level, and HBeAg-positive CHB is defined as CHB in the presence of a detectable HBeAg 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, 5–23 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 10^4 copies/mL to 10^5 copies/mL; and 6 (9.7%) achieved clearance of HBsAg (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 viremia. 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 HBsAg 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, 3–21 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 source of infection (table 2).

At the end of the follow-up period, 86 children (79.6%) had experienced HBeAg seroclearance. This clearance was associated with development of anti-HBe and an HBV DNA level $<10^5$ 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 (75%) 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

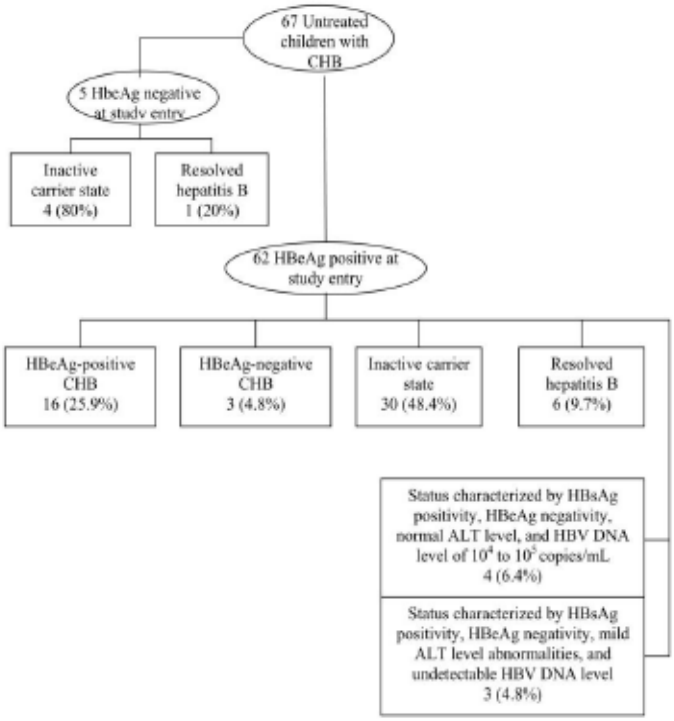

Figure 2. Flow diagram of the outcome in 67 untreated children with chronic hepatitis B (CHB). Hepatitis B early antigen (HBeAg)–negative CHB is defined as CHB in the absence of a detectable HBeAg level, and HBeAg-positive CHB is defined as CHB in the presence of a detectable HBeAg level. ALT, alanine 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; ~8% of the patients achieved a detectable anti-HBsAg 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 HBsAg level. The authors explained that this lower rate of HBsAg 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 HBsAg clearance rate was 17.5% [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,

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

Outcome	Route of infection, no. (%) of patients	
	Vertical (n = 46)	Horizontal (n = 28)
Clearance of HBeAg and acquisition of anti-HBe	35 (76.1)	25 (89.3)
Persistence of detectable HBeAg levels	9 (19.6)	2 (7.1)
HBeAg-negative chronic hepatitis B	2 (4.3)	1 (3.6)

NOTE. Hepatitis B 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.

it is possible that a percentage of these patients will experience seroconversion at a later time. All inactive carriers had serum HBV DNA levels $<10^4$ copies/mL, but a group of these patients had a detectable HBV DNA level by PCR. Other authors reported that a sizable percentage of patients with an undetectable HBeAg level had a detectable HBV DNA level by PCR [24, 25]. These low levels of viremia probably reflect a low viral replication persisting for several years in patients with CHB, even after remission of liver disease and clearance of HBsAg. 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-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, Bortolotti 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 mutant, none of the patients in our study who had HBeAg-negative CHB showed clinical, laboratory, or echographic signs of severe liver damage.

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.

Outcome	No (%) of IFN-treated patients (n = 41)	No. (%) of untreated patients (n = 67)[a]
Clearance of HBeAg and acquisition of anti-HBe	27 (65.9)	38 (56.7)
Resolved hepatitis B	4 (9.7)	7 (10.4)
Persistence of detectable HBeAg levels	6 (14.8)	16 (23.9)
HBeAg-negative chronic hepatitis B	2 (4.8)	3 (4.5)
Fluctuating ALT levels, HBV DNA level $<10^5$ copies/mL, and an undetectable HBeAg level	2 (4.8)	3 (4.5)

NOTE. Hepatitis B 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.

[a] Five of 67 untreated children already had undetectable HBeAg levels at study entry.

HBsAg 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., adefovir, 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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References

1. Lee WM. Hepatitis B virus infection. N Engl J Med **1997**; 337:1733–45.
2. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol **2005**; 5:215–29.
3. Sokal EM, Bortolotti F. Update on prevention and treatment of viral hepatitis in children. Curr Opin Pediatr **1999**; 11:384–9.
4. Chang MH. Chronic hepatitis virus infection in children. J Gastroenterol Hepatol **1998**; 13:541–8.
5. Pattovich G. Natural history of hepatitis B. J Hepatol **2003**; 39:S50–8.
6. Pattovich G, Rugge M, Brollo L, et al. Clinical, virologic and histologic outcome following seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. Hepatology **1986**; 6:167–72.
7. Bortolotti F, Jara P, Crivellaro C, et al. Outcome of chronic hepatitis B in Caucasian children during a 20-year observation period. J Hepatol **1998**; 29:184–90.
8. Hsu YS, Chien RN, Yeh CT, et al. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology **2002**; 35:1522–7.
9. Bortolotti F, Cadrobbi P, Crivellaro C, et al. Long-term outcome of chronic type B hepatitis in patients who acquire hepatitis B virus infection in childhood. Gastroenterology **1990**; 99:805–10.
10. Chang MH, Chen PJ, Chen JY, et al. Hepatitis B virus integration in hepatitis B virus-related hepatocellular carcinoma in childhood. Hepatology **1991**; 13:316–20.
11. Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med **1997**; 336:1855–9.
12. Ni YH, Chang MH, Wang KJ, et al. Clinical relevance of hepatitis B virus genotype in children with chronic infection and hepatocellular carcinoma. Gastroenterology **2004**; 127:1733–8.
13. Sokal EM, Conjeevaram HS, Roberts EA, et al. Interferon alfa therapy for chronic hepatitis B in children: a multinational randomized controlled trial. Gastroenterology **1998**; 114:988–95.
14. Barbera C, Bortolotti F, Crivellaro C, et al. Recombinant interferon-alpha 2a hastens the rate of HBeAg clearance in children with chronic hepatitis B. Hepatology **1994**; 20:287–90.
15. Bortolotti F, Jara P, Barbera C, et al. Long term effect of alpha interferon in children with chronic hepatitis B. Gut **2000**; 46:715–8.
16. Jonas MM, Mizerski J, Badia IB, et al. Clinical trial of lamivudine in children with chronic hepatitis B. N Engl J Med **2002**; 346:1706–13.
17. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. Hepatology **2004**; 39:1147–71.
18. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology **2007**; 45:507–39 (erratum: Hepatology 2007; 45:1347).
19. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. Adv Data **2000**; 1–27.
20. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol **1995**; 22:696–9.
21. Fujisawa T, Komatsu H, Inui A, et al. Long-term outcome of chronic hepatitis B in adolescents or young adults in follow-up from childhood. J Pediatr Gastroenterol Nutr **2000**; 30:201–6.
22. Bortolotti F, Guido M, Bartolacci S, et al. Chronic hepatitis B in children after e antigen seroclearance: final report of a 29-year longitudinal study. Hepatology **2006**; 43:556–62.
23. Manno M, Camma C, Schepis F, et al. Natural history of chronic HBV carriers in northern Italy: morbidity and mortality after 30 years. Gastroenterology **2004**; 127:756–63.
24. Ruiz-Moreno M, Otero M, Millan A, et al. Clinical and histological outcome after hepatitis B e antigen to antibody seroconversion in children with chronic hepatitis B. Hepatology **1999**; 29:572–5.
25. Bortolotti F, Wirth S, Crivellaro C, Alberti A, Martine U, de Moliner L. Long-term persistence of hepatitis B virus DNA in the serum of children with chronic hepatitis B after hepatitis B e antigen to antibody seroconversion. J Pediatr Gastroenterol Nutr **1996**; 22:270–4.
26. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology **1999**; 29:971–5.
27. Lau DT, Everhart J, Kleiner DE, et al. Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. Gastroenterology **1997**; 113:1660–7.
28. Hsu YS, Chien RN, Yeh CT, et al. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology **2002**; 35:1522–7.
29. Murata K, Sugimoto K, Shiraki K, Nakano T. Relative predictive factors for hepatocellular carcinoma after HBeAg seroconversion in HBV infection. World J Gastroenterol **2005**; 11:6848–52.
30. Shneider BL, Gonzalez-Peralta R, Roberts EA. Controversies in the management of pediatric liver disease: hepatitis B, C and NAFLD: summary of a single topic conference. Hepatology **2006**; 44:1344–54.

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Is liver biopsy mandatory in children with chronic hepatitis C?

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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 evaluation 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.

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

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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 fibrosis together with recognition of architectural disturbances[1]. 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[1].

Liver biopsy represents, however, an invasive procedure associated with discomfort and risk to the patient[1]. In pediatric age, it has been reported that liver biopsy is burden 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[3-6].

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[7-9]. 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[10]. 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[11,12].

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[13-16]. 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[17,18]. Furthermore, these data about the severe course of pediatric HCV infection obtained from studies performed in tertiary or quaternary referral centres, do not provide an adequate perception of the real prevalence of worsening prognosis[17,18]. 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*[13] 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 ± 51.9 mo after the first observation of increased transaminases. The authors reported a rate of 0.8% of cirrhosis. Vogt *et al*[14], 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. Jara *et al*[15], 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 4 wk 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 Liver Unit of the University "Federico II" (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 changes (staged 0-6), in accordance with the methodology of Ishak *et al*[19]. 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 218 000 IU/mL, range 40 000-146 7000 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[16].

In conclusion, liver histology investigation 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?

REFERENCES

1 **Rockey DC**, Bissell DM. Noninvasive measures of liver fibrosis. *Hepatology* 2006; **43**: S113-S120

2 **Scheimann AO**, Barrios JM, Al-Tawil YS, Gray KM, Gilger MA. Percutaneous liver biopsy in children: impact of ultrasonography and spring-loaded biopsy needles. *J Pediatr Gastroenterol Nutr* 2000; **31**: 536-539

3 **Pasha T**, Gabriel S, Therneau T, Dickson ER, Lindor KD. Cost-effectiveness of ultrasound-guided liver biopsy. *Hepatology* 1998; **27**: 1220-1226

4 **Regev A**, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, Reddy KR, Schiff ER. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; **97**: 2614-2618

5 **Colloredo G**, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. *J Hepatol* 2003; **39**: 239-244

6 **Bedossa P**, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449-1457

7 **Wirth S**, Lang T, Gehring S, Gerner P. Recombinant alfa-interferon plus ribavirin therapy in children and adolescents with chronic hepatitis C. *Hepatology* 2002; **36**: 1280-1284

8 **Gonzalez-Peralta RP**, Kelly DA, Haber B, Molleston J, Murray KF, Jonas MM, Shelton M, Mieli-Vergani G, Lurie Y, Martin S, Lang T, Baczkowski A, Geffner M, Gupta S, Laughlin M. Interferon alfa-2b in combination with ribavirin for the treatment of chronic hepatitis C in children: efficacy, safety, and pharmacokinetics. *Hepatology* 2005; **42**: 1010-1018

9 **Schwarz KB**, Mohan P, Narkewicz MR, Molleston JP, Nash SR, Hu S, Wang K, Gries JM. Safety, efficacy and pharmacokinetics of peginterferon alpha2a (40 kd) in children with chronic hepatitis C. *J Pediatr Gastroenterol Nutr* 2006; **43**: 499-505

10 **Strader DB**, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **39**: 1147-1171

11 **Bacon BR**. Treatment of patients with hepatitis C and normal serum aminotransferase levels. *Hepatology* 2002; **36**: S179-S184

12 **Jacobson IM**, Ahmed F, Russo MW, Lebovics E, Dieterich DT, Esposito SP, Bach N, Klion F, Tobias H, Antignano L, Brown RS Jr, Gabbaizadeh D, Geders J, Levendoglu H. Interferon alfa-2b [correction of alpha-2b]and ribavirin for patients with chronic hepatitis C and normal ALT. *Am J Gastroenterol* 2004; **99**: 1700-1705

13 **Guido M**, Rugge M, Jara P, Hierro L, Giacchino R, Larrauri J, Zancan L, Leandro G, Marino CE, Balli F, Bagni A, Timitilli A, Bortolotti F. Chronic hepatitis C in children: the pathological and clinical spectrum. *Gastroenterology* 1998; **115**: 1525-1529

14 **Vogt M**, Lang T, Frosner G, Klingler C, Sendl AF, Zeller A, Wiebecke B, Langer B, Meisner H, Hess J. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. *N Engl J Med* 1999; **341**: 866-870

15 **Jara P**, Resti M, Hierro L, Giacchino R, Barbera C, Zancan L, Crivellaro C, Sokal E, Azzari C, Guido M, Bortolotti F. Chronic hepatitis C virus infection in childhood: clinical patterns and evolution in 224 white children. *Clin Infect Dis* 2003; **36**: 275-280

16 **Iorio R**, Giannattasio A, Sepe A, Terracciano LM, Vecchione R, Vegnente A. Chronic hepatitis C in childhood: an 18-year experience. *Clin Infect Dis* 2005; **41**: 1431-1437

17 **Rumbo C**, Fawaz RL, Emre SH, Suchy FJ, Kerkar N, Morotti RA, Shneider BL. Hepatitis C in children: a quaternary referral center perspective. *J Pediatr Gastroenterol Nutr* 2006; **43**: 209-216

18 **Barshes NR**, Udell IW, Lee TC, O'Mahony CA, Karpen SJ, Carter BA, Goss JA. The natural history of hepatitis C virus in pediatric liver transplant recipients. *Liver Transpl* 2006; **12**: 1119-1123

19 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699

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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.**

***Methods*: Fifteen tertiary care centers, belonging to a national Observatory established in 1998, retrospectively/prospectively recruited 806 consecutive HCV-infected, otherwise healthy, children seen from 1990 to 2004.**

***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 ($p < 0.001$) 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 anti retroviral 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/retrospective 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 (SIGEP, now SIGENP) 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 seen 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 diseases, or coinfection 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 ELISAs. HCV RNA seropositivity was required for the diagnosis in children born of infected mothers and aged 18 months or less, given the possibility of passive antibody transmission from mother to newborn. HCV RNA was investigated 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 χ^2 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. None was a referral center 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 2000–2004s versus those recruited from 1995 to 1999. Conversely, the number of foreign children, though small, has increased significantly ($p < 0.0001$) in recent years.

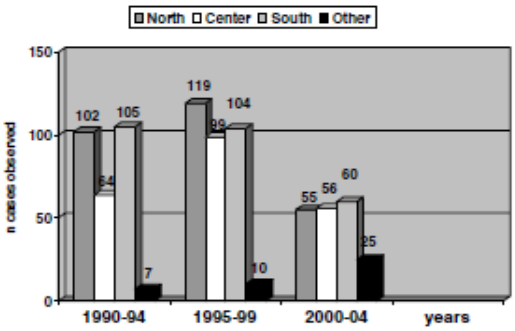

Fig. 1. Distribution of the 806 cases by year of first visit at the referral centers ($p < 0.001$).

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 88% (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
Demographic features and mode of recruitment of the 764 Italian children enrolled in the study, in relation to the geographic area of origin

	Geographic origin of children				
	Total	North	Center	South	*p*
N cases	764	276	219	269	–
M/F	0.88	0.79	1.00	0.93	0.38
Mean age at first visit (±SD years)[a]	5.8 (±4.6)	5.2 (±4.7)	5.5 (±4.7)	6.7 (±4.4)	0.002
Mode of recruitment: *N* cases (%)					
Symptoms/signs	67	18 (6.5)	26 (12)	23 (9)	0.11
Maternal screening[b]	324	143 (52)	105 (48)	76 (28)	0.0001
Post transfusion screening[c]	106	32 (11.5)	25 (11)	49 (18)	0.03
Screening for adoption	28	11 (4)	10 (5)	7 (3)	0.48
Intercurrent disease	124	48 (17)	32 (15)	44 (16)	0.70
Other/unknown[d]	115	24 (9)	21 (9)	70 (26)	0.0001

[a] $p = 0.0002$ South vs North; $p = 0.0046$ South vs Center.
[b] $p = 0.0001$ South vs North; $p = 0.0001$ South vs Center.
[c] $p = 0.04$ South vs North; $p = 0.04$ 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

Type of exposure	Putative time of exposure				
	N (%)				*p*
	Before 1990	1990–1994	1995–1999	2000–2004	
Transfusions[a]	200 (55)	17 (10)	0	0	0.0001
Other parenteral exposure[b]	40 (11)	10 (5)	3 (1.5)	0	0.0001
Infected mother[c]	82 (22)	123 (66)	152 (84)	52 (93)	0.0001
Infected family contact	7 (2)	3 (1)	1 (0.5)	0	0.48
Unknown	26 (10)	24 (18)	20 (14)	4 (7)	0.02
Total cases	355	177	176	56	

None of the children with a history of blood transfusions had received blood after 1992.
[a] $p = 0.0001$ before 1990 vs 1990–1994.
[b] $p = 0.05$ before 1990 vs 1990–1994; $p = 0.0001$ before 1990 vs 1995–1999.
[c] $p = 0.0001$ before 1990 vs other time periods.

and transfused children, with a rate of 0.6 in the former and 1.3 in the latter. The putative source of infection was a family contact only in 11 cases.

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, while 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 Ib 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 607% 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 nad maternal HIV coinfection. The proportion of drug abusers tended to decline over the years, albeit without reaching a statistically significant difference. Data on HIV seropositivity among infected mothers were only partial: there is no doubt, however, that coinfection with HIV was strongly associated with drug abuse.
(b) Information on type of delivery (vaginal or cesarean) was available for 261 non-HIV coinfected mothers. The frequency of cesarean 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

Type of exposure	Geographic origin				
	Total	North	Center	South	*p*
Transfusions[a] (%)	217 (28)	63 (23)	47 (21.5)	107 (40)	0.0001
Other parenteral exposure (%)	53 (7)	18 (6.5)	9 (4)	26 (9.5)	0.052
Infected mother[b] (%)	409 (54)	173 (62.5)	137 (62.5)	99 (36.5)	0.0001
Infected family contact (%)	11 (1)	4 (1.5)	2 (1)	5 (2)	0.68
Unknown (%)	74 (10)	18 (6.5)	24 (11)	32 (12)	0.08
Total	764	276	219	269	

[a] Blood transfusion South vs North: $p = 0.0001$; South vs Center: $p = 0.0001$.
[b] Anti HCV + mothers South vs North: $p = 0.0001$; South vs Center: $p = 0.0001$.

Table 4
Distribution of HCV genotypes in 462 children in relation to area of origin and time of putative exposure

	HCV genotype, *N* (%)					
	1a	1b	2	3	4	Other
Geographic origin[a]						
North: 158	35 (22)	56 (35)	28 (18)	26 (16)	12 (8)	1 (1)
Center: 136	30 (22)	47 (35)	21 (15)	25 (18)	8 (6)	5 (4)
South: 168	25 (15)	83 (50)	37 (22)	9 (5)	5 (3)	9 (5)
$p=$	0.16	0.01	0.32	0.001	0.17	0.04
Time of exposure[b]						
Before 1990: 226	40 (18)	106 (47)	46 (20)	17 (8)	9 (4)	8 (3)
1990–1994: 110	28 (25)	37 (34)	23 (21)	17 (15)	2 (2)	3 (3)
1995–1999: 102	17 (17)	38 (37)	13 (13)	20 (20)	11 (10)	3 (3)
2000–2004: 24	5 (21)	5 (21)	4 (17)	6 (25)	3 (12)	1 (4)
$p=$	0.32	0.016	0.35	0.0035	0.008	0.96

[a] 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.
[b] Genotype 1b: $p=0.02$, Exposed before 1990 vs 1990–1994; $p=0.016$, before 1990 vs 2000–2004. Genotype 3: $p=0.038$, Exposed before 1990 vs 1990–1994; $p=0.003$: before 1990 vs 1995–1999; $p=0.014$, before 1990 vs 2000–2004. Genotype 4: $p=0.003$, before 1990 vs 1995–1999; $p=0.008$: 1990–1994 vs 1995–1999.

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 (=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.

Table 5
Distribution of HCV infected children whose mothers were drug abusers and non-drug abusers, in relation to geographic origin, time of exposure nad maternal HIV coinfection

	N (%)	
	Drug user mother[a] (154)	Non-drug user mother (139)
Geographic origin		
North (146)	100 (65)	46 (33)
Center (87)	43 (28)	44 (32)
South (60)	11 (7)	49 (35)
	$p=0.0001$	
Time of exposure		
Before 1990 (55)	33 (21)	22 (16)
1990–1994 (82)	44 (29)	38 (27)
1995–1999 (112)	58 (38)	54 (39)
2000–2004 (44)	19 (12)	25 (18)
	$p=0.41$	
Maternal HIV coinfection	56/115 (49)	0/96
	$p=0.0001$	

[a] Drug user South vs North: $p=0.0001$; South vs Center: $p=0.0001$; Center vs North: $p=0.006$.

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 not 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-coinfected pregnant women and the efficacy of treatment with IFN and ribavirin 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 [8,12,23,24] 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. [26], however, investigating the families of 2856 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 use of nondisposable 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 transfusion, 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 hormone 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 breast-feed 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 immigrated or adopted children in our study rose from 3% in the Nineties to 13% 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 recruited 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 admits 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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References

[1] Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;5:556–558.
[2] Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion transmitted viral infections. The Retrovirus Donor Epidemiology Study:N Engl J Med 1996;334:1685–1690.
[3] Gibb DM, Neave PE, Tookey PA, Ramsay M, Harris H, Balogun K, et al. Active surveillance of hepatitis C infection in the UK and Ireland. Arch Dis Child 2000;82:286–291.
[4] Wasley A, Alter MJ. Epidemiology of hepatitis C:geographic differences and temporal trends. Semin Liver Dis 2000;20:1–16.
[5] McHutchison JG. Understanding hepatitis C. Am J Manag Care 2004;10:S21–S29.
[6] El-Kamary SS, Servint JR, Joffe A, Santosham M, Duggan AK. Prevalence of hepatitis C virus infection in urban children. J Pediatr 2003;143:54–59.
[7] Otho H, Terasawa S, Sasaki N, Hino K, Ishiwata C, Kako M, et al. Transmission of hepatitis C virus from mothers to infants. The Vertical Transmission of Hepatitis C Virus Study Group. N Engl J Med 1994;330:744–750.
[8] Resti M, Azzari C, Mannelli F, Moriondo M, Novembre E, De Martino M, et al. Mother to child transmission of the hepatitis C virus:prospective study of risk factors and timing of infection in children born to women seronegative for HIV 1. BMJ 1998;317:437–441.
[9] European Pediatric Hepatitis C Virus Infection Network. Tovo PA, Pembrey LJ, Newell ML. Persistence rate and progression of vertically acquired hepatitis C infection. J Infect Dis 2000;181:419–424.
[10] Mast EE, Hwang L-WR, Seto DSY, Nolte FS, Nainan OV, Wurtzel H, et al. Risk factors for perinatal transmission of hepatitis C virus (HCV) and the natural history of HCV infection acquired in infancy. J Infect Dis 2005;192:1880–1889.
[11] Roberts E, Yeung L. Maternal-infant transmission of hepatitis C virus infection. Hepatology 2002;36:S106–S113.
[12] Conte D, Fraquelli M, Prati D, Colucci A, Minola E. Prevalence and clinical course of chronic hepatitis C (HCV) infection and rate of HCV vertical transmission in a cohort of 15,250 pregnant women. Hepatology 2000;31:751–755.
[13] Romanò L, Azara A, Chiaramonte M, De Mattia D, Giammanco A, Moschen ME, et al. Low prevalence of anti-HCV antibodies among Italian children. Infection 1994;22:350–351.
[14] Bortolotti F, Iorio R, Resti M, Verucchi G, Giacchino R, Vegnente A, et al. An epidemiological survey of hepatitis C virus infection in Italian children in the decade 1990-1999. J Pediatr Gastroenterol Nutr 2001;32:562–566.
[15] Bortolotti F, Resti M, Marcellini M, Giacchino R, Verucchi G, Nebbia G, et al. Hepatitis C virus (HCV) genotypes in 373 Italian children with HCV infectionxchanging distribution and correlation with clinical features and outcome. Gut 2005;54:852–857.
[16] Matera G, Lamberti A, Quirino A, Foca D, Giancotti A, Barreca GS, et al. Changes in the prevalence of hepatitis C virus (HCV) genotype 4 in Calabria, Southern Italy. Diagn Microbiol Infect Dis 2002;42:69–73.
[17] Bellentani S, Miglioli L, Masutti F, Saccoccio G, Tiribelli C. Epidemiology of hepatitis C virus infection in Italy:the slowly unraveling mystery. Microbes and infection 2000;2:1753–1757.
[18] Mazzeo C, Azzaroli F, Giovannelli S, Dormi A, Festi D, Colecchio A, et al. Ten year incidence of HCV infection in Northern Italy and frequency of spontaneous viral clearance. Gut 2003;52:1030–1034.
[19] Ansaldi F, Bruzzone B, Salmaso S, Rota MC, Durando P, Gasparini R, et al. Different seroprevalence and molecular epidemiology patterns of hepatitis C virus infection in Italy. J Med Virol 2005;76:327–332.
[20] Gonzales M, Règine V, Piccinini V, Vulcano F, Giampaolo A, Hassan HJ. Residual risk of transfusion-transmitted human immunodeficiency virus, hepatitis C virus, and hepatitis B virus infections in Italy. Transfusion 2000;45:1670–1675.
[21] Schroter M, Zollner B, Schafer P, Reimer A, Feucht HH. Epidemiological dynamics of hepatitis C virus among 747 German individuals:new subtypes on the advance. J Clin Microbiol 2002;40:1866–1870.
[22] Gérard C, Delwaide J, Vaira D, Bastens B, Servais B, Wain E, et al. Evolution over a 10 year period of the epidemiological profile of 1,726 newly diagnosed HCV patients in Belgium. J Med Virol 2005;76:503–510.
[23] Ceci O, Margiotta M, Marello F, Francavilla R, Loizzi P, Francavilla A, et al. Vertical transmission of hepatitis C virus in a cohort of 2,447 HIV-seronegative pregnant women: a 24-month prospective study. J Pediatr Gastroenterol Nutr 2001;33:570–575.
[24] Dal Molin G, D'Agaro P, Ansaldi F, Ciana G, Fertz C, Alberico S, et al. Mother-to-infant transmission of hepatitis C virus: rate of infection and assessment of viral load and IgM anti HCV as risk factors. J Med Virol 2002;67:137–142.
[25] Del Corno G, Civardi E. Intrafamilial transmission of hepatitis B and C viruses in an Italian local health district. An Ig 2006;18:287–295.
[26] Minola E, Baldo V, Baldovin T, Trivello R, Floreani A. Intrafamilial transmission of hepatitis C virus infection. Eur J Epidemiol 2006;21:293–297.

[27] Mok J, Pembrey L, Tovo P-A, Newell M-L. When does mother to child transmission of hepatitis C virus occur? Arch Dis Child Fetal Neonatal Ed 2005;90:156–160.
[28] Guadagnino V, Stroffolini T, Rapicetta M, Costatino A, Kondili LA, Menniti-Ippolito F, et al. Prevalence, risk factors, and genotype distribution of hepatitis C virus infection in the general population: A community-based survey in Southern Italy. Hepatology 1997;26:106–111.
[29] Osella AR, Misciagna G, Leone A, Di Leo A, Fiore G. Epidemiology of hepatitis C virus infection in an area of Southern Italy. J Hepatol 1997;27:30–35.
[30] European pediatric hepatitis C virus network. A significant sex – but not elective cesarean section – effect on mother-to-child transmission of hepatitis C virus infection. J Infect Dis 2005;192:1972–1979.
[31] Resti M, Bortolotti F, Vajro P, Maggiore G. Committee of Hepatology of the Italian Society of Pediatric Gastroenterology and Hepatology. Dig Liver Dis 2003;35:453–457.
[32] American Academy of Pediatrics. Hepatitis C virus infection. 1998;101:481–485.
[33] Jhaveri R, Grant W, Kauf T, McHutchison J. The burden of hepatitis C virus infection in children: estimated direct medical costs over a 10-year period. J Pediatr 2006;148: 353–358.
[34] Bortolotti F, Guido M, Zancan L, Gussetti N. Long-term outcome of hepatitis C in children. Hepatology 2004;39:1455.
[35] Consensus Conference: Lo screening per l'infezione da virus dell'epatite C negli adulti in Italia. Rome: Istituto Superiore di Sanità (ISS);2005.
[36] Centers for disease control and prevention. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease: MMWR Recomm Rep 1998;47(RR-19):1–39.
[37] Donati S, Grandolfo ME, Andreozzi S. Do Italian mothers prefer cesarean delivery? Birth 2003;30:89–93.
[38] Quintero Romero S, Bernal R, Barbiero C, Passamonte R, Cattaneo A. A rapid ethnographic study of brestfeeding in the North and South of Italy. International Brestfeeding Journal 2006;1:14–25.
[39] Data from the Italian National Institute of Statistics (ISTAT). 2005. Available from: www.istat.it.

ticipation in physical activity by those patients with reduced exercise capacity does not necessarily require "strict monitoring ensured 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 submaximal exertion. Requiring cardiologist 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 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 meaningful 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.[4,5]

Indeed, reduced lean mass, small cross-sectional muscle fiber area, and increased triglyceride deposition in skeletal muscle in conjunction with dysfunctional/reduced lipolysis can result in reduced cardiorespiratory 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 the evidence for exercise training as a therapy for NAFLD and also presents the principles for prescribing exercise as a therapeutic intervention. Future studies will endeavor to better determine the relative contributions of IR, adiposity, and NAFLD to reduced fitness.

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References

1. Krasnoff JB, Painter PL, Wallace JP, Bass NM, Merriman RB. Health-related fitness and physical activity in patients with nonalcoholic fatty liver disease. HEPATOLOGY 2008;47:1158-1166.
2. Nobili V, Manco M, Raponi M, Marcellini M. Case management in children affected by non-alcoholic fatty liver disease. J Paediatr Child Health 2007;43:414.
3. Nobili V, Marcellini M, Devito R, Ciampalini P, Piemonte F, Comparcola D, et al. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. HEPATOLOGY 2006;44:458-465.
4. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, et al. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 1999;107:450-455.
5. Marchesini G, Marzocchi R. Metabolic syndrome and NASH. Clin Liver Dis 2007;11:105-117, ix.

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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-naïve children with chronic hepatitis C (CHC) were investigated.[1] The authors reported that inflammation was minimal in 42% 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 inflammation that did not reach the statistical significance ($P = 0.058$). In addition, no significant correlation was found between duration of infection and fibrosis. Nevertheless, 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[2] was not considered. Our study 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 (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 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.[3,4] In another study regarding adults infected as young military recruits, the frequency of cirrhosis after 50 years was only 6%.[5] No linear correlation was found between duration and severity of disease.[5] Furthermore, it seems that higher degrees of fibrosis were related with age at infection being more frequent in patients ≥65 years regardless the duration of infection.[6] Finally, it has been shown that the majority of fibrosis progression occurred in patients aged 50 years or older.[7]

In conclusion, on the basis of the majority of studies concerning the long-term outcome of HCV infection in children[1,2] and adults,[3-7] we think that the conclusive sentence 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

1. Goodman ZD, Makhlouf HR, Liu L, Balistreri W, Gonzalez-Peralta RP, Haber B, et al. Pathology of chronic hepatitis C in children: liver biopsy findings in the Peds-C Trial. HEPATOLOGY 2008;47:836-843.
2. Iorio R, Giannattasio A, Sepe A, Terracciano LM, Vecchione R, Vegnente A. Chronic hepatitis C in childhood: an 18-year experience. Clin Infect Dis 2005;41:1431-1437.
3. Alter HJ. HCV natural history: the retrospective and prospective in perspective. J Hepatol 2005;43:550-552.
4. Seeff LB, Hoofnagle JH. National Institutes of Health Consensus Development Conference: management of hepatitis C: 2002. HEPATOLOGY 2002;36(5 Suppl 1):S1-S2.
5. Seeff LB, Miller RN, Rabkin CS, Buskell-Bales Z, Straley-Eason KD, Smoak BL, et al. 45-year follow-up of hepatitis C virus infection in healthy young adults. Ann Intern Med 2000;132:105-111.
6. Thabut D, Le Calvez S, Thibault V, Massard J, Munteanu M, Di Martino V, et al. Hepatitis C in 6,865 patients 65 yr or older: a severe and neglected curable disease? Am J Gastroenterol 2006;101:1260-1267.
7. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis c. J Hepatol 2001;34:730-739.

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Reply:

We thank Iorio et al. for their interest in our work and for making us aware of their study,[1] which included some information on liver biopsies in 64 children. Their findings support those of our and other series, namely that chronic hepatitis C in children is generally histologically mild but may occasionally result in advanced fibrosis and cirrhosis. By design, our own study excluded children with decompensated cirrhosis, so if anything, our collection of liver biopsies could have been biased toward more mild disease. None of the patients in the study by Iorio et al. initially had cirrhosis, but it is noteworthy that three children (5.2%) in that series, ages 2.1, 2.7, and 13.6 years, already had Ishak stage 4 fibrosis, which is defined as marked bridging fibrosis.[2] Furthermore, one child in that series, who was initially stage 2, progressed to stage 5 (incomplete 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 acquired in childhood.[3,4] All observers agree that a minority of patients with hepatitis C will progress to cirrhosis and its complications, and 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

1. Iorio R, Giannattasio A, Sepe A, Terracciano LM, Vecchione R, Vegnente A. Chronic hepatitis C in childhood: an 18-year experience. Clin Infect Dis 2005;41:1431-1437.
2. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-699.
3. Strickland DK, Jenkins JJ, Hudson MM. Hepatitis C infection and hepatocellular carcinoma after treatment of childhood cancer. J Pediatr Hematol Oncol 2001;23:527-529.
4. Gonzalez-Peralta RP, Langham ML, Andres JM, Colombani P, Mohan P, Schwarz K. Hepatocellular carcinoma in two adolescents with hepatitis C virus infection. J Pediatr Gastroenterol Nutr. In press.

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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 oh 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 hepatotopic 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 herpatitis 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 macrovescicular 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

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

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

1. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006;43: S99-S112.
2. Marion AW, Baker AJ, Dhawan A. Fatty liver disease in children. Arch Dis Child 2004;89:648-52.
3. Roberts EA. Pediatric nonalcoholic fatty liver disease (NAFLD): a "growing" problem? J Hepatol 2007;46:1133-42.
4. Thomopoulos KC, Arvaniti V, Tsamantas AC, et al. Prevalence of liver steatosis in patients with chronic hepatitis B: a study of associated factors and of relationship with fibrosis. Eur J Gastroenterol Hepatol 2006;18: 233-237.
5. Bondini S, Kallman J, Wheeler A, et al. Impact of non-alcoholic fatty liver disease on chronic hepatitis B. Liver Int 2007;27:607-11.
6. Hwang SJ, Luo JC, Chu CW, et al. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. J Gastroenterol Hepatol 2001;16:190-95.
7. Gordon A, McLean CA, Pedersen JS, et al. Hepatic steatosis in chronic hepatitis B and C: predictors, distribution and effect on fibrosis. J Hepatol 2005;43:38-44.
8. Adinolfi LE, Gambardella M, Andreana A, et al. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001;33:1358-64.
9. Poynard T, Ratziu V, McHutchison J, et al. Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. Hepatology 2003;38:75-85.
10. Patton HM, Patel K, Behling C, et al. The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients. J Hepatol 2004;40:484-90.
11. Castera L, Chouteau P, Hezode C, et al. Hepatitis C virus induced hepatocellular steatosis. Am J Gastroenterol 2005;100:711-15.
12. Hourigan LF, Macdonald GA, Purdie D, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology 1999;29:1215-19.
13. Castéra L, Hézode C, Roudot-Thoraval F, et al. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. Gut 2003;52:288-92.
14. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States Adv Data 2000;314: 1-27.

http://www.cdc.gov/nccdphp/dnpa/growthcharts/sas.htm

15. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-19.
16. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-99.
17. Brunt EM, Janney CG, Di Bisceglie AM, et al. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467-74.
18. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-21.
19. Keasler VV, Lerat H, Madden CR, et al. Increased liver pathology in hepatitis C virus transgenic mice expressing the hepatitis B virus X protein. Virology 2006;347:466-75.
20. Kumar D, Farrell GC, Fung C, et al. Hepatitis C virus genotype 3 is cytopathic to hepatocytes: Reversal of hepatic steatosis after sustained therapeutic response. Hepatology 2002;36:1266-72.
21. Muzzi A, Leandro G, Rubbia-Brandt L, et al. Insulin resistance is associated with liver fibrosis in non-diabetic chronic hepatitis C patients. J Hepatol 2005;42:41-46.
22. Akuta N, Suzuki F, Tsubota A, et al. Efficacy of interferon monotherapy to 394 consecutive naive cases infected with hepatitis C virus genotype 2a in Japan: therapy efficacy as consequence of tripartite interaction of viral, host and interferon treatment-related factors. J Hepatol 2002;37:831-36.

2.6 Publications

1. **Giannattasio A**, Spagnuolo MI, Sepe A, Valerio G, Vecchione R, Vegnente A, Iorio R. Is HCV infection associated with liver steatosis also in children? J Hepatol 2006;45:350-54.

2. **Giannattasio A**, Cirillo F, Terlizzi V, Liccardo D, Vecchione R, Iorio R. Hepatic steatosis is uncommon in children with chronic hepatitis B. J Clin Virol 2009;46:360-62.

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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.**

***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 hyperlipidaemia) in genotype 1-infected patients, while it is principally 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

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Abbreviations: CHC, chronic hepatitis C; HCV, hepatitis C virus; BMI, body mass index; IFN, interferon; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; HOMA, homeostasis model assessment.

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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 Pediatrics, 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/m^2, were evaluated at the time of liver biopsy. Overweight was defined by BMI $\geqslant$ 90th percentile for age and obesity by BMI $\geqslant$ 95th percentile for age, according to CDC standards [13]. Laboratory data of all children, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (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 frozen serum samples. 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 [14].

2.3. Histological evaluation

Liver specimens were formalin-fixed and paraffin embedded for histological evaluation. Liver histology was evaluated according to Ishak scoring (necroinflammation 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 χ^2-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 macrovescicular and located in the periportal area rather than in the centrilobular area. Histopathologic 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

Table 1
Clinical, laboratory and histological features in 64 children with CHC according to presence of steatosis at liver biopsy

	Absence of steatosis ($n = 48$)	Presence of steatosis ($n = 16$)	P value
Male/female	22/26	7/9	N.S.
Route of HCV infection, *n* (%)			
Blood transfusion	23 (47.9)	7 (43.7)	N.S.
Vertical transmission	15 (31.2)	6 (37.5)	N.S.
Minor surgery	7 (14.6)	1 (6.3)	N.S.
Unknown	3 (6.3)	2 (12.5)	N.S.
HCV genotype			
1, *n* (%)	29 (60.4)	10 (62.6)	N.S.
2, *n* (%)	12 (25)	2 (12.5)	N.S.
3, *n* (%)	2 (4.2)	1 (6.2)	N.S.
Others, *n* (%)	5 (10.4)	3 (18.7)	N.S.
Median age at liver biopsy (years) (range)	8.3 (2.5–14)	7.6 (2.7–13.6)	N.S.
Median duration of HCV infection before liver biopsy (years) (range)	6.8 (2–14)	7.2 (2.5–11.3)	N.S.
Mean relative BMI (%) ± SD	106.2 ± 15.3	112.7 ± 15.4	N.S.
Median ALT values (IU/l) (range)	95 (40–350)	117 (50–300)	N.S.
Median HCV RNA serum levels (IU/ml) (range)	218,000 (40,000–1,467,000)	220,000 (40,200–587,000)	N.S.
Median GGT levels (U/l) (range)	17 (8–79)	22 (7–62)	N.S.
Median cholesterol levels (mg/dl) (range)	150 (93–231)	146.5 (116–201)	N.S.
Median triglycerides levels (mg/dl) (range)	69.5 (39–132)	62.5 (35–99)	N.S.
Median fasting glucose (mmol/l) (range)	4.7 (3.1–5.4)	4.8 (4–5.4)	N.S.
Median insulin (mU/ml) (range)	9.0 (2.1–22)	10.15 (4.8–22)	N.S.
HOMA	2.1 (0.5–5.2)	2.2 (1.0–4.8)	N.S.
Histology			
Median necroinflammation score (range)	4 (2–8)	4 (2–7)	N.S.
Median fibrosis score (range)	2 (0–4)	2 (1–4)	N.S.
Median period of observation (years) (range) in patients treated with interferon	7.9 (3–14.2)	7.9 (5.5–9.4)	N.S.

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 (23%) 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

[1] Hwang SJ, Luo JC, Chu CW, Lai CR, Lu CL, Tsay SH, et al. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. J Gastroenterol Hepatol 2001;16:190–195.
[2] Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology 1999;29:1215–1219.
[3] Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001;33:1358–1364.
[4] Kumar D, Farrell GC, Fung C, George J. Hepatitis C virus genotype 3 is cytopathic to hepatocytes: reversal of hepatic

steatosis after sustained therapeutic response. Hepatology 2002;36:1266–1272.
[5] Hezode C, Roudot-Thoraval F, Zafrani ES, Dhumeaux D, Pawlotsky JM. Different mechanisms of steatosis in hepatitis C virus genotypes 1 and 3 infections. J Viral Hepat 2004;11:455–458.
[6] Rubbia-Brandt L, Quadri R, Abid K, Giostra E, Male PJ, Mentha G, et al. Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. J Hepatol 2000;33:106–115.
[7] Castera L, Chouteau P, Hezode C, Zafrani ES, Dhumeaux D, Pawlotsky JM. Hepatitis C virus-induced hepatocellular steatosis. Am J Gastroenterol 2005;100:711–715.
[8] Poynard T, Ratziu V, McHutchison J, Manns M, Goodman Z, Zeuzem S, et al. Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. Hepatology 2003;38:75–85.
[9] Patton HM, Patel K, Behling C, Bylund D, Blatt LM, Vallee M, et al. The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients. J Hepatol 2004;40:484–490.
[10] Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, et al. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression. Gastroenterology 2003;125:1695–1704.
[11] Ratziu V, Munteanu M, Charlotte F, Bonyhay L, Poynard T, LIDO Study Group. Fibrogenic impact of high serum glucose in chronic hepatitis C. J Hepatol 2003;39:1049–1055.
[12] Castera L, Hezode C, Roudot-Thoraval F, Bastie A, Zafrani ES, Pawlotsky JM, et al. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. Gut 2003;52:288–292.
[13] Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. Adv Data 2000;314:1–27 (http://www.cdc.gov/nccdphp/dnpa/growthcharts/sas.htm/).
[14] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–419.
[15] Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–699.
[16] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
[17] Mihm S, Fayyazi A, Hartmann H, Ramadori G. Analysis of histopathological manifestations of chronic hepatitis C virus infection with respect to virus genotype. Hepatology 1997;25:735–759.
[18] Westin J, Nordlinder H, Lagging M, Norkrans G, Wejstal R. Steatosis accelerates fibrosis development over time in hepatitis C virus genotype 3 infected patients. J Hepatol 2002;37:837–842.
[19] Rubbia-Brandt L, Fabris P, Paganin S, Leandro G, Male PJ, Giostra E, et al. Steatosis affects chronic hepatitis C progression in a genotype specific way. Gut 2004;53:406–412.
[20] Muzzi A, Leandro G, Rubbia-Brandt L, James R, Keiser O, Malinverni R, et al. Insulin resistance is associated with liver fibrosis in non-diabetic chronic hepatitis C patients. J Hepatol 2005;42:41–46.
[21] Barba G, Harper F, Harada T, Kohara M, Goulinet S, Matsuura Y, et al. Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Natl Acad Sci USA 1997;94:1200–1205.
[22] Lerat H, Honda M, Beard MR, Loesch K, Sun J, Yang Y, et al. Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. Gastroenterology 2002;122:352–365.
[23] Akuta N, Suzuki F, Tsubota A, Suzuki Y, Someya T, Kobayashi M, et al. Efficacy of interferon monotherapy to 394 consecutive naive cases infected with hepatitis C virus genotype 2a in Japan: therapy efficacy as consequence of tripartite interaction of viral, host and interferon treatment-related factors. J Hepatol 2002;37:831–836.

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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 2 (4%) 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.

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.[1] As for the role of chronic viral hepatitis, the relationship between chronic hepatitis C virus infection and liver steatosis it is well documented.[2–5] In hepatitis C virus (HCV)-infected children the prevalence of histological evidence of hepatic steatosis accounts for 25–27%.[3,4] Liver steatosis impacts on the degree of inflammation and fibrosis and may influence the long-term prognosis of chronic hepatits 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 histology in adults with CHB ranges from 18% to 76%[6,7]; it seems especially correlated with metabolic parameters (such as fasting glucose and overweight), while no correlation has been found with HBV DNA titre and HBeAg status.[6,7] 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 paediatric patients have not so many co-factors responsible for steatosis such as alcohol intake, dyslipidaemia, 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 anthropometrical, 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 Paediatric 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 (HBsAg) 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, dyslipidaemia, thyroid diseases)

Abbreviations: HCV, hepatitis C virus; CHC, chronic hepatitis C; CHB, chronic hepatitis B; HBsAg, hepatitis B surface antigen; BMI, body mass index; AST, aspartate aminotranferase; ALT, alanine aminotranferase; GGT, gamma-glutamyl transpeptidase.

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Table 1
Demographic, anthropometric, biochemical, virological and histological features at liver biopsy in 56 children with chronic hepatitis B.

	All patients (N = 56)	Patients with steatosis (N = 2)	Patients without steatosis (N = 54)
Males (%)	38 (68%)	2 (100%)	36 (67%)
Median age in years (range)	8.1 (2.2–17.3)	9.3 (5.6–13)	8 (2.2–17.3)
Route of infection (%)			
Vertical transmission	29 (52%)	2 (100%)	27 (50%)
Horizontal transmission	7 (12%)	0	7 (13%)
Unknown	20 (36%)	0	20 (37%)
Relative BMI (±SD)	110 ± 15.7	132 ± 9	109 ± 15.3
Median fasting glucose in mg/dl (range)	85 (69–110)	85.5 (78–93)	85 (69–110)
Median triglycerides levels in mg/dl (range)	72 (29–195)	95 (68–123)	72 (29–195)
Median cholesterol levels in mg/dl (range)	151 (101–219)	192 (174–210)	151 (101–219)
Median ALT values in IU/l (range)	97 (21–488)	133 (102–164)	93 (21–488)
Median GGT value in U/l (range)	14.5 (8–49)	20 (14–26)	14.5 (8–49)
Median serum HBV DNA in copies/ml (range)[a]	104,710 (330–854,660)	231,494	101,880 (330–854,660)
HBeAg positivity[b]	48 (91%)	2 (100%)	46 (93.8%)
Histology			
Median necroinflammation score (range)	5 (0–9)	4 in both cases	5 (0–9)
Median fibrosis score (range)	2 (0–5)	1 in both cases	2 (0–5)

[a] One patient with steatosis and one patient without steatosis had undetectable HBV DNA in serum at the time of liver biopsy.
[b] In 5 cases HBeAg status was unknown.

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/m^2, 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 a BMI > 95th percentile for age, according to CDC standards.[8] The relative BMI was calculated and expressed as percentage of individual BMI/median BMI value for age and sex, according to age reference values.[9] 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 plasma glucose, cholesterol and triglycerides serum levels, viral markers (HBsAg/HBsAb, HBeAg/HBeAb, 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 (Amplicor HBV-Monitor kit, Roche Diagnostic System, NJ, USA). Values obtained with different HBV DNA quantification assays were compared (1 pg HBV DNA = 283,000 copies).[11]

Histological analysis of the liver sections was performed by an experienced pathologist (R.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.[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 3 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%; CI 0–8) children (grade 1 in both cases). In both cases, steatosis was macrovescicular and located in the periportal 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 (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, 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%) 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 CHB[3,4,6,7] 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).[7] 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.[6,7] 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 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 can not be explained with HBV infection and should be carefully investigated to rule out the multiple causes of paediatric fatty liver.

References

1. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;**118**:1388–93.
2. Hwang SJ, Luo JC, Chu CW, Lai CR, Lu CL, Tsay SH, et al. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. *J Gastroenterol Hepatol* 2001;**16**:190–5.
3. Giannattasio A, Spagnuolo MI, Sepe A, Valerio G, Vecchione R, Vegnente A, et al. Is HCV infection associated with liver steatosis also in children? *J Hepatol* 2006;**45**:350–4.
4. Guido M, Bortolotti F, Jara P, Giacomelli L, Fassan M, Hierro L, et al. Liver steatosis in children with chronic hepatitis C. *Am J Gastroenterol* 2006;**101**:2616–8.
5. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001;**33**:1358–64.
6. Thomopoulos KC, Arvaniti V, Tsamantas AC, Dimitropoulou D, Gogos CA, Siagris D, et al. Prevalence of liver steatosis in patients with chronic hepatitis B: a study of associated factors and of relationship with fibrosis. *Eur J Gastroenterol Hepatol* 2006;**18**:233–7.
7. Gordon A, McLean CA, Pedersen JS, Bailey MJ, Roberts SK. Hepatic steatosis in chronic hepatitis B and C: predictors, distribution and effect on fibrosis. *J Hepatol* 2005;**43**:38–44.
8. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. *Adv Data* 2000;**8**:1–27.
9. Poskitt EM. Defining childhood obesity: the relative body mass index (BMI). European Childhood Obesity group. *Acta Paediatr* 1995;**84**:961–3.
10. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 1976;**51**:170–9.
11. Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 2001;**34**:1225–41.
12. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;**22**:696–9.
13. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;**41**:1313–21.

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 wad 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

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

§ 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).

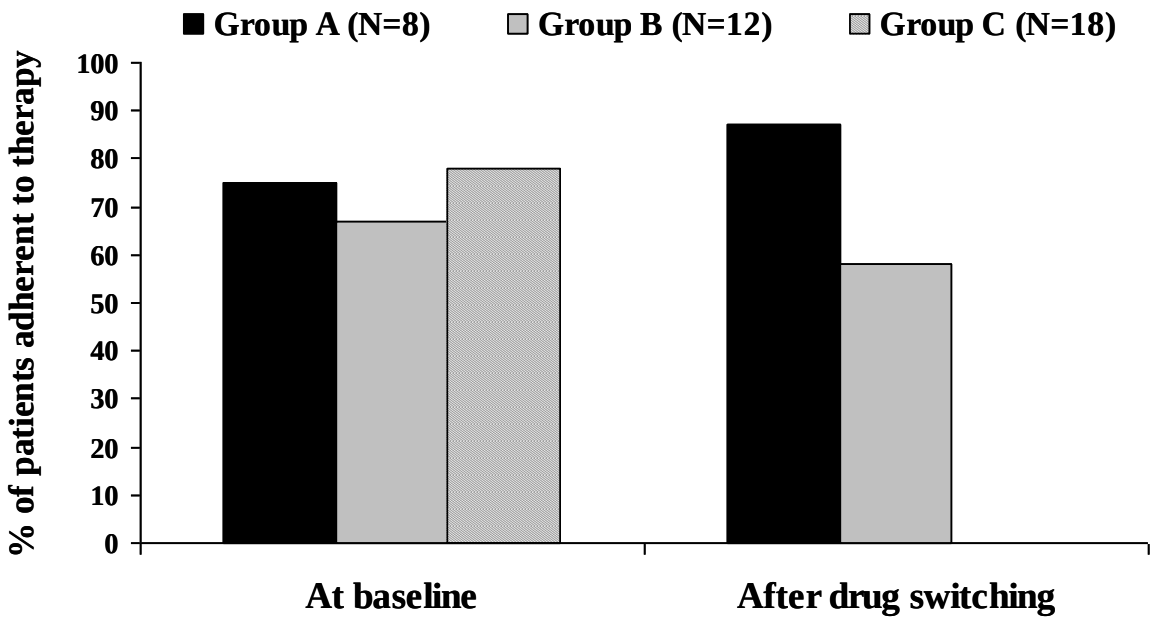

Figure 1. Percentage of patients adherent to therapy at baseline (for all groups) and after drug switching (for groups A and B)

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

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

§ 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 to11.55).

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.

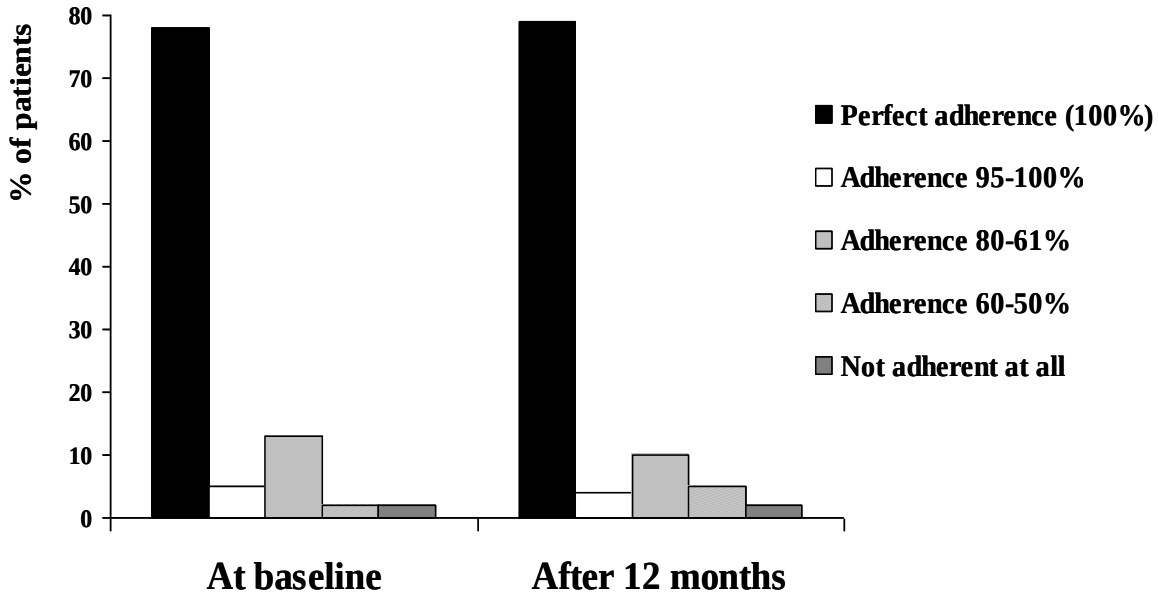

Figure 2. Rates of adherence in 112 HIV-infected children according to key informants

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.

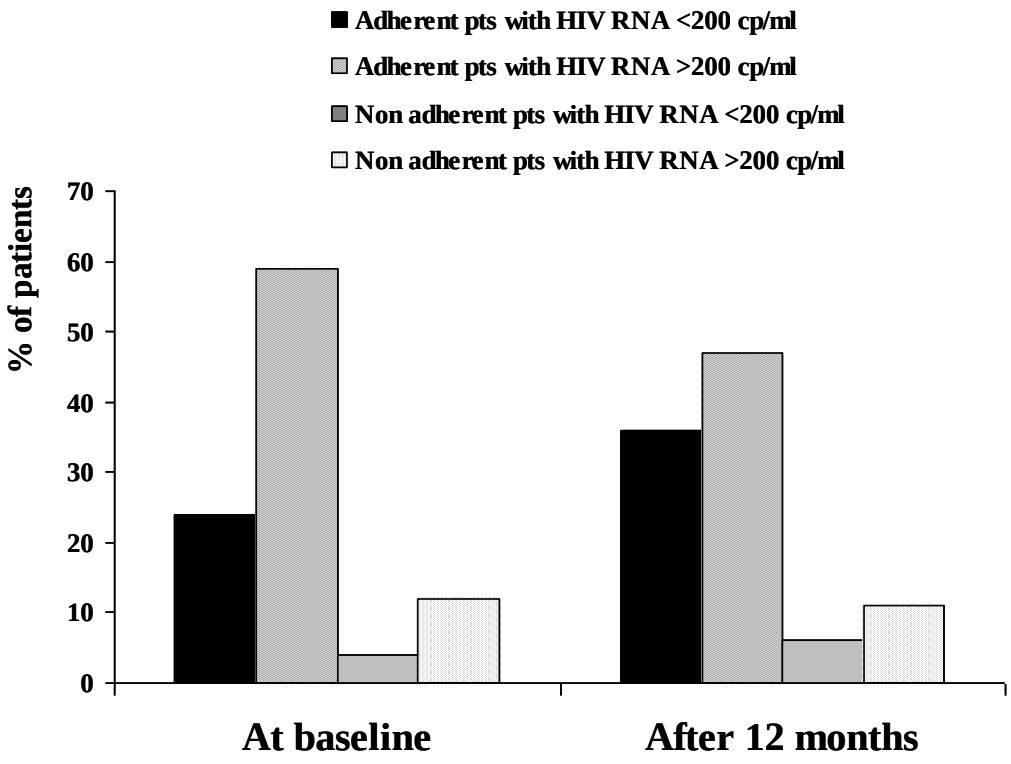

Figure 3. Rate of adherence in HIV-infected children with detectable or undetectable HIV RNA

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

No. of doses/day	At baseline			After 12 months		
	Adherent	***Non adherent***	***p***	***Adherent***	***Non adherent***	***p***
<6	44 (75%)	15 (25%)	0.01	32 (73%)	12 (27%)	0.03
≥6	49 (92%)	4 (8%)		61 (90%)	7 (10%)	

Data are shown as n (%)

Table 4. Adherence according to the type of caregiver

Type of caregiver	At baseline			After 12 months		
	Adherent	***Non adherent***	***p***	***Adherent***	***Non adherent***	***p***
Biological parents	61 (78%)	17 (22%)	0.05	49 (79%)	13 (21%)	
Foster parents	32 (93%)	2 (7%)		31 (97%)	1 (3%)	0.02
Self-administered	0	0		13 (72%)	5 (28%)	

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 complaint 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

1. Gortmaker SL, Hughes M, Cervia J, et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 2001;345:1522-28.
2. Murphy EL, Collier AC, Kalish LA, et al. Highly active antiretroviral therapy decreases mortality and morbidity in patients with advanced HIV disease. Ann Intern Med 2001;135:17-26.
3. Sullivan JL, Luzuriaga K. The changing face of pediatric HIV-1 infection. N Engl J Med 2001;345:1568-9.
4. Melvin AJ, Frenkel LM. Pediatric human immunodeficiency virus type 1 infection: updates on prevention and management. AIDS Clin Rev 2000-2001:63-83.
5. Chesney MA. Factors affecting adherence to antiretroviral therapy. Clin Infect Dis 2000;30:S171-6.
6. Steele RG, Grauer D. Adherence to antiretroviral therapy for pediatric HIV infection: review of the literature and recommendations for research. Clin Child Fam Psychol Rev 2003;6:17-30.
7. Friedland GH, Williams A. Attaining higher goals in HIV treatment: the central importance of adherence. AIDS 1999;13:S61-72.
8. Paterson DL, Swindells S, Mohr J, et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Ann Intern Med 2000;133:21-30.
9. Van Dyke RB, Lee S, Johnson GM, et al. Reported adherence as a determinant of response to highly active antiretroviral therapy in children who have human immunodeficiency virus infection. Pediatrics 2002;109:1-7.
10. Goode M, McMaugh A, Crisp J, et al. Adherence issues in children and adolescents receiving highly active antiretroviral therapy. AIDS Care 2003;15:403-8.
11. Pontali E, Feasi M, Toscanini F, et al. Adherence to combination antiretroviral treatment in children. HIV Clin Trials 2001;2:466-73.
12. Murphy DA, Wilson CM, Durako SJ, et al. Antiretroviral medication adherence among the REACH HIVinfected adolescent cohort in the USA. AIDS Care 2001;13:27-40.
13. Mellins CA, Brackis-Cott E, Dolezal C, et al. The role of psychosocial and family factors in adherence to antiretroviral treatment in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2004;23:1035-41.

14. Giacomet V, Albano F, Starace F, et al. Adherence to antiretroviral therapy and its determinants in children with human immunodeficiency virus infection: a multicentre, national study. Acta Paediatr 2003;92:1398-02.
15. Williams PL, Storm D, Montepiedra G, et al. Predictors of adherence to antiretroviral medications in children and adolescents with HIV infection. Pediatrics 2006;118:e1745-57.
16. Chesney MA, Morin M, Sherr L. Adherence to HIV combination therapy. Soc Sci Med 2000;50:1599-605.
17. Guidelines for the use of antiretroviral agents in pediatric HIV infection. http://AIDSinfo.nih.gov
18. Italian Register for HIV Infection in Children. Italian guidelines for antiretroviral therapy in children with human immunodeficiency virus-type 1 infection. Acta Paediatr 1999; 88: 228-32.
19. Chiappini E, Galli L, Tovo PA, et al. Virologic, immunologic, and clinical benefits from early combined antiretroviral therapy in infants with perinatal HIV-1 infection. AIDS 2006;20:207-15.
20. Chiappini E, Galli L, Gabiano C, et al. Early triple therapy vs mono or dual therapy for children with perinatal HIV infection. JAMA 2006;295:626-28.
21. Chiappini E, Galli L, Tovo PA, et al. Changing patterns of clinical events in perinatally HIV-1-infected children during the era of HAART. AIDS 2007;21:1607-15.
22. Sherr L, Lampe F, Norwood S, et al. Successive switching of antiretroviral therapy is associated with high psychological and physical burden. Int J STD AIDS 2007;18:700-04.
23. Albano F, Giacomet V, De Marco G, et al. Adherence to antiretroviral therapy in children: a comparative evaluation of caregiver reports and physician judgment. AIDS Care 2007;19:764-66.
24. Merzel C, Vandevanter N, Irvine M. Adherence to antiretroviral therapy among older children and adolescents with HIV: a qualitative study of psychosocial contexts. AIDS Patient Care STDS 2008;22:977-87.
25. Martini M, Nasta P, Ricci E, et al. Perceptions of disease and therapy are factors influencing adherence to antiretroviral therapy. Sex Transm Infect 2000;76:496-97.
26. Wrubel J, Moskowitz JT, Richards TA, et al. Pediatric adherence: perspectives of mothers of children with HIV. Soc Sci Med 2005;61:2423-33.

27. Billups SJ, Malone DC, Carter BL. The relationship between drug therapy non compliance and patient characteristics, health-related quality of life, and health care costs. Pharmacotherapy 2000;20:941-49.
28. Sharkness CM, Snow DA. The patient's view of hypertension and compliance. Am J Prev Med 1992;8:141-46.
29. Wagner GJ, Rabkin JG. Measuring medication adherence: are missed doses reported more accurately then perfect adherence? AIDS Care 2000;12:405-08.
30. Miller LG, Hays RD. Adherence to combination antiretroviral therapy: synthesis of the literature and clinical implications. AIDS Read 2000;10:177-85.
31. Shalansky SJ, Levy AR. Effect of number of medications on cardiovascular therapy adherence. Ann Pharmacother 2002;36:1532-39.

3.3 Publications

1. **Giannattasio A**, Barbarino A, Lo Vecchio A, Bruzzese E, Mango C, Guarino A.
   Effects of antiretroviral drug recall on perception of therapy benefits and on adherence to antiretroviral treatment in HIV-infected children.
   Current HIV Research 2009;7:468-72.

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)

3. Galli L, Puliti D, Chiappini E, and the Italian Register for HIV Infection in Children.
   Is the interruption of antiretroviral treatment during pregnancy an additional major risk factor for mother-to-child transmission of HIV type 1?.
   Clin Infect Dis 2009;48:1310-17.

Effects of Antiretroviral Drug Recall on Perception of Therapy Benefits and on Adherence to Antiretroviral Treatment in HIV-Infected Children

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Abstract: In June 2007, the European Medicines Agency announced the recall by Roche of nelfinavir 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 nelfinavir 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 nelfinavir 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 foster parents than 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 nelfinavir 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, non-adherence 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].

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Therefore, an array of heterogeneous conditions affect treatment adherence in AIDS. 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 [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 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. After this unpredictable event, we decided to investigate the effect of switching therapies because of nelfinavir 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

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; $p<0.0001$), 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

	Group A (N = 8)	Group B (N = 12)	Group C (N = 18)	Total (N = 38)
Males/females	3/5	6/6	4/14	13/25
Mean age in years ± SD	11.2 ± 3.1	12.6 ± 3.9	10.4 ± 4.4	12.1 ± 6.7
Caregiver:*				
Biological parents	7 (87%)	4 (33%)	10 (56%)	21 (55%)
Relatives	0	3 (25%)	4 (22%)	7 (19%)
Foster parents	1 (13%)	5 (42%)	4 (22%)	10 (26%)
Caregiver school education:§				
< 8 years	7 (87.5%)	5 (45%)	11 (65%)	22 (61%)
> 8 years	1 (12.5%)	6 (55%)	6 (95%)	14 (39%)
Mean value of CD4+ cells in cells/mm^3 ± SD	827 ± 232	795 ± 335	940 ± 511	870 ± 410
Mean value of HIV RNA in copies/ml in patients with detectable HIV-RNA ± SD	16495 ± 27806	33672 ± 42253	4912 ± 7784	21774 ± 33572
Number of patients with viral load less than 40 copies/ml (%)	5 (62%)	5 (42%)	14 (78%)	24 (63%)

SD = standard deviation.

Differences between the groups was assessed by the chi square test and Fisher's exact test for dichotomous variables (e.g. sex). ANOVA test was used to compare continuous variables.

*Primary caregivers of group A were more frequent 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 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 **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±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; $p>0.05$).

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 38 HIV-Infected Children

	Group A (N = 8)	Group B (N = 12)	Group C (N = 18)	Total (N = 38)
How do you consider health status of your child?				
- Good	6 (75%)	10 (83%)	16 (89%)	32 (84%)
- Poor	2 (25%)	2 (17%)	2 (11%)	6 (16%)
Do you considered HAART necessary and effective?				
- Yes	8 (100%)	12 (100%)	18 (100%)	38 (100%)
- No	0	0	0	0
*Do you trust therapy?§ **				
- Yes	8 (100%)	9 (75%)	18 (100%)	35 (92%)
- No	0	3 (25%)	0	3 (8%)
If no, why? §				
- Side effects of drugs	-	1 (33%)	-	1 (33%)
- Long-term efficacy	-	2 (67%)	-	2 (67%)
- Other reasons	-	0	-	0
Was it a problem to take the therapy? §				
- Never a problem	2 (25%)	4 (33%)	7 (39%)	13 (34%)
- Hardly ever a problem	5 (62%)	5 (42%)	8 (44%)	18 (47%)
- Frequent problem	1 (13%)	3 (25%)	3 (17%)	7 (19%)
If it was a frequent problem, what was the reason? §				
- Formulation of drugs (taste, pill size…)	1 (100%)	1 (33%)	3 (100%)	5 (72%)
- Schedule of therapy (meals, sleep, school…)	0	1 (33%)	0	1 (14%)
- Side effects	0	0	0	0
- Social context	0	1 (34%)	0	1 (14%)
Did your child comply with the drug timing? §				
- Never	0	0	0	0
- Sometimes	3 (37%)	5 (42%)	2 (12%)	10 (26%)
- Often	2 (25%)	3 (25%)	8 (44%)	13 (35%)
- Always	3 (38%)	4 (33%)	8 (4%)	15 (39%)
Number of patients adherent to therapy (%) §	6 (75%)	8 (67%)	14 (78%)	28 (74%)

§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 ($p = 0.03$).

Table 3. Reaction to Drug Shifting, Perception of Antiviral Treatment and Adherence to Treatment in 20 HIV-Infected Children who Shifted Therapy

	Group A (N = 8)	Group B (N = 12)	Total (N = 20)
What was your feeling when your child needed to change therapy?			
- Reassurance	0	2 (17%)	2 (10%)
- Indifference	1 (12%)	0	1 (5%)
- Concern	6 (75%)	9 (75%)	15 (75%)
- Anxiety	1 (13%)	1 (8%)	2 (10%)
Why did you feel worried?			
- Side effects	2 (29%)	5 (50%)	7 (42%)
- Drug toxicity	2 (29%)	0	2 (11%)
- Inefficacy of the new therapy	3 (42%)	5 (50%)	8 (47%)
Do you trust new therapy?			
- Yes	7 (87.5%)	12 (100%)	19 (95%)
- No	1 (12.5%)	0	1 (5%)
If no, why?			
- Side effects	1 (100%)	-	1 (100%)
- Long-term efficacy	0	-	0
- Other reasons	0	-	0
Number of patients adherent to the new therapy (%) §	7 (87%)	7 (58%)	14 (70%)

§Adherence to the new treatment was evaluated during the 4th 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 26 patients who received drugs from a caregiver, although the difference was not statistically significant (percentage of adherent patients 83% *vs* 64%, respectively: $p>0.05$).

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 changes 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 nelfinavir recall, 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 [19]. 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.

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ABBREVIATIONS

HAART = Highly active antiretroviral therapy

EMEA = European Medicines Agency

SD = Standard Deviation

REFERENCES

[1] Rotheram-Borus MJ, Flannery D, Rice E, *et al.* Families living with HIV. AIDS Care 2005; 17: 978-87.

[2] Laurence J. Adhering to antiretroviral therapies. AIDS Patient Care STDS 2001; 15: 107-8.

[3] Friedland GH, Williams A. Attaining higher goals in HIV treatment: the central importance of adherence. AIDS 1999; 13: S61-72.

[4] McNabb J, Ross JW, Abriola K, Turley C, Nightingale CH, Nicolau DP. Adherence to highly active antiretroviral therapy predicts virologic outcome at an inner-city human immunodeficiency virus clinic. Clin Infect Dis 2001; 33: 700-5.

[5] Paterson DL, Swindells S, Mohr J, *et al.* Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Ann Intern Med 2000; 133: 21-30.

[6] Carrieri P, Cailleton V, Le Moing V, *et al.* The dynamic of adherence to highly active antiretroviral therapy: results from the French National APROCO cohort. J Acquir Immune Defic Syndr 2001; 28: 232-9.

[7] Gibb DM, Goodall RL, Giacomet V, McGee L, Compagnucci A, Lyall H. Adherence to prescribed antiretroviral therapy in human immunodeficiency virus-infected children in the PENTA 5 trial. Pediatr Infect Dis J 2003; 22: 56-62.

[8] Mellins CA, Brackis-Cott E, Dolezal C, Abrams EJ. The role of psychosocial and family factors in adherence to antiretroviral treatment in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2004; 23: 1035-41.

[9] Giacomet V, Albano F, Starace F, *et al.* Adherence to antiretroviral therapy and its determinants in children with human immunodeficiency virus infection: a multicentre, national study. Acta Paediatr 2003; 92: 1398-402.

[10] Singh N, Squier C, Sivek C, Wagener M, Nguyen MH, Yu VL. Determinants of compliance with antiretroviral therapy in patients with human immunodeficiency virus: prospective assessment with implications for enhancing compliance. AIDS Care 1996; 8: 261-9.

[11] Sherr L, Lampe F, Norwood S, *et al.* Successive switching of antiretroviral therapy is associated with high psychological and physical burden. Int J STD AIDS 2007; 18: 700-4.

[12] Chesney MA, Morin M, Sherr L. Adherence to HIV combination therapy. Soc Sci Med 2000; 50: 1599-605.

[13] Italian Register for HIV Infection in Children. Italian guidelines for antiretroviral therapy in children with human immunodeficiency virus-type 1 infection. Acta Paediatr 1999; 88: 228-32.

[14] Chiappini E, Galli L, Tovo PA, *et al.* Virologic, immunologic, and clinical benefits from early combined antiretroviral therapy in infants with perinatal HIV-1 infection. AIDS 2006; 20: 207-15.

[15] Albano F, Giacomet V, De Marco G, *et al.* Adherence to antiretroviral therapy in children: a comparative evaluation of caregiver reports and physician judgement. AIDS Care 2007; 19: 764-6.

[16] Van Dyke RB, Lee S, Johnson GM, *et al.* Reported adherence as a determinant of response to highly active antiretroviral therapy in children who have human immunodeficiency virus infection. Pediatrics 2002; 109: e61.

[17] Campo RE, Narayanan S, Clay PG, *et al.* Factors influencing the acceptance of changes in antiretroviral therapy among HIV-1-infected patients. AIDS Patient Care STDS 2007; 21: 329-38.

[18] Altice FL, Mostashari F, Friedland GH. Trust and the acceptance of and adherence to antiretroviral therapy. J Acquir Immune Defic Syndr 2001; 28: 47-58.

[19] Martini M, Nasta P, Ricci E, Parazzini F, Agnoletto V. Perceptions of disease and therapy are factors influencing adherence to antiretroviral therapy. Sex Transm Infect 2000; 76: 496-7.

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Expert Opinion

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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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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 and nine 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

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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 [1-4]. Nearly perfect adherence (95%) is required to achieve and sustain viral suppression, to slow disease progression and to obtain optimal long-term benefits [5]. 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 metanalyses suggest that efforts to improve adherence to antiretroviral therapy among adults can be effective [6,7]. 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%)

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

Dose taken (% of total prescribed)	Baseline n (%)	After 12 months n (%)	Definition
100%	87 (78)	88 (79)	Adherent
> 95% to < 100%	6 (5)	5 (4)	
80%	14 (12)	8 (7)	Non-adherent
75%	1 (1)	3 (3)	
60%		2 (2)	
50%	2 (2)	4 (4)	
0	2 (2)	2 (2)	

of 129 children with HIV infection failed to adhere to treatment [8]. In addition, we found that psychosocial rather than demographic or clinical features were major determinants of adherence [8]. 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. Children grow and their behaviour changes. Consequently, we have investigated the changing pattern of adherence to HAART.

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 [9,10]. 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 paediatric HIV-infection as 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 standardized scripted dialogue was given to persons in charge of drug administration (caregivers). Data on sociodemographic 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 numeric 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 results 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 instrument is a reliable surrogate marker for monthly adherence and was used in the previous study [11,12]. 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 as 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 χ^2-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 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 'persistently non-adherent'. Conversely, nine 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 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.

Characteristics	Baseline				After 12 months			
	All	Adherent	Non-adherent	p	All	Adherent	Non-adherent	p
Clinical class								
N	4	4	0		2	2	0	
A	30	24	6		24	21	3	
B	38	31	7		37	32	5	
C	40	34	6		49	49	11	
$CD4^+$ T cells								
< 15%	21 (18.7)	17 (81.0)	4 (19.0)	NS	14 (12.5)	11 (78.6)	3 (21.4)	NS
> 15%	91 (81.3)	76 (83.5)	15 (16.5)		98 (87.5)	82 (83.7)	16 (16.3)	
Viral load:								
< 200 copies/ml	32 (28.6)	27 (84.4)	5 (15.6)	NS	47 (42.0)	40 (85.1)	7 (14.9)	NS
> 200 copies/ml	80 (71.4)	66 (82.5)	14 (17.5)		65 (58.0)	53 (81.5)	12 (18.5)	

Data are shown as n (%).

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

Antiretroviral regimen	Baseline				After 12 months			
	All	Adherent	Non-adherent	p	All	Adherent	Non-adherent	p
HAART	87 (78.0)	76 (87.3)	11 (12.6)	0.03	92	79 (85.9)	13 (14.1)	NS
No HAART	25 (22.0)	17 (68.0)	8 (32.0)		20	14 (70.0)	6 (30.0)	
No. of doses/day:								
< 6	59 (52.7)	44 (74.6)	15 (25.4)	0.01	44	32 (72.7)	12 (27.3)	0.03
≥ 6	53 (47.3)	49 (92.4)	4 (7.5)		68	61 (89.7)	7 (10.3)	

Data are shown as n (%).
HAART: Highly active antiretroviral therapy.

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. 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 and number of patients with undetectable viral load (**Table 2**). 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 – 100,000) 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 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. Out 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

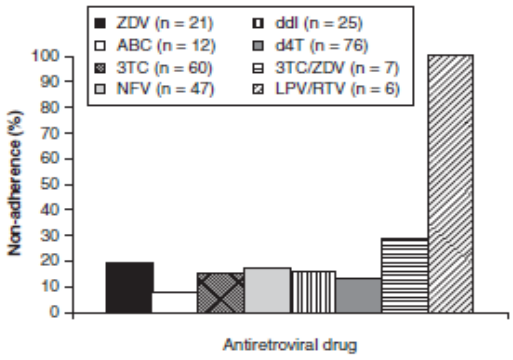

Figure 1. Adherence of HIV-infected children to individual antiretroviral drugs.

Data for individual antiretroviral drugs taken by more than five patients or with a percentage of non-adherence more than 5% are reported. Adherence rate was 100% in the case of ritonavir (n = 16), efavirenz (n = 4) and indinavir (n = 16) (data not reported in figure).

3TC: Lamivudine; 3TC/ZDV: Lamivudine/zidovudine combination; ABC: Abacavir; d4T: Stavudine; ddI: Aidanosine; LPV/RTV: 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 caregiver, 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 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. 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 [13,14]. 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 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 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, 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/ml), there was a significant trend for decreased level of serum HIV RNA in adherent patients compared with non-adherent subjects.

Overall, our results confirm that psychosocial features of caregivers and children play a major role in adherence and that foster parents are more reliable caregivers than are 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 is, 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 [11,15].

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.

Type of caregiver	Baseline				After 12 months			
	All	Adherent	Non-adherent	p	All	Adherent	Non-adherent	p
Biological parents	78	61 (78.2)	17 (21.8)	0.05	62	49 (79.0)	13 (21.0)	0.02
Foster parents	34	32 (92.9)	2 (7.1)		32	31 (96.8)	1 (3.2)	0.01
Self-administered	0				18	13 (72.3)	5 (27.7)	

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 sicker adults with chronic diseases taking several drugs seemed to be highly complaint with therapy [16-20]. However, in these studies, 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 doses are more compliant with therapy than patients taking fewer 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.

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

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Laurence J. Adhering to antiretroviral therapies. AIDS Patient Care STDS 2001;15:107-8
2. McNabb J, Ross JW, Abriola K, et al. Adherence to highly active antiretroviral therapy predicts virologic outcome at an inner-city human immunodeficiency virus clinic. Clin Infect Dis 2001;33:700-5
3. Paterson DL, Swindells S, Mohr J, et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Ann Intern Med 2000;133:21-30
4. Gibb DM, Goodall RL, Giacomet V, et al. Adherence to prescribed antiretroviral therapy in human immunodeficiency virus-infected children in the PENTA 5 trial. Pediatr Infect Dis J 2003;22:56-62
   • **In this multicentre study social factors were detected as main factors affecting non-adherence to antiretroviral therapy.**
5. Guidelines for the use of antiretroviral agents in pediatric HIV infection. Available from: http://AIDSinfo.nih.gov
6. Amico KR, Harman JJ, Johnson BT. Efficacy of antiretroviral therapy adherence interventions: a research synthesis of trials, 1996 to 2004. J Acquir Immune Defic Syndr 2006;41:285-97
7. Simoni J, Pearson CR, Pantalone DW, et al. Efficacy of interventions in improving highly active antiretroviral therapy adherence and HIV-1 RNA viral load: a metaanalytic review of randomized controlled trials. J Acquir Immune Defic Syndr 2006;43:S23-35
   • **This is a recent meta-analysis including a large number of HIV-infected adults (19 randomized controlled trials with a total of 1839 participants). The results of the meta-analysis indicate that HAART adherence behavioural interventions can be efficacious to improve adherence rate in HIV-infected adults.**
8. Giacomet V, Albano F, Starace F, et al. Adherence to antiretroviral therapy and its determinants in children with human immunodeficiency virus infection: a multicentre, national study. Acta Paediatr 2003;92:1398-402
   • **In this multicentre study, including 129 HIV-infected children, psychological rather than clinical or sociodemographic features were found as major determinants of adherence to antiretroviral treatment.**
9. Byakika-Tusiime J, Crane J, Oyugi JH, et al. Longitudinal antiretroviral adherence in HIV+ Ugandan parents and their children initiating HAART in the MTCT-Plus family treatment model: role of depression in declining adherence over time. AIDS Behav 2009;13:82-91
   • **This study, which includes 177 participants living in Uganda, shows that depression was significantly associated with incomplete adherence. Furthermore, there was a significantly declined of adherence over time on therapy.**
10. Davies MA, Boulle A, Fakir T, et al. Adherence to antiretroviral therapy in young children in Cape Town, South Africa, measured by medication return and caregiver self-report: a prospective cohort study. BMC Pediatr 2008;8:34
11. Van Dyke RB, Lee S, Johnson GM, et al. Reported adherence as a determinant of response to highly active antiretroviral therapy in children who have human immunodeficiency virus infection. Pediatrics 2002;109:e61

- 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.

12. Albano F, Giacomet V, De Marco G, et al. Adherence to antiretroviral therapy in children: a comparative evaluation of caregiver reports and physician judgment. AIDS Care 2007;19:764-6

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

13. Mernel C, Vandevanter N, Irvine M. Adherence to antiretroviral therapy among older children and adolescents with HIV: a qualitative study of psychosocial contexts. AIDS Patient Care STDS 2008;22:977-87

14. Mellins CA, Brackis-Cott E, Dolezal C, Abrams EJ. The role of psychosocial and family factors in adherence to antiretroviral treatment in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2004;23:1035-41

- This study shows that caregiver/family factors, as higher caregiver or child stress, worse parent–child communication and lower caregiver quality of life, are 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.

15. Wruhel J, Moskowitz JT, Richards TA, et al. Pediatric adherence: perspectives of mothers of children with HIV. Soc Sci Med 2005;61:2423-33

16. Billups SJ, Malone DC, Carter BL. The relationship between drug therapy non-compliance and patient characteristics, health-related quality of life, and health care costs. Pharmacotherapy 2000;20:941-9

- The study investigates potential indicators of compliance to medication in 1054 adults with chronic diseases. The authors found that compliance was significantly related to the number of drugs in regimen, being higher in patients taking several drugs.

17. Sharkness CM, Snow DA. The patient's view of hypertension and compliance. Am J Prev Med 1992;8:141-6

18. Wagner GJ, Rablen JG. Measuring medication adherence: are missed doses reported more accurately then perfect adherence? AIDS Care 2000;12:405-8

19. Miller LG, Hays RD. Adherence to combination antiretroviral therapy: synthesis of the literature and clinical implications. AIDS Read 2000;10:177-85

20. Shalansky SJ, Levy AR. Effect of number of medications on cardiovascular therapy adherence. Ann Pharmacother 2002;36:1532-9

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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 experts' 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 937 children who were perinatally exposed to HIV-1 to estimate adjusted odds ratios for confounding factors on mother-to-child transmission, including maternal interruption of ART.

Results. Among 937 pregnant women infected with HIV-1, ART was interrupted in 81 (8.6%) 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], 5–6 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 1.3% (95% confidence interval [CI], 0.7%–2.3%), whereas it was 4.9% (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, maternal mono- or double therapy, delivery by a mode other than elective cesarean delivery, and a viral load at delivery >4.78 $\log_{10}$ copies/mL were independently associated with an increased 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 restarted 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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(MTCT) of HIV-1 [2–5]. In developed countries, the synergic effect of ART in the mother and newborn, cesarean delivery, and avoidance of breast-feeding 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 experts' 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 instituted in 1985. Data come from a network of 106 participating pediatric centers located throughout Italy, representing the overall population of exposed infants in Italy [3]. 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 infectious status were considered [3]. Data with regard to mother-infant pairs were collected as described elsewhere [3, 9]. Detailed data on the type of regimen(s), gestational age at beginning and end of ART (for each single regimen if switches occurred during pregnancy), and ART administered intrapartum and/or to the newborn were included. Maternal 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 2002, only children born in 2002 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 definition. Infection in children [3, 9, 10], elective cesarean delivery [2], 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 2 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 interruption of ART was 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 2005 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 χ^2 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 (<2.6, 2.6–4.00, 4.01–5.78, >5.78 $\log_{10}$ copies/mL, or unknown), $CD4^+$ cell count at delivery (<200, 200–499, ≥500 cells/μL, 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), intrapartum ART (yes, no, or unknown), and neonatal ART (yes, no, or unknown). Because

no child was breast-fed, the mode of feeding was not included in the regression analyses. To better evaluate interaction between quantitative variables potentially associated with the rate of MTCT, an additional logistic regression model with $\log_{10}$ plasma viral RNA load and $\log_{10}$ $CD4^+$ cell count as continuous variables was performed for a subgroup of mother-child pairs with both variables known.

RESULTS

Mother-infant pairs. Overall, 937 of 1016 mother-child pairs receiving ART entered the study; 79 (7.8%) of the exposed infants were excluded because they were lost to follow-up before ascertainment of infectious status. The characteristics of the 937 mothers and children are shown in table 1. Intrapartum and neonatal prophylaxis consisted of zidovudine in all but 8 cases in which nevirapine was given. Maternal plasma viral load was known in 630 (67.2%) of the 937 mother-child pairs. Of the 630 women with known viral load, the majority (453 [71.9%]) showed an undetectable plasma viral load (tested by an assay with a cutoff value of 50 copies/mL [$n = 340$] and 400 copies/mL [$n = 113$]). Characteristics of mother-child pairs were compared according to known or unknown maternal plasma viral load. Frequency of intrapartum ART administration was higher when maternal viral load was known (586 [93.0%] of 630 women vs. 261 [85.0%] of 307 women; $\chi^2 = 15.21.5$; $P < .001$), and frequency of prepartum HAART administration was higher when maternal viral load was known (454 [72.1%] of 630 women vs. 197 [64.2%] of 307 women; $\chi^2 = 6.07$; $P = .014$); no difference was observed in the frequency of mode of elective cesarean delivery (580 [92.1%] of 630 women vs. 280 [91.2%] of 307 women; $\chi^2 = 0.20$; $P = .653$), the use of neonatal ART (614 [97.5%] of 630 infants vs. 301 [98.0%] of 307 infants; $\chi^2 = 0.31$; $P = .579$), and the infant's infectious status (8 [1.3%] of 630 cases vs. 4 [1.3%] of 307 cases; $\chi^2 = 0.002$; $P = .966$). The maternal viral load decreased as the complexity of the ART regimen increased. The mean plasma viral load was 2.6 $\log_{10}$ copies/mL (95% CI, 2.2–2.9 $\log_{10}$ copies/mL), 2.4 $\log_{10}$ copies/mL (95% CI, 2.2–2.6 $\log_{10}$ copies/mL), and 1.9 $\log_{10}$ copies/mL (95% CI, 1.8–2.0 $\log_{10}$ copies/mL) in women receiving monotherapy, double therapy, or HAART, respectively (analysis of variance, $F = 17.2$; $P < .001$). The median gestational age at the beginning of treatment was 18 weeks (IQR, 13–35 weeks), 18 weeks (IQR, 13–25 weeks), and 13 weeks (IQR, 6–22 weeks) in women receiving monotherapy, double therapy, or HAART, respectively, thus decreasing with the increase in the ART complexity ($\chi^2 = 36.8$; $P < .001$). The proportion of women with detectable viral load did not differ according to trimester at start of ART when monotherapy was given ($n = 50$; 78.6% during the first trimester, 55.0% during the second trimester, and 56.3% during the third trimester; $\chi^2 = 2.27$; $P = .321$) In addition, the pro-

Table 1. Characteristics of 937 mother-child pairs in our study of mother-to-child transmission of HIV-1 infection.

Variable	No. (%) of mother-child pairs
Mother	
Type of ART	
Monotherapy	99 (10.6)
Double therapy	187 (20.0)
HAART	651 (69.5)
Type of ART before interruption ($n = 81$)	
Nevirapine-based HAART	27 (33.3)
Protease inhibitor-based HAART	27 (33.3)
Other regimen of HAART	19 (23.5)
Nonspecified HAART	2 (2.5)
Double therapy	3 (3.7)
Monotherapy	3 (3.7)
Type of ART after interruption ($n = 81$)	
Nevirapine-based HAART	33 (40.7)
Protease inhibitor–based HAART	33 (40.7)
Other regimen of HAART	6 (7.4)
Nonspecified HAART	2 (2.5)
Double therapy	3 (3.7)
Monotherapy	4 (4.9)
Type of delivery	
Elective cesarean	860 (91.8)
Cesarean other than elective	51 (5.4)
Vaginal	20 (2.1)
Unknown	6 (0.6)
Viral load at delivery, $\log^{10}$ copies/mL	
<2.6	453 (48.3)
2.6–4.00	133 (14.2)
4.01–4.78	C36 (3.8)
>4.78	8 (0.9)
Unknown	307 (32.8)
$CD4^+$ cell count at delivery, cells/μL	
<200	43 (4.6)
200–499	267 (28.5)
≥500	265 (28.3)
Unknown	362 (38.6)
Intrapartum ART	
Yes	847 (90.4)
No	90 (9.6)
Child	
Sex	
Male	482 (51.4)
Female	455 (48.6)
Gestational age	
<37 weeks	187 (20.0)
≥37 weeks	745 (79.5)
Unknown	5 (0.5)
Neonatal ART	
Yes	915 (97.7)
No	22 (2.3)
Infectious status	
Infected	12 (1.3)
Uninfected	925 (98.7)

NOTE. HAART was defined as ≥3 antiretroviral drugs. ART, antiretroviral therapy.

Table 2. Characteristics of the 12 mother-child pairs in which the infants were infected.

Identifier	Maternal ART regimen			Interruption of ART		Maternal HIV-1 RNA load at delivery, copies/mL	$CD4^+$ cell count at delivery, cells/μL	Gestational age, weeks	Intrapartum ART	Neonatal ART
	At beginning of pregnancy	After interruption	After switching without interruption	During first trimester	During third trimester					
1	ddI+TDF+T20	ddI+TDF+NFV	…	Yes	No	28,110	74	33	Yes	Yes
2	ddI+d4T+LPV/RIT	…	AZT	No	No	Unknown	Unknown	Unknown	Yes	Yes
3	AZT+3TC+NVP	AZT+3TC+NVP	…	Yes	No	780	781	38	Yes	Yes
4	AZT+3TC+NFV	…	…	No	No	1710	839	38	Yes	Yes
5	AZT	…	…	No	No	Unknown	Unknown	31	No	Yes
6	d4T+TDF+LPV/RIT	AZT+3TC+NVP	…	Yes	No	<50	326	27	No	Yes
7	d4T+3TC+NFV	d4T+3TC+NFV	…	No	Yes	600,000	410	31	Yes	Yes
8	AZT+ 3TC	AZT + 3TC	…	Yes	No	500,000	200	37	Yes	No
9	AZT+3TC+NVP	…	…	No	No	16,900	468	39	Yes	Yes
10	AZT+3TC	…	…	No	No	54	390	38	Yes	Yes
11	AZT+3TC+NVP	AZT+3TC+NVP	…	No	Yes	Unknown	Unknown	21	Yes	Yes
12	AZT	…	…	No	No	2800	141		Yes	Yes

NOTE. All deliveries were elective cesarean deliveries. AZT, zidovudine; ddI, didanosine; d4T, stavudine; LPV/RIT, lopinavir-ritonavir; NFV, nelfinavir; NVP, nevirapine; TDF, tenofovir; T20, enfuvirtide; 3TC, lamivudine.

portion of women with detectable viral load did not differ according to the trimester during which ART was initiated when double therapy was given ($n = 126$; 38.1% during the first trimester, 37.3% during the second trimester, and 47.1% during the third trimester; $\chi^2 = 0.56$; $P = .756$). However, the proportion of women with detectable viral load was significantly higher when HAART (given to 454 women) was started during the third trimester (45.9%), compared with during the second (17.5%) or first trimester (17.8%; $\chi^2 = 25.81$; $P < .001$). Women treated with HAART who had a known plasma viral load had similar mean plasma viral loads when 281 women who were treated with a PI-based regimen (1.95 $\log_{10}$ copies/mL; 95% CI, 1.77–2.07 $\log_{10}$ copies/mL) were compared with 328 women treated with a nevirapine-based regimen (1.85 $\log_{10}$ copies/mL; 95% CI, 1.65–1.92 $\log_{10}$ copies/mL; Student's t test, 1.29; $P = .197$).

Discontinuation and subsequent restart of ART was reported in 81 (8.6%) of the 937 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 mother-child pairs in the third trimester (median time at suspension, 32 weeks [IQR, 23–36 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 both 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 (3 [1.6%] of 187 women) or with monotherapy (4 [4.0%] of 99 women; χ^2 test, 20.50; $P < .001$).

We checked whether women switched therapy after interruption to a less or more active regimen, but the proportions of women treated with a nevirapine- or PI-based HAART regimen before or after treatment interruption were similar (table 1). Treatment was restarted with the same class of antiretroviral drugs in 47 (58.0%) of the 81 women, among whom 19 (23.5%) received the same nevirapine-based regimen that they received prior to the interruption.

Among women whose viral load at delivery was known, the mean plasma viral load was similar in those who had treatment interrupted in the first trimester (2.04 $\log_{10}$ copies/mL; 95% CI, 1.81–2.26 $\log_{10}$ copies/mL) and in those who did not have treatment interrupted (2.04 $\log_{10}$ copies/mL; 95% CI, 1.95–2.14 $\log_{10}$ copies/mL; Student's t test, −0.04; $P = .967$). Similarly, the mean $CD4^+$ cell count at delivery was similar in women who had treatment interrupted (2.63 $\log_{10}$ cells/μL; 95% CI, 2.57–2.69 $\log_{10}$ cells/μL) and in those who did not have treatment interrupted (2.65 $\log_{10}$ cells/μL; 95% CI, 2.65–2.67 $\log_{10}$ cells/μL; Student's t test, −0.40; $P = .689$). Among HAART-treated women who had therapy interrupted and who had known plasma viral load, we found no difference in mean plasma viral load between 29 women treated with a PI-based regimen (2.06 $\log_{10}$ copies/mL; 95% CI, 1.70–2.42 $\log_{10}$ copies/mL) and 28 women treated with a nevirapine-based regimen (1.85 $\log_{10}$ copies/mL; 95% CI, 1.69–2.01 $\log_{10}$ copies/mL; Student's t test, 1.12; $P = .268$).

MTCT rate and confounding factors. Overall, the rate of MTCT in the whole cohort was 1.3% (95% CI, 0.7%–2.3%). The rate of MTCT among children born to mothers who had ART interrupted in the first trimester was 4.9% (95% CI, 1.9%–13.2%), and the rate of MTCT among children born to mothers who had ART interrupted in the third trimester was 18.2% (95% CI, 4.5%–72.7%). Characteristics of the 12 mother-child pairs in which the children were infected are shown in table 2.

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, 34.1). The probability of infection in children

Table 3. Univariate and logistic regression analysis of risk factors for mother-to-child transmission of HIV-1 infection.

Factor	No. of infected infants/total no. of infants	Univariate analysis		Multivariate analysis	
		OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Type of maternal ART					
Monotherapy	5/99	Reference		Reference	
Double	1/187	0.10 (0.01–0.88)	.038	0.21 (0.02–2.21)	.192
HAART	6/651	0.18 (0.05–0.58)	.005	0.17 (0.04–0.80)	.025
Trimester at start of ART					
First	5/463	Reference		Reference	
Second	4/321	1.16 (0.31–4.34)	.830	0.54 (0.10–3.06)	.487
Third	3/149	1.88 (0.44–7.97)	.391	0.92 (0.15–5.51)	.929
Unknown	0/4	NA		NA	
Interruption of ART					
During the first trimester					
No	8/856	Reference		Reference	
Yes	4/81	5.51 (1.62–18.70)	.006	10.33 (2.02–52.91)	.005
During the third trimester					
No	10/926	Reference		Reference	
Yes	2/11	20.36 (3.89–106.42)	<.001	46.86 (4.28–512.64)	.002
Type of delivery					
Elective cesarean	10/860	Reference		Reference	
Cesarean other than elective	2/51	3.47 (0.74–16.27)	.115	5.9 (0.93–37.28)	.059
Vaginal	0/20	NA		NA	
Unknown	0/6	NA		NA	
Viral load at delivery, $\log_{10}$ copies/mL					
<2.6	2/453	Reference		Reference	
2.61–4.00	3/133	5.20 (0.86–31.48)	.073	4.78 (0.65–34.98)	.124
4.01–4.78	1/36	6.44 (0.57–72.82)	.132	7.41 (0.52–105.87)	.140
>4.78	2/8	75.17 (9.03–625.60)	<.001	29.19 (1.80–473.81)	.018
Unknown	4/307	2.98 (0.54–16.35)	.210	7.69 (0.64–92.97)	.109
$CD4^+$ cell count at delivery, cells/μL					
<200	1/43	Reference		Reference	
200–499	5/267	0.80 (0.09–7.03)	.842	0.99 (0.09–11.09)	.994
>500	2/265	0.32 (0.03–3.60)	.356	0.88 (0.06–12.59)	.923
Unknown	4/362	0.47 (0.05–4.30)	.503	0.25 (0.01–4.44)	.341
Sex of child					
Female	5/455	Reference		Reference	
Male	7/482	1.33 (0.42–4.21)	.632	1.02 (0.25–4.13)	.983
Intrapartum ART					
No	2/90	Reference		Reference	
Yes	10/847	0.53 (0.11–2.44)	.411	0.53 (0.09–3.11)	.481
Neonatal ART					
No	1/22	Reference		Reference	
Yes	11/915	0.26 (0.03–2.07)	.201	0.84 (0.04–18.30)	.912

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 regained by periodical cross-matches 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 46.86 (95% CI, 4.28–512.64; $P = .002$) in the whole cohort, with the inclusion of maternal viral load as a category with missing values. When only cases with known maternal viral load were included, the adjusted OR for interruption of ART in the first trimester was 11.45 (95% CI, 1.58–83.08; $P = .016$) and in the third trimester was 24.33 (95% CI, 0.77–765.92; $P = .070$). When maternal viral load was excluded, the adjusted OR for interruption of ART in the first trimester was 8.32 (95% CI, 1.88–36.90; $P = .005$) and in the third trimester was 40.52 (95% CI, 4.24–387.64; $P = .001$) in the whole cohort.

Finally, a logistic regression model on a subgroup ($n = 538$) of mother-child pairs with both known plasma viral load and known $CD4^+$ cell count was performed, including these factors as continuous variables. Treatment interruption in the first trimester was associated with an adjusted OR of 10.70 (95% CI, 1.48–77.18), and treatment interruption in the third trimester was associated with an adjusted OR of 30.38 (95% CI, 0.98–941.66). Plasma viral load was associated with an adjusted OR of 2.50 (95% CI, 1.27–4.91), and $CD4^+$ cell count was associated with an adjusted OR of 1.87 (95% CI, 0.07–53.26). 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 $\log_{10}$ copies/mL 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 viremic rebound [12, 13] 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, maybe because the viremic rebound is stronger if the virological 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 fear of drug toxicity in the embryo. 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 plasma viral RNA load at delivery. When viral load increased by 1 $\log_{10}$ copies/mL, 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 predelivery 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 nonnucleoside reverse-transcriptase inhibitor–based regimen, as recently was reported by the European 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 nonnucleoside 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 in 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 mother-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 intolerance of treatment are the most probable reasons for ART discontinuation, and if so, it would be unlikely that women who had ART suspended were a priori at 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 do not systematically measure HIV-1 RNA or DNA load during the first 24–48 h of life.

We investigated 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 intrapartum and neonatal therapy and the rate of MTCT, but the numbers of mother-child pairs who were not receiving intrapartum and neonatal 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, in women who do not require ART for their own health, ART for MTCT 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.

OTHER PARTICIPANTS OF THE ITALIAN REGISTER FOR HIV INFECTION IN CHILDREN

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References

1. Connor EM, Sperling RS, Gelber R, et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med **1994**; 331:1173–80.
2. The International Perinatal HIV Group. The mode of delivery and the risk of vertical transmission of human immunodeficiency virus type 1: a meta-analysis of 15 prospective cohort studies. N Engl J Med **1999**; 340:977–87.
3. The Italian Register for Human Immunodeficiency Virus Infection in Children. Determinants of mother-to-infant human immunodeficiency virus 1 transmission before and after the introduction of zidovudine prophylaxis. Arch Pediatr Adolesc Med: **2002**; 156:915–21.
4. Mandelbrot L, Landreau A, Rekacevicz C, et al. Lamivudine-zidovudine combination for prevention of maternal-infant transmission of HIV-1. JAMA **2001**; 285:2083–93.
5. Cooper ER, Charurat M, Mofenson LM, et al. Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission. J Acquir Immune Defic Syndr **2002**; 29:484–94.
6. European Collaborative Study. Mother-to-child transmission of HIV

infection in the era of highly active antiretroviral therapy. Clin Infect Dis **2005**; 40:458–65.
7. Perinatal HIV Guidelines Working Group. Public health service task force recommendations for use of antiretroviral drugs in pregnant HIV-infected women for maternal health and interventions to reduce perinatal HIV transmission in the United States. 8 July **2008**:1–98. Available at: http://aidsinfo.nih.gov/ContentFiles/PerinatalGL.pdf. Accessed 9 March 2009.
8. Antiretroviral Pregnancy Registry Steering Committee. Antiretroviral Pregnancy Registry international interim report for 1 January 1989–31 July 2005. Wilmington, NC: Registry Coordinating Center, **2005**. Available at: http://www.APRegistry.com. Accessed 9 March 2009.
9. Galli L, Puliti D, Chiappini E, et al.; the Italian Register for HIV Infection in Children. Lower mother-to-child HIV-1 transmission in boys is independent of type of delivery and antiretroviral prophylaxis. J Acquir Immune Defic Syndr **2005**; 40:479–85.
10. Centers for Disease Control and Prevention. 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. **1994**. Available at: http://www.cdc/mmwr/preview/mmwrhtml/00032890.htm. Accessed March 20, 2009.
11. de Martino M, Tovo PA, Balducci M, et al. Reduction in mortality with availability of antiretroviral therapy for children with perinatal HIV-1 infection. JAMA **2000**; 284:190–7.
12. Bucceri AM, Somigliana E, Matrone R, et al. Discontinuing combination antiretroviral therapy during the first trimester of pregnancy: insights from plasma human immunodeficiency virus–1 RNA viral load and CD4 cell count. Am J Obstet Gynecol **2003**; 189:545–51.
13. The Strategies for Management of Antiretroviral Therapy (SMART) Study Group. $CD4^+$ count–guided interruption of antiretroviral treatment. N Engl J Med **2006**; 355:2283–96.
14. Garcia PM, Kalish LA, Pitt J, et al.; the Women and Infants Transmission Study Group. Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. New Engl J Med **1999**; 341:394–402.
15. European Collaborative Study. Maternal viral load and vertical transmission of HIV-1: an important factor but not the only one. AIDS **1999**; 13:1377–85.
16. Duran AS, Losso MH, Salomon H, et al. Drug resistance among HIV-infected pregnant women receiving antiretrovirals for prophylaxis. AIDS **2007**; 21:199–205.
17. Lockman S, Shapiro RL, Smeaton LM, et al. Response to antiretroviral therapy after a single, peripartum dose of nevirapine. N Engl J Med **2007**; 356:135–47.
18. European Collaborative Study. Time to undetectable viral load after highly active antiretroviral therapy initiation among HIV-infected pregnant women. Clin Infect Dis **2007**; 44:1647–56.
19. Dunn DT, Brandt CD, Krivine A, et al. The sensitivity of HIV-1 DNA polymerase chain reaction in the neonatal period and the relative contributions of intrauterine and intrapartum transmission. AIDS **1995**; 9:F7–11.

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

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

* 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

Impairments	Patients with impairments, n (%)
Thorax*	1 (2.4)
Immune system**	13 (32)
Endocrine system***	2 (5)
Skin problems/fat distribution §	11 (27)

* 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

Impairments	Patients with impairments, n (%)
Eye function*	7 (17)
Gastrointestinal function**	6 (15)

* 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

Barriers	Families reporting the barriers, n (%)
Insufficient availability of:	
Food	7 (17)
Drugs	6 (15)
Daily consumption products	8 (19)
Transportation facilities	5 (12)
Inadequate support by:	
Other family members	11 (27)
Relatives and friends	22 (54)
Care providers (health, education or social)	34 (83)
Inadequate approach by:	
Other family members	1 (2.4)
Relatives and friends	8 (19.5)
Care providers (health, education or social)	6 (15)

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

Domains of activity limitations and participation restrictions	Families reporting limitations and restrictions, n (%)
Relationship with relatives and friends	4 (10)
Learning in informal setting	5 (12)
Learning at school	7 (17)

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

1. Andrews G, Skinner D, Zuma K. Epidemiology of health and vulnerability among children orphaned and made vulnerable by HIV/AIDS in sub-Saharan Africa. AIDS Care 2006;18:269-76.
2. Atwine B, Cantor-Graae E, Bajunirwe F. Psychological distress among AIDS orphans in rural Uganda. Soc Sci Med 2005;61:555-64.
3. Thurman TR, Brown L, Richter L, et al. Sexual risk behavior among South African adolescents: is orphan status a factor? AIDS Behav 2006;10:627-35.
4. Nagler SF, Adnopoz J, Forsyth BWC. Uncertainty, stigma and secrecy: psychological aspects of AIDS for children and adolescents. In: Geballe S, Gruendel J, Andiman W., editors. Forgotten children of the AIDS epidemic. New Haven:Yale University Press; 1995, pp 71-82.
5. Mellins CA, Ehrhardt AA. Families affected by pediatric acquired immunodeficiency sindrome: sources of stress and coping. J Dev Behav Pediatr 1994;15:S54-60.
6. Wiener L, Mellins CA, Marhefka S, et al. Disclosure of an HIV diagnosis to children: history, current research, and future directions. J Dev Behav Pediatr 2007;28:155-66.
7. King E, De Silva M, Stein A, et al. Interventions for improving the psychosocial well-being of children affected by HIV and AIDS. Cochrane Database Syst Rev 2009;2:CD006733.
8. Wiener L, Septimus A, Grady C. Psychosocial support an ethical issues for the child and family. In: Pizzo P, Wilfert K., editors. Pediatric AIDS: the challenge of HIV infection in infants, children, and adolescents. Baltimore, MD: Williams and Wilkins, 1998, pp 703-27.
9. Simeonsson RJ, Scarborough AA, Hebbeler KM. ICF and ICD codes provide a standard language of disability in young children. J Clin Epidemiol 2006;59:365-73.
10. Rosenbaum P, Stewart D. The World Health Organization International Classification of Functioning, Disability, and Health: a model to guide clinical thinking, practice and research in the field of cerebral palsy. Semin Pediatr Neurol 2004;11:5-10.
11. Ustün TB, Chatterji S, Bickenbach J, et al. The International Classification of Functioning, Disability and Health: a new tool for understanding disability and health. Disabil Rehabil 2003;25:565-71.
12. Guidelines for the use of antiretroviral agents in pediatric HIV infection. http://AIDSinfo.nih.gov

13. Chiappini E, Galli L, Tovo PA, et al. Virologic, immunologic, and clinical benefits from early combined antiretroviral therapy in infants with perinatal HIV-1 infection. AIDS 2006;20:207-15.
14. Chiappini E, Galli L, Gabiano C, et al. Early triple therapy vs mono or dual therapy for children with perinatal HIV infection. JAMA 2006;295:626-28.
15. Chiappini E, Galli L, Tovo PA, et al. Changing patterns of clinical events in perinatally HIV-1-infected children during the era of HAART. AIDS 2007;21:1607-15.
16. Arkela-Kautiainen M, Haapasaari J, Kautiainen H, et al. Functioning and preferences for improvement of health among patients with juvenile idiopathic arthritis in early adulthood using the WHO ICF model. J Rheumatol 2006;33:1369-76.
17. Battaglia M, Russo E, Bolla A, et al. International Classification of Functioning, Disability and Health in a cohort of children with cognitive, motor, and complex disabilities. Dev Med Child Neurol 2004;46:98-106.
18. Schneider M, Manabile E, Tikly M. Measuring chronic health condition and disability as distinct concepts in national surveys of school-aged children in Canada: a comprehensive review with recommendations based on the ICD-10 and ICF. Disabil Rehabil 2003;25:922-39.
19. Westby C. Application of the ICF in children with language impairments. Semin Speech Lang 2007;28:265-72.
20. Campbell WN, Skarakis-Doyle E. School-aged children with SLI: the ICF as a framework for collaborative service delivery. J Commun Disord 2007;40:513-35.
21. Goldstein DN, Cohn E, Coster W. Enhancing participation for children with disabilities: application of the ICF enablement framework to pediatric physical therapist practice. Pediatr Phys Ther 2004;16:114-20.
22. Østensjø S, Bjorbaekmo W, Carlberg EB, et al. Assessment of everyday functioning in young children with disabilities: an ICF-based analysis of concepts and content of the Pediatric Evaluation of Disability Inventory (PEDI). Disabil Rehabil 2006;28:489-04.
23. Ogonowski J, Kronk R, Rice C, et al. Inter-rater reliability in assigning ICF codes to children with disabilities. Disabil Rehabil 2004;26:353-61.

3.6 Publications

Psychosocial issues in children and adolescents growing with HIV infection evaluated with an age-specific descriptor system

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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

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

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).

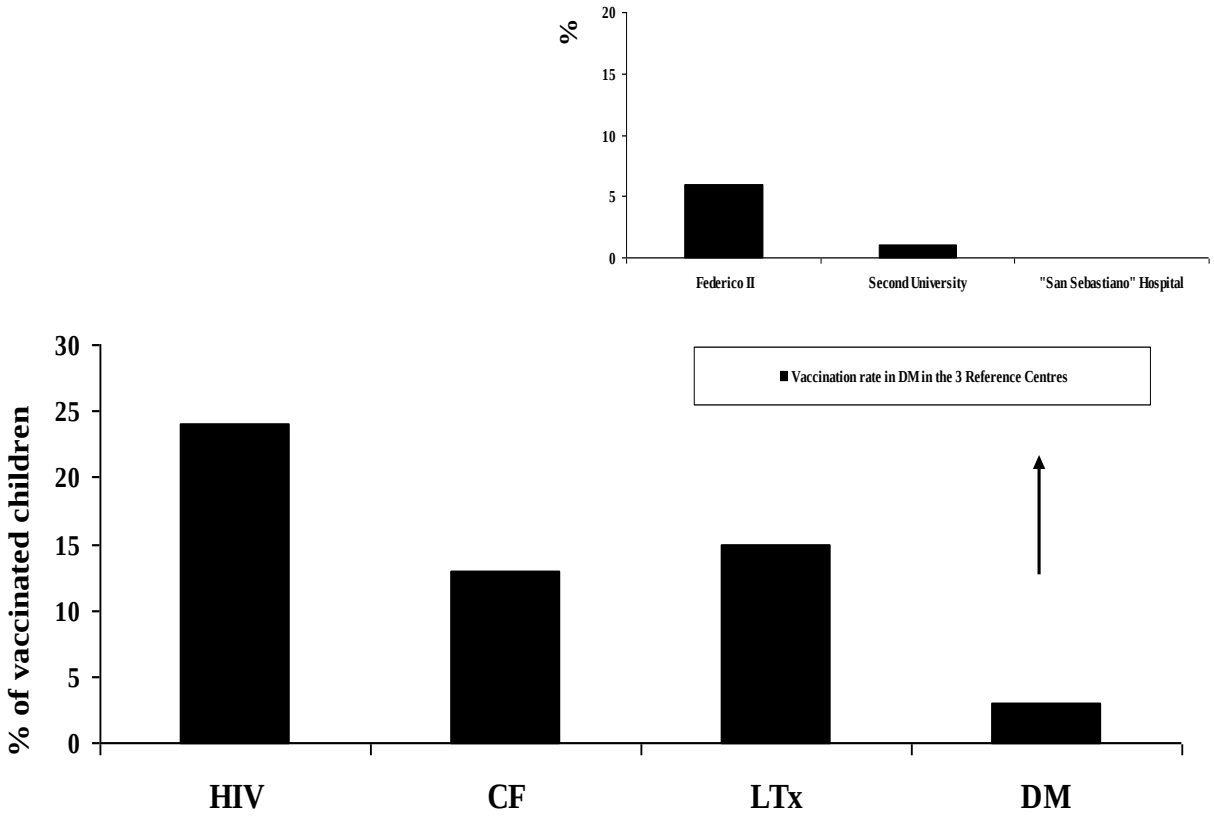

Figure 1. Pneumococcal vaccination rates in 343 high risk children

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.

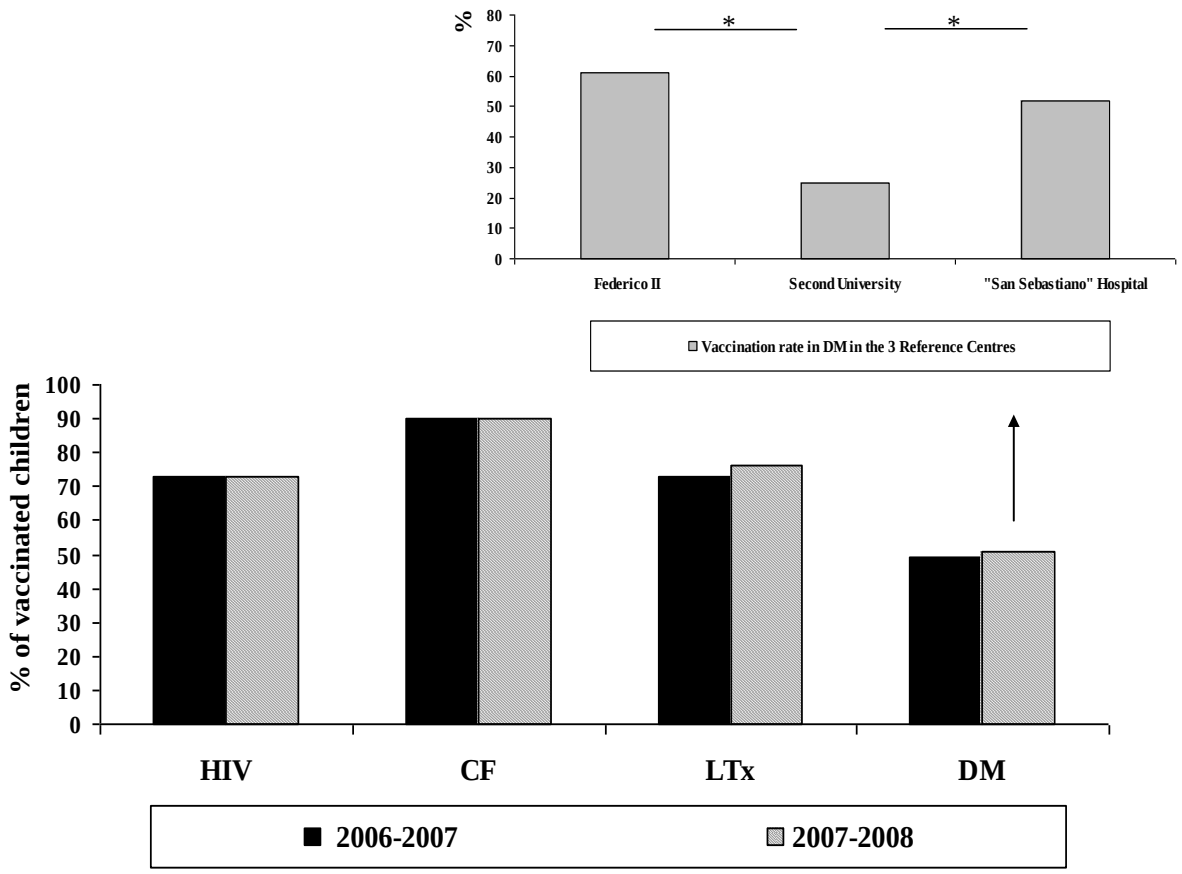

Figure 2. Influenza vaccination rates in 343 high risk children

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.

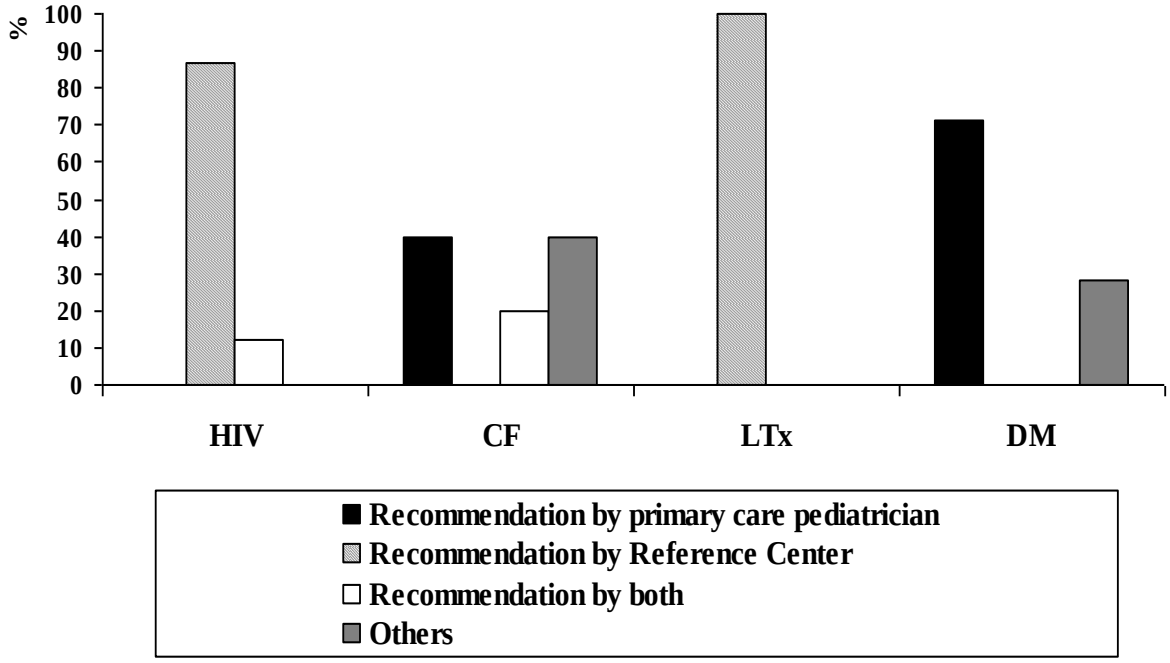

Figure 3. Reasons for receiving pneumococcal vaccination in 343 high-risk children

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.

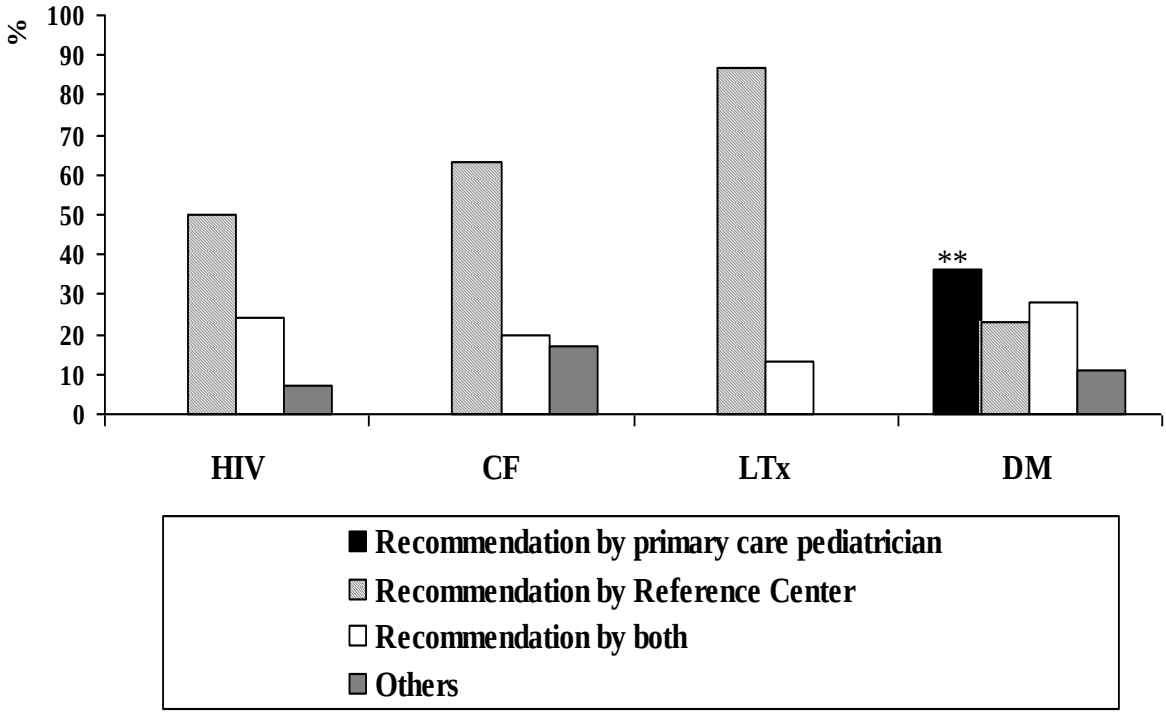

Figure 4. Reasons for receiving influenza vaccination in 343 high risk children

** 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**).

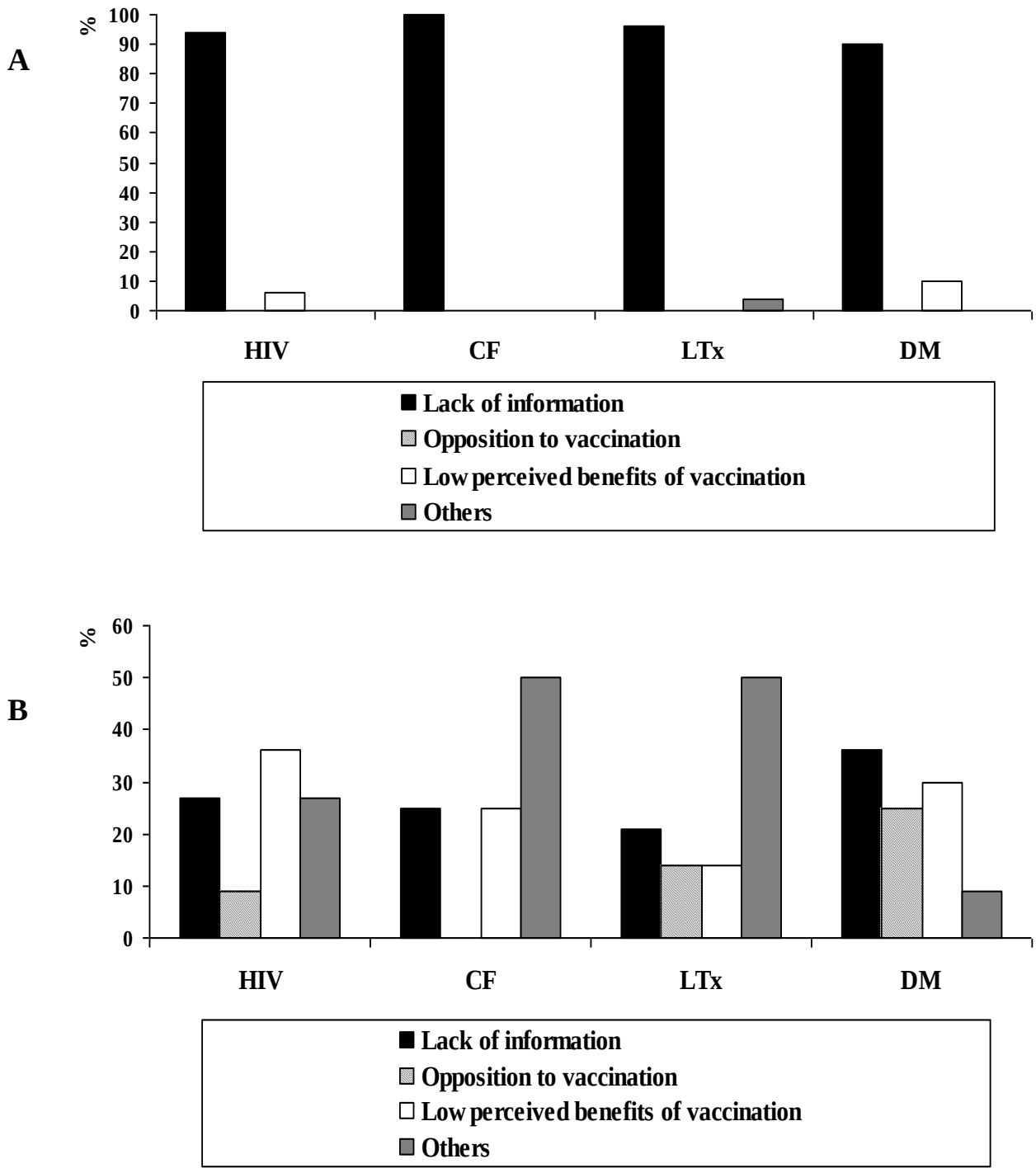

Figure 5. Reasons for not having pneumococcal or influenza vaccinations in 343 high risk children

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 considered 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 from on having three categories of physicians, namely disease specialists, primary care physicians and vaccination service physicians, equally encharged 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 has 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

1. Advisory Committee on Immunisation Practices. Preventing pneumococcal disease among infants and young children. Recommendations of the Advisory Committee on Immunisation Practices (ACIP) MMWR Recomm Rep 2000;49:1-35.
2. Ajani UA, Ford ES, Okoro CA, et al. Low prevalence of influenza vaccination among people with cardiovascular disease--BRFSS. Am J Prev Med 2005;29:31-35.
3. Bhat N, Wright JG, Broder KR, et al. Influenza-associated deaths among children in the United States, 2003-2004. N Engl J Med 2005;2559-67.
4. Bonanni P. Vaccination and risk groups: how can we really protect the weakest? Hum Vaccin 2007;3:217-19.
5. Cartwright K. Pneumococcal disease in western Europe: burden of disease, antibiotic resistance and management. Eur J Pediatr 2002;161:188-95.
6. Centers for Disease Control and Prevention (CDC). Influenza and pneumococcal vaccination coverage among persons aged > 65 years and persons aged 18-64 years with diabetes or asthma--United States, 2003. MMWR Morb Mortal Wkly Rep 2004;53:1007-12.
7. Daley MF, Barrow J, Pearson K, et al. Identification and recall of children with chronic medical conditions for influenza vaccination. Pediatrics 2004;113:e26-33.
8. Davies P, Nwokoro C, Leigh M. Vaccinations against influenza and pneumococcus in children with diabetes: telephone questionnaire survey. BMJ 2004;328.
9. Davis MM, McMahon SR, Santoli JM, et al. A national survey of physician practices regarding influenza vaccine. J Gen Intern Med 2002;17:670-76.
10. De Marco G, Ummarino D, Giannetti E, et al. Impact of mass communication in the implementation of influenza vaccination for infants. Arch Pediatr Adolesc Med 2005;159:596.
11. Esposito S, Marchisio P, Droghetti R, et al. Influenza vaccination coverage among children with high-risk medical conditions. Vaccine 2006;24:5251-55.
12. Esposito S, Tremolati E, Bellasio M, et al. Attitudes and knowledge regarding influenza vaccination among hospital health workers caring for women and children. Vaccine 2007;25:5283-89.
13. Evans MR, Watson PA. Why do older people not get immunised against influenza? A community survey. Vaccine 2003;2:2421-27.

14. Fiore AE, Shay DK, Broder K, et al. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2008;57:1-60.
15. Fiore AE, Shay DK, Haber P, et al. Prevention and control of influenza: recommendations of the Advisory Committee on Immunisation Practices (ACIP). MMWR Recomm Rep 2007;56:1-54.
16. Fredrickson K, McLaren RP, Enger KS, et al. Influenza vaccination coverage among children aged 6-23 months - six immunisation information system sentinel sites, United States, 2005-06 influenza season. MMWR Morb Mortal Wkly Rep 2006;55:1329-30.
17. Istituto Superiore di Sanità-Gruppo di lavoro ICONA. ICONA 2003: indagine nazionale sulla copertura vaccinale infantile vii,116 p. Rapporti ISTISAN 03/37.
18. Levine OS, Farley M, Harrison LH, et al. Risk factors for invasive pneumococcal disease in children: a population-based case-control study in North America. Pediatrics 1999;103:E28.
19. Nakamura MM, Lee GM. Influenza vaccination in adolescents with high-risk conditions. Pediatrics 2008;122:920-28.
20. Neuzil KM, Wright PF, Mitchel EF Jr, et al. The burden of influenza illness in children with asthma and other chronic medical conditions. J Pediatr 2000;137:856-64.
21. Nuorti JP, Martin SW, Smith PJ, et al. Uptake of pneumococcal conjugate vaccine among children in the 1998-2002 United States birth cohorts. Am J Prev Med 2008;34:46-53.
22. O'Brien MA, Uyeki TM, Shay DK, et al. Incidence of outpatient visits and hospitalisations related to influenza in infants and young children. Pediatrics 2004;113:585-93.
23. Ompad DC, Galea S, Vlahov D. Distribution of influenza vaccine to high-risk groups. Epidemiol Rev 2006;28:54-70.
24. Overturf GD. Pneumococcal vaccination of children. Semin Pediatr Infect Dis 2002;13:155-64.
25. Piano Sanitario Nazionale 2005-2008. Available at: http://www.ministerosalute.it/resources/static/psn/documenti/psn_2005-2008.PDF.
26. Shinall MC Jr, Plosa EJ, Poehling KA. Validity of parental report of influenza vaccination in children 6 to 59 months of age. Pediatrics 2007;20:e783-87.
27. Vázquez-Fernández del Pozo S, Hernández-Barrera V, Carrasco-Garrido P, et al. Influenza vaccination coverage and related factors among Spanish children. J Infect 2007;54:483-89.

28. Zimmerman RK, Raymund M, Janosky JE, et al. Sensitivity and specificity of patient self-report of influenza and pneumococcal polysaccharide vaccinations among elderly outpatients in diverse patient care strata. Vaccine 2003;2:1486-91.

4.3 Publications

1. Giacomet V, Tarallo L, De Marco G, **Giannattasio A**, De Martino M, Guarino A.
   Preparing for an influenza pandemic in Italy: resources and procedures in paediatric hospital units.
   Eurosurveillance 2007;12:E7-8.

2. Pandolfi E, Marino MG, Gesualdo F, Romano M, **Giannattasio A**.
   Vaccinare i bambini con patologia cronica? E' meno difficile di quello che sembra!
   RIAP 02/2009: 15-21.

3. Marino MG, Pandolci E, Carloni E, Ciofi degli Atti M, Tozzi AE e il gruppo di lavoro V+.
   V+: strategie per il miglioramento della copertura vaccinale nei bambini con patologia cronica.
   Ig Sanità Pubbl 2009; 65: 183-192.

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

5. **Giannattasio A***, Lo Vecchio A*, Franzese A*, Prisco F§, Femiano P§§, Guarino A*.
   Pathways and determinants of vaccination rates against influenza and pneumococcus in children with type 1 diabetes
   * Department of Paediatrics, University Federico II, Naples, Italy
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   §§ Department of Paediatrics, "Sant'Anna e San Sebastiano" Hospital, Caserta, Italy.
   (submitted)

4.4 Influenza and pneumococcal vaccinations in HIV-infected children: assessment of methological 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

Study [type of article]*	**Domain scores (%)**						**Mean score (%)**
	Scope/ purpose	Stakeholder involvement	Rigor of development	Clarity and presentation	Applicability	Editorial independence	
Kaplan JE [G] (2)	83.3	58.3	56	81.3	30.6	16.7	**54.4**
Fiore AE [G] (8)	52.8	33.3	52.4	70.8	30.6	8.3	**41.4**
NACI [CS] (9)	52.8	31.3	33.3	58.3	25	20.8	**36.9**
Overturf GD [CS] (31)	75	29	38.1	46	28	17	**38.8**
AAP [CS] (32)	78	23	38.1	77	28	8.3	**42.1**
NY State Dept. of Health 2003 [G] (33)	38.9	33.3	19	35.4	11.1	25	**27.2**
Geretti [G] (34)	52.8	27.1	27.4	72.9	19.4	16.7	**36.0**
ECDC [CS] (35)	72.2	22.9	19	43.8	0	8.3	**27.7**
NY 2006 [G] (36)	30.6	22.9	26.2	48.3	16.7	45.8	**31.7**
Mean score	**59.6**	**31.2**	**34.4**	**59.3**	**21.0**	**18.5**	

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

Agreement, %	**N. of items (agreement calculated by Fleiss test)**
0-20	0
21-40	0
41-60	11 (48%)
61-80	12 (52%)
>81	0

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 3. Quality assessment of systematic reviews on influenza and pneumococcal vaccinations in HIV-infected children

Author	Scope and purpose	Search methods stated	Search comprehensive	Study quality assessment	Combine findings	Overall scientific quality
Overton ET (1)	C	NA	NA	NA	NA	-
Anema A (23)	B	A	A	B	B	++
Zanetti AR (24)	C	NA	NA	NA	NA	-
Neuzil KM (25)	B	NA	NA	NA	NA	-
Brydak LB (26)	A	B	B	NA	NA	+
Destefano F (27)	B	A	A	B	B	++
Bliss SJ (28)	B	A	C	B	A	++
Spencer DC (29)	C	NA	NA	NA	NA	-
Pai VB (30)	B	B	C	B	NA	+

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 pneumococcus 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 ≤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, al 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

1. Overton ET. An overview of vaccinations in HIV. Curr HIV/AIDS Rep 2007;4:105-13.
2. Guidelines for prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children. 2008. Available from: http://www.aidsinfo.nih.gov/
3. Lin JC, Nichol KL. Excess mortality due to pneumonia or influenza during influenza seasons among persons with acquired immunodeficiency syndrome. Arch Intern Med 2001;161:441-6.
4. Klein MB, Lu Y, DelBalso L, et al. Influenzavirus infection is a primary cause of febrile respiratory illness in HIV-infected adults, despite vaccination. Clin Infect Dis 2007;45:234-40.
5. Levine OS, Farley M, Harrison LH, et al. Risk factors for invasive pneumococcal disease in children: a population-based case-control study in North America. Pediatrics 1999;103:E28.
6. Cartwright K. Pneumococcal disease in western Europe: burden of disease, antibiotic resistance and management. Eur J Pediatr 2002;161:188-95.
7. Piano Sanitario Nazionale 2005-2008. Available from: http://www.ministerosalute.it/resources/static/psn/documenti/psn_2005-2008.PDF.
8. Fiore AE, Shay DK, Broder K, et al. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. MMWR Recomm Rep 2008;57:1-60.
9. National Advisory Committee on Immunization (NACI). Statement on influenza vaccination for the 2008-2009 season. Canada Communicable Disease Report; Volume 34, July 2008. Available from: http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/08vol34/acs-3/index-eng.php
10. Shaneyfelt TM, Mayo-Smith MF, Rothwangl J. Are guidelines following guidelines? The methodological quality of clinical practice guidelines in the peer-reviewed medical literature. JAMA 1999;281:1900-05.
11. Cluzeau FA, Littlejohns P, Grimshaw JM, et al. Development and application of a generic methodology to assess the quality of clinical guidelines. Int J Qual Health Care 1999;11:21-8.
12. Boluyt N, Lincke CR, Offringa M. Quality of evidence-based pediatric guidelines. Pediatrics 2005;115:1378-91.

13. Lopez-Olivo MA, Kallen MA, Ortiz Z, et al. Quality appraisal of clinical practice guidelines and consensus statements on the use of biologic agents in rheumatoid arthritis: a systematic review. Arthritis Rheum 2008;59:1625-38.
14. Potting C, Mistiaen P, Poot E, et al. A review of quality assessment of the methodology used in guidelines and systematic reviews on oral mucositis. J Clin Nurs 2009;18:3-12.
15. Villari P, Manzoli L, Boccia A. Methodological quality of studies and patient age as major sources of variation in efficacy estimates of influenza vaccination in healthy adults: a meta-analysis. Vaccine 2004;22:3475-86.
16. Campbell F, Dickinson HO, Cook JV, et al. Methods underpinning national clinical guidelines for hypertension: describing the evidence shortfall. BMC Health Serv Res 2006;6:47.
17. Field M, Lohr K. Institute of Medicine Committee to advice the Public Health Service on clinical practice guidelines. Clinical practice guidelines: directions for a new program. Washington (DC): National Academy Press;1990.
18. Institute of Medicine. Guidelines for Clinical Practice: From Development to Use. Washington, DC: National Academy Press;1992.
19. The Cochrane Collaboration. Glossary of Cochrane Collaboration and research terms. Available from: http://www.cochrane.org/resources/glossary.htm
20. AGREE Collaboration. Development and validation of an international appraisal instrument for assessing the quality of clinical practice guidelines: the AGREE project. Qual Saf Health Care 2003;12:18-23.
21. Scottish Intercollegiate Guidelines Network. SIGN 50. A guideline developer's handbook. Edinburgh: SIGN 2008. Available from: http://www.sign.ac.uk/guidelines/fulltext/50/index.html
22. Randolph JJ. Free-Marginal Multirater Kappa: an alternative to Fleiss'Fixed Marginal Multirater Kappa. Presented at the Joensuu University Learning and Instruction Symposium 2005, Joensuu, Finland, October 14-15th, 2005. Available from: http://www.eric.ed.gov/ERICDocs/data/ericdocs2sql/content_storage_01/0000019b/80/1b/c3/27.pdf
23. Anema A, Mills E, Montaner J, et al. Efficacy of influenza vaccination in HIV-positive patients: a systematic review and meta-analysis. HIV Med 2008;9:57-61.
24. Zanetti AR, Amendola A, Besana S, et al. Safety and immunogenicity of influenza vaccination in individuals infected with HIV. Vaccine 2002;20:B29-32.

25. Neuzil KM, Griffin MR, Schaffner W. Influenza vaccine: issues and opportunities. Infect Dis Clin North Am 2001;15:123-41.
26. Brydak LB, Machala M. Humoral immune response to influenza vaccination in patients from high risk groups. Drugs 2000;60:35-53.
27. Destefano F, Pfeifer D, Nohynek H. Safety profile of pneumococcal conjugate vaccines: systematic review of pre- and post-licensure data. Bull World Health Organ 2008;86:373-80.
28. Bliss SJ, O'Brien KL, Janoff EN, et al. The evidence for using conjugate vaccines to protect HIV-infected children against pneumococcal disease. Lancet Infect Dis 2008;8:67-80.
29. Spencer DC. Preventing bacterial disease in the HIV-infected of sub-Saharan Africa: the role of cotrimoxazole and the pneumococcal vaccines. Curr HIV/AIDS Rep 2007;4:141-46.
30. Pai VB, Heyneman CA, Erramouspe J. Conjugated heptavalent pneumococcal vaccine. Ann Pharmacother 2002;36:1403-13.
31. Overturf GD. American Academy of Pediatrics. Committee on Infectious Diseases. Technical report: prevention of pneumococcal infections, including the use of pneumococcal conjugate and polysaccharide vaccines and antibiotic prophylaxis. Pediatrics 2000;106:367-76.
32. American Academy of Pediatrics, Committee on Infectious Diseases. Policy Statement: Recommendations for the prevention of pneumococcal infections, including the use of pneumococcal conjugate vaccine (Prevnar), pneumococcal polysaccharide vaccine, and antibiotic prophylaxis. Pediatrics 2000;106:362-66.
33. New York State Department of Health. Immunologic considerations in HIV-infected children. New York State Department of Health 2003:20. Available from: http://www.hivguidelines.org/GuidelineDocuments/p-imm.pdf
34. Geretti AM. British HIV Association Guidelines for Immunization of HIV infected Adults 2008. HIV Medicine 12/16/2008. Available from: http://www.medscape.com/viewarticle/583957
35. European Centre for Disease Prevention and Control (ECDC). Priority risk groups for influenza vaccination. Stockholm, August 2008.
36. Prevention of secondary disease: preventive medicine. Available from: http://www.hivguidelines.org/GuideLine.aspx?pageID=260&guideLineID=86

37. Skiest DJ, Machala T. Comparison of the effects of acute influenza infection and Influenza vaccination on HIV viral load and CD4 cell counts. J Clin Virol 2003;26:307-15.
38. Harpole LH, Kelley MJ, Schreiber G, et al. Assessment of the scope and quality of clinical practice guidelines in lung cancer. Chest 2003;123:7S-20S.
39. Nast A, Spuls P, Ormerod A, Reytan N, Saiag P, Smith C, Rzany B. A critical appraisal of evidence-based guidelines for the treatment of psoriasis vulgaris: 'AGREE-ing' on a common base for European evidence-based psoriasis treatment guidelines. J Eur Acad Dermatol Venereol 2009;23:782-87.
40. Vlayen J, Aertgeerts B, Hannes K, et al. A systematic review of appraisal tools for clinical practice guidelines: multiple similarities and one common deficit. Int J Qual Health Care 2005;17:235-42.
41. Burgers JS, Fervers B, Haugh M, et al. International assessment of the quality of clinical practice guidelines in oncology using the Appraisal of Guidelines and Research and Evaluation Instrument. J Clin Oncol 2004;22:2000-07.

4.5 Publications

1. Guarino A, De Marco G, Lo Vecchio A, **Giannattasio A**, Morciano C.
   Implementation of guidelines is the key of success to reduce inappropriate hospitalization. Post Publication peer reviews to Reducing inappropriate hospital use on a general pediatric inpatient unit.
   Pediatrics 2008;121:e1068-e1073.
   http://pediatrics.aappublications.org/cgi/eletters/121/5/e1068

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 o 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 has 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

1. Iorio R, **Giannattasio A**, Vespere G, Vegnente A.
   LKM-1 antibody and Interferon therapy in children with chronic hepatitis C.
   J Hepatol 2001;35:685-87.
2. Guarino A, **Giannattasio A**, De Marco G.
   Terapie non convenzionali per le malattie infettive.
   Prospettive in Pediatria 2003;33:199-208.
3. Iorio R, Sepe A, **Giannattasio A**, Vecchione R, Vegnente A.
   Lack of benefit of gluten-free diet on autoimmune hepatitis in a boy with celiac disease.
   J Pediatr Gastroenterol Nutr 2004;39:207-10.
4. Guarino A, **Giannattasio A**, Di Caro S.
   Terapie non convenzionali per le malattie infettive.
   Area Pediatrica 2004;5:7-12.
5. Iorio R, Sepe A, **Giannattasio A**, Cirillo F.
   Approccio al lattante colestatico.
   Bollettino SIGENP 2005;13:23-24.
6. Iorio R, Sepe A, **Giannattasio A**, Cirillo F, Vegnente A.
   Hypertransaminasemia in childhood as a marker of genetic liver disorders.
   J Gastroenterology 2005;40:820-26.
7. Iorio R, **Giannattasio A**, Sepe A, Terracciano LM, Vecchione R, Vegnente A.
   Chronic hepatitis C in childhood: a 18-year experience.
   Clin Infect Dis 2005;41:1431-37.
8. Iorio R, Sepe A, **Giannattasio A**, Cirillo F, Spagnuolo MI, Franzese A, Fontana S, Aufiero D, Perna F, Vegnente A, Matarese G.
   Immune phenotype and serum leptin in children with obesity-related liver disease.
   J Clin Endocrinol Metab 2006;91:341-44.
9. **Giannattasio A**, D'Ambrosi M, Volpicelli M, Iorio R.
   Steroid therapy for a case of severe drug-induced cholestasis.
   Ann Pharmacother 2006;40:1196-99.
10. Iorio R, **Giannattasio A**, Lamberti E, Della Corte C, Nicastro E, Spagnuolo MI.
    Hyper-gamma-glutamyltransferase is commonly present in non-breast-fed infants with biliary atresia successfully treated with portoenterostomy.
    Clin Chem 2006;52:1430.

11. **Giannattasio A**, Spagnuolo MI, Sepe A, Valerio G, Vecchione R, Vegnente A, Iorio R.
Is HCV infection associated with liver steatosis also in children?
J Hepatol 2006;45:350-54.
12. Cirillo F, **Giannattasio A**, D'Alessandro L, Iorio R.
Un commento al BET: diversi punti di vista.
Bollettino SIGENP 2006;14:20-21
13. **Giannattasio A**, Spagnuolo MI, Sepe A, Valerio G, Vecchione R, Vegnente A, Iorio R.
Is HCV infection associated with liver steatosis also in children?
(Letter to the Editor) J Hepatol 2006;45:758-59.
14. **Giannattasio A**, Cirillo F, Liccardo D, Farina A.
Epatite cronica HCV correlata in età correlata in età pediatrica: qual'è il reale rischio di epatotrapainto?
Bollettino SIGENP 2006;14:18-19.
15. Iorio R, **Giannattasio A**.
Le vaccinazioni opportune. Epatite A.
Collana monografica Società Italiana Pediatria. Pacini Editore 2006, 229-36.
16. **Giannattasio A**, Lenta S, Capuano G, Iorio R, Vajro P.
Novità in epatologia pediatrica.
Prospettive in Pediatria, 2006:108-09.
17. Bortolotti F, Jorio R, Resti M, Cammà C, Marcellini M, Giacchino R, Marazzi MG, Verucchi G, Zancan L, Barbera C, Maggiore G, Vajro P, **Giannattasio A**, Bartolacci S.
Epidemiological profile of 806 Italian children with hepatitis C virus infection over a 15-year period.
J Hepatol 2007;46:783-90.
18. Iorio R, **Giannattasio A**, Cirillo F, D'Alessandro L, Veggente A.
Long-term outcome of children with chronic hepatitis B: a 24-year observation period.
Clin Infect Dis 2007;45:943-49.
19. Iorio R, Verrico A, **Giannattasio A**.
Is liver biopsy mandatory in children with chronic hepatitis C?
World J Gastroenterol 2007;713:4025-26.
20. Giacomet V, Tarallo L, De Marrco G, **Giannattasio A**, De Martino M, Guarino A.
Preparing for an influenza pandemic in Italy: resources and procedures in paediatric hospital units.
Eurosurveillance 2007;12:E7-8.

21. Guarino A, **Giannattasio A**, Volpicelli M, Squeglia V, Barbarino A.
Le gastroenteriti: terapia e prevenzione.
Pediatria preventiva e sociale 2007;2:119-20.

22. **Giannattasio A**, Cirillo F, Liccardo D, Russo M, Vallone G, Iorio R.
Diagnostic role of US for biliary atresia.
Radiology 2008;247:912.

23. Iorio R, , Cirillo F, Telizzi V, **Giannattasio A**.
Children with chronic hepatitis C: what future?
Hepatology 2008;48:691-92.

24. **Giannattasio A**, Lo Vecchio A, Guarino A.
Impatto delle vaccinazioni su larga scala: influenza e Rotavirus.
Vnews vaccination update 2008; vol 2:2-9.

25. Guarino A, De Marco G, Lo Vecchio A, **Giannattasio A**, Morciano C.
Implementation of guidelines is the key of success to reduce inappropriate hospitalization.
Post Publication peer reviews to Reducing inappropriate hospital use on a general pediatric inpatient unit.
Pediatrics 2008;121:e1068-e1073
http://pediatrics.aappublications.org/cgi/eletters/121/5/e1068

26. A Guarino, **A Giannattasio**, E Bruzzese.
La prevenzione in gastroenterologia pediatrica.
Collana monografica Società Italiana Pediatria. Pacini Editore 2008, 80-95

27. Galli L, Puliti D, Chiappini E, Gabiano C, Ferraris G, Mignone F, Viganò A, Giaquinto C, Genovese O, Anzidei G, Badolato R, Buffolano W, Maccabruni A, Salvini F, Cellini M, Ruggeri M, Manzionna M, Bernardi S, Tovo P, de Martino M; Italian Register for HIV Infection in Children.
Is the interruption of antiretroviral treatment during pregnancy an additional major risk factor for mother-to-child transmission of HIV type 1?.
Clin Infect Dis 2009;48:1310-17.

28. Pandolfi E, Marino MG, Gesualdo F, Romano M, **Giannattasio A**.
Vaccinare i bambini con patologia cronica? E' meno difficile di quello che sembra!
RIAP 02/2009:15-21.

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.
Ig Sanità Pubbl 2009; 65: 183-192.

30. **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

31. **Giannattasio A**, Barbarino A, Lo Vecchio A, Bruzzese E, Mango C, Guarino A. Effects of antiretroviral drug recall on perception of therapy benefits and on adherence to antiretroviral treatment in HIV-infected children.
Curr HIV Res 2009;7:468-72.

32. **Giannattasio A**, Cirillo F, Terlizzi V, Liccardo D, Vecchione R, Iorio R. Hepatic steatosis is an unusual finding in children with chronic hepatitis B.
J Clin Virol 2009;46:360-62.

33. **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)

6.2 Grants

1. Nuovi modelli assistenziali per l'ottimizzazione della copertura vaccinale in bambini a rischio in Regione Campania.
   Progetto in convenzione con la Provincia di Caserta approvato in data 19 ottobre 2006.

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.