MAJOR ARTICLE

Long-Term Benefit of Hepatitis B Vaccination among Children in Thailand with Transient Hepatitis B Virus Infection Who Were Born to Hepatitis B Surface Antigen–Positive Mothers

Yong Poovorawan,[1] Voranush Chongsrisawat,[1] Apiradee Theamboonlers,[1] Karthik Srinivasa,[3] Yanee Hutagalung,[2] Hans L. Bock,[4] and Bernard Hoet[4]

[1]Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University, and [2]GlaxoSmithKline Biologicals, Bangkok, Thailand; [3]GlaxoSmithKline Biologicals, Bangalore, India; [4]GlaxoSmithKline Biologicals, Rixensart, Belgium

Background. Transmission of hepatitis B virus (HBV) from carrier mothers to their babies appears to be one of the most important factors influencing the prevalence of chronic HBV infection in areas of high hepatitis B endemicity.

Methods. Infants born to HBV surface antigen (HBsAg)–positive mothers who were or were not positive for HBV e antigen (HBeAg) or to mothers who were negative for both HBsAg and HBeAg have been followed for 17 years for serological evidence of HBV infection. These infants were divided into 2 groups on the basis of their hepatitis B vaccination protocols: group 1 received vaccine at birth and 1, 2, and 12 months later, and group 2 received vaccine at birth and 1 and 6 months later. Follow-up involved annual clinic visits, during which a blood sample was taken and analyzed for the presence of HBsAg, antibody to HBsAg, and antibody to HBV core antigen (HBcAg). Selected blood samples that tested positive for HBV markers during $\geq$2 consecutive visits separated by a long interval were further investigated by polymerase chain reaction to detect HBV DNA.

Results. Transient presence of HBsAg or transient and/or long-term presence of antibody to HBcAg suggested that this population was heavily exposed to HBV during the follow-up period. Despite these findings, no new cases of chronic HBV infection were observed. None of the subjects with transient presence of HBsAg had any clinical symptoms of liver disease.

Conclusions. This study demonstrates the efficacy of the HBV vaccine and its ability to protect against symptomatic disease.

Hepatitis B is recognized as a serious public health problem worldwide. It is estimated that 2 billion people have serological evidence of hepatitis B virus (HBV) infection and that 350 million people are chronically infected with HBV [1–3]. Epidemiologic studies in the 1970s and 1980s demonstrated the correlation between chronic HBV infection and chronic liver disease, including hepatocellular carcinoma [4, 5]. The frequency and pattern of HBV transmission vary markedly across the world. In areas of high hepatitis B endemicity, such as Southeast Asia, the prevalence of HBV carriage is high, and infection during infancy and early childhood is common [6]. The risk of perinatal infection is higher when the mother, at the time of delivery, is serologically positive for HBV e antigen (HBeAg), which is linked with high serum concentrations of HBV DNA [7]. If a mother is positive for both HBV surface antigen (HBsAg) and HBeAg at the time of delivery, her children have a 70%–90% risk of becoming chronically infected [8]. Consequently, the long-term sequelae of

Received 29 June 2008; accepted 27 January 2009; electronically published 27 May 2009.

Potential conflicts of interest: Y.P., V.C., and A.T. received financial support from and K.S., Y.H., H.L.B, and B.H. are current employees of GlaxoSmithKline Biologicals.

Presented in part: 12th International Symposium on Viral Hepatitis and Liver Disease, 1–5 July 2006, Paris, France (poster P522).

Financial support: GlaxoSmithKline Biologicals (trial numbers 103860/271, 103860/272, and 103860/273); Commission of Higher Education, Ministry of Education, Thailand (to Y.P., V.C., and A.T.).

Reprints or correspondence: Prof. Yong Poovorawan, Center of Excellence in Clinical Virology, Dept. of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand (Yong.P@chula.ac.th).

The Journal of Infectious Diseases 2009; 200:33–8

0022-1899/2009/20001-0007$15.00
DOI: 10.1086/599331

Table 1. Typical interpretation of serological test results for hepatitis B virus (HBV) infection.

Test results, by antigen			
HBsAg	Total anti-HBc	Anti-HBs	Interpretation
Negative	Negative	Negative	Never infected
Positive	Negative	Negative	Early acute infection; transient up to 18 days after infection
Positive	Positive	Negative	Acute or chronic infection depending on the timing of the cohort study or the supportive anti-HBc IgM result
Negative	Positive	Positive or negative	Acute resolving infection
Negative	Positive	Positive	Recovered from past infection and immune
Negative	Positive	Negative	False positive (i.e., susceptible); past infection; low-level chronic infection or passive transfer of anti-HBc to infant born to HBsAg-positive mother
Negative	Negative	Positive	Immune if anti-HBs concentration is ⩾10 mIU/mL; passive transfer after HBV immunoglobulin administration

NOTE. Data are from a report by Mast and Ward [7]. Anti-HBc, antibody to HBV core antigen; anti-HBs, antibody to HBsAg; HBsAg, HBV surface antigen; IgM, immunoglobulin M.

chronic HBV infection (i.e., chronic active hepatitis, cirrhosis, and hepatocellular carcinoma) are major causes of morbidity and mortality among adults in hepatitis B–endemic regions of the world [9, 10].

All major health authorities agree that the most effective approach to reducing the burden of hepatitis B is to prevent HBV infection. Since 1992, when the World Health Organization recommended that HBV vaccine be integrated into national immunization programs by 1997, there has been a worldwide effort to reduce the transmission of HBV [11].

We report data obtained during long-term follow-up (duration, 15–17 years) from 3 ongoing prospective studies to determine the protection induced by a monovalent hepatitis B vaccine (Engerix-B; GlaxoSmithKline Biologicals) administered to neonates born to mothers classified on the basis of HBsAg and HBeAg serostatus. Data obtained previously from these trials have been published elsewhere [12–16].

SUBJECTS, MATERIALS, AND METHODS

Healthy infants born to HBsAg-positive mothers who were or were not positive for HBeAg or to mothers who were negative for both HBsAg and HBeAg were enrolled in the 3 neonatal primary vaccination studies (i.e., HBV-064, HBV-115, and HBV-143) after written informed consent was obtained from their mothers. All 3 studies were conducted in Thailand; approved by the ethics committee of the Faculty of Medicine, Chulalongkorn Hospital; and conducted in accordance with the Declaration of Helsinki and the standards of Good Clinical Practice.

The primary studies aimed to evaluate the antibody response associated with different vaccination schedules, which, for a subset of infants, included administration of HBV immunoglobulin at birth. Infants received a vaccine containing 10 μg of recombinant HBV ⩽12 h after birth and again 1, 2, and 12 months later or 1 and 6 months later. During the primary phase in the 2 studies (HBV-064 and HBV-115) for which the final primary dose was administered 12 months after birth, blood samples were taken at the time the first dose was administered and 2, 6, 9, 12, and 13 months later. For the third study (HBV-143), in which participants were vaccinated at birth and 1 and 6 months later, blood samples were obtained at the time the first dose was administered and 1, 2, 6, and 9 months later. Follow-up is ongoing and scheduled to end 20 years after receipt of the first vaccine dose. Participants annually visit the study site, where blood samples are taken and assayed for the presence of HBV markers. The present article describes the observations made on pooled data collected during 15–17 years of follow-up. Data from 15 years of follow-up were available for 117 subjects, data from 16 years of follow-up were available for 20, and data from 17 years of follow-up were available for 45.

Serum samples were analyzed for the presence of HBsAg, antibodies to HBsAg (anti-HBs), and antibodies to HBV core antigen (anti-HBc) at all time points during which blood samples were collected, using commercial immunoassay kits. Auszyme II/AusriaII was used to detect HBsAg, AxSYM CORE was used to detect total anti-HBc, and AUSAB was used to detect anti-HBs (all 3 assays were produced by Abbott Diagnostics). For blood samples that tested positive for HBsAg and/or anti-HBc, polymerase chain reaction (PCR) analysis was used for detection of HBV DNA.

Data were reviewed to investigate whether, regardless of their mother's HBV antigen status, their primary vaccination schedule, or their receipt of HBV immunoglobulin at birth, subjects who were exposed to HBV developed clinically apparent HBV infection. The course of HBV infection is typically tracked using the serological markers shown in table 1. Serological data in this study were manually reviewed, and resulting immunolog-

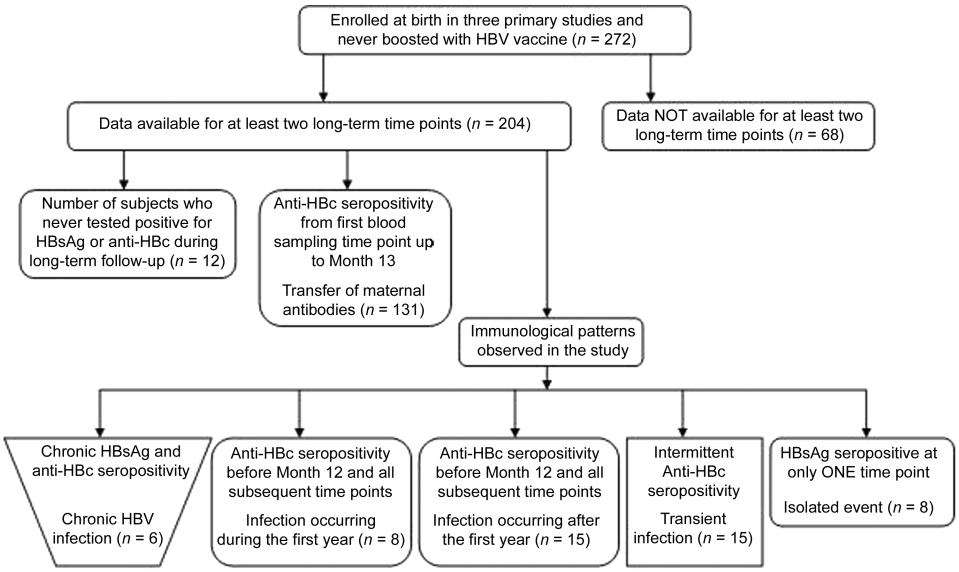

Figure 1. Flow chart depicting immunological patterns observed among participants in a study to determine the long-term benefit of hepatitis B vaccination. anti-HBc, antibody to HBV core antigen; HBsAg, HBV surface antigen; HBV, hepatitis B virus.

ical patterns were classified as outlined in figure 1. Positive results of serological tests for detection of HBsAg and anti-HBc were used as indicators of HBV infection. Seroconversion was defined as the appearance of a serological marker in the serum of subjects who had previously been seronegative. A seronegative subject was classified as one who had no detectable serum markers. An unexpected increase in the anti-HBs level between 2 consecutive follow-up visits (neither of which was associated with receipt of vaccine) was defined as a 4-fold increase in the anti-HBs concentration, when the concentration in the first sample was <100 mIU/mL, and as a 2-fold increase, when the concentration in the first sample was ⩾100 mIU/mL.

The following patterns were identified in the study: (1) chronic HBV infection: seropositivity for anti-HBc and HBsAg at all time points; (2) infection during the first year of life: seroconversion for anti-HBc during the first year of life and seropositivity at all consecutive time points; (3) infection occurring after the first year of life: seroconversion for anti-HBc after the first year of life and seropositivity for anti-HBc at all consecutive time points; (4) isolated anti-HBc seropositivity; (5) isolated HBsAg seropositivity; and (6) not infected: no test positive for HBsAg or anti-HBc, except for subjects with isolated anti-HBc positivity up to month 13 of follow-up, for whom a test positive for anti-HBc was considered indicative of passive transfer of maternal antibodies.

RESULTS

Of the 428 subjects enrolled in the 3 primary studies, 272 did not receive an HBV vaccine booster and were considered for this report. Of these 272 subjects, 204 had data from at least 2 long-term follow-up visits (i.e., visits after the primary vaccination phase) and were eligible for inclusion in the current review. Table 2 shows the characteristics of the cohort, including HBV vaccination history and maternal serological status. The mean age ($\pm$SD) of subjects who returned for the follow-up visits at years 15–17 ranged from 14.5 $\pm$ 0.5 years to 16.6 $\pm$ 0.5 years. More than 10,110 serological readouts were available from the follow-up visits.

Of the 204 subjects with data available, 131 had either no signs of HBV exposure or had isolated anti-HBc positivity limited to the first 13 months of age. This profile is compatible with a passive transfer of anti-HBc antibodies from mother to child and was primarily observed in infants born to HBsAg-positive mothers.

Six subjects were seropositive for HBsAg and anti-HBc at all available time points during or after the first year of life, as previously reported [12, 13]. These subjects were considered to have acquired chronic HBV infection at birth or before completing the vaccination course, an hypothesis that accords with previously published reports of chronic HBV infection [17]. Mothers of all 6 chronically infected subjects were positive for both HBsAg and HBeAg. Only 2 of 6 subjects had responded to vaccination by achieving an anti-HBs concentration of ⩾10 mIU/mL, the concentration considered protective against disease.

An additional 53 subjects were seropositive for anti-HBc at some time point after month 12. Despite detection of anti-HBc at all time points in some subjects and intermittent detection of anti-HBc in others, none had clinical signs of infection. PCR

Table 2. Characteristics of groups in primary studies to determine the long-term benefit of hepatitis B vaccination.

Primary study, group	Enrolled subjects, no.	Vaccination schedule	Mother's HBV antigen serostatus		HBV Ig detected at birth	Duration of follow-up (no. of subjects considered for review)
			HBsAg	HBeAg		
HBV-064		0, 1, 2, 12 months				17 years (82)
1	46	…	Positive	Positive	No	…
2	52	…	Positive	Negative	No	…
3	24	…	Negative	Negative	No	…
HBV-115		0, 1, 2, 12 months				16 years (55)
1	66	…	Positive	Positive	Yes	…
HBV-143		0, 1, 6 months				15 years (67)
1	43	…	Positive	Positive	Yes	…
2	41	…	Positive	Positive	No	…

NOTE. HBeAg, HBV e antigen; HBsAg, HBV surface antigen; HBV, hepatitis B virus; Ig, immunoglobulin.

findings were available for 28 of these subjects. Twenty-two tested negative, and only 6 tested positive for HBV DNA.

Eight subjects were seropositive for HBsAg at 1 or 2 isolated time points. For 4 subjects, this result was associated with detection of HBV DNA by PCR, whereas for the other 4, no other HBV markers were detected. No interpretable results of HBsAg testing were available for 45 subjects.

Serological signs of HBV infection were not consistently associated with increases in levels of circulating anti-HBs. Also, some subjects showed serological evidence of HBV infection despite having an anti-HBs level of up to >10,000 mIU/mL. Furthermore, increases in the anti-HBs concentration were observed independent of vaccine administration or other changes in immunological patterns suggestive of infection or challenge with HBV. Among the 204 subjects included in the analyses, 61 cases (involving 56 subjects) of unexpected increase in anti-HBs were observed. Evaluation of the serological status in terms of anti-HBc test results, HBsAg test results, and PCR results at the time points immediately before, immediately after, and at the time of the unexpected increase in the anti-HBs concentration showed no obvious correlation with any other serological signs of infection (table 3).

This study was not designed to analyze the patterns observed as a function of the primary vaccination schedules. However, there was no indication that the patterns observed differed according to the primary vaccination schedule.

DISCUSSION

The purpose of this study was to observe the protection induced by HBV vaccine when administered during infancy to subjects born in an area of high hepatitis B endemicity to HBsAg-positive mothers who were or were not positive for HBeAg or to mothers who were negative for both HBsAg and HBeAg. This study was not designed to analyze the patterns observed as a function of the vaccination schedules. However, there was no indication that the patterns observed differed according to the primary vaccination schedule. Likewise, no obvious differences were observed between subjects who received HBV immunoglobulin at birth and those who did not. Furthermore, children of HBsAg- and HBeAg-positive mothers were more likely to have maternal antibodies detected than were those whose mothers had a different serostatus.

Data encompassing up to 17 years of follow-up showed that 6 of 204 teenagers evaluated had serological evidence of chronic HBV infection but that none of the 6 had developed clinical illness by the final time point of observation. Factors that were associated with most of the chronic HBV infections were inability to respond to vaccination with a protective level of HBV IgG antibodies and having a mother who was positive for both HBsAg and HBeAg.

Furthermore, among an additional 53 subjects who were seropositive for anti-HBc either consistently or intermittently (evidence of natural infection), no clinical symptoms were experienced, with intermittent viremia detected only in a few. These cases could have represented mild, aborted, or occult infections. This suggests that the duration of the viremic phase of the natural infection was short, which further suggests little

Table 3. Detection of hepatitis B virus (HBV) serological markers before, at the time of, and after the unexpected increase in the level of antibody to HBV surface antigen (HBsAg).

Marker	Cases detected, %		
	Before increase	At the time of increase	After increase
HBsAg	3.3	1.6	1.6
Anti-HBc	14.7	22.9	18.0
HBV DNA[a]	1.6	0	0

NOTE. Data are for 61 cases of an unexpected increase (involving 56 subjects). Anti-HBc, antibody to HBV core antigen.

[a] Detected by polymerase chain reaction.

risk for transmission to others and no risk for long-term viremia (i.e., chronic disease).

Detection of HBsAg and HBV DNA in isolated samples possibly indicated rapidly eliminated infection. Occult hepatitis is defined by the persistent presence of HBV DNA in serum or liver in the absence of HBsAg [18, 19]. Because only HBsAg-positive and/or anti-HBc–positive samples were analyzed for HBV DNA, this study did not allow the detection of persistent HBV DNA in subjects who lacked other immunological signs of HBV infection.

The observed increases in the anti-HBs concentration in subjects who had not recently received vaccine, who had no other changes in serological HBV markers, and/or who lacked clinical symptoms of acute hepatitis are believed to represent natural challenges with HBV in this population. The presence of HBsAg-specific memory cells after HBV vaccination is suggested by epidemiological data in a number of studies that showed the absence of disease in vaccinated populations and is proven by demonstration of an anamnestic response after revaccination [20, 21]. These data indicate that this study population has had frequent challenges from HBV, including brief viral incubation, aborted infections, and immunological patterns compatible with acute infection. Of primary interest was that no clinical symptoms of HBV disease were observed over the extended follow-up period.

The results of this study indicate that HBV vaccination confers persistent clinical protection against HBV infection to recipients for at least 17 years and have important implications for older adolescents and young adults who received postexposure prophylaxis against HBV infection as infants in areas of high endemicity. Data imply that there is no need for booster doses of HBV vaccine in such areas, which is especially important in resource-poor countries. Although these results may not be universally applicable to areas of low endemicity, they are very encouraging. It must also be recognized that isolated anti-HBc and/or HBsAg positivity could have been false-positive results and thus must be interpreted cautiously. However, other studies involving long-term follow-up (duration, 10–18 years) of infants, children, and adults from industrialized and developing counties who were vaccinated against HBV have shown that detection of HBsAg was rare and, if present, only transient. Six vaccine recipients acquired a chronic carrier state in the first year of life, as described elsewhere [22–24]. During the 17 years of postvaccination follow-up, no new cases of chronic HBV carriage were reported.

From this ongoing long-term study that will terminate after 20 years of follow-up, additional information will be gathered on the duration of the protective effects of HBV vaccination in subjects whose immune response is repeatedly challenged through contact with HBV.

Acknowledgments

We thank the staff of the Center of Excellence in Clinical Virology, Chulalongkorn University (Bangkok, Thailand), for taking care of the subjects during the long-term follow-up period; the Commission of Higher Education, Ministry of Education (Bangkok), for their support; Vidya Virajith, Janice Beck, and Veronique Delpire, for providing technical assistance with and coordinating the preparation of the manuscript on behalf of GSK Biologicals; and Jeanne-Marie Jacquet and Maarten Leyssen, for their critical review of the manuscript.

References

1. Beasley RP, Hwang LY. Hepatocellular carcinoma and hepatitis B virus. Semin Liver Dis **1984**; 4:113–21.
2. Poovorawan Y, Theamboonlers A, Vimolket T, et al. Impact of hepatitis B immunization as part of the EPI. Vaccine **2000**; 19:943–9.
3. Goldstein ST, Zhou F, Hadler SC, et al. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. Int J Epidemiol **2005**; 34:1329–39.
4. Beasley RP, Hwang LY. Overview of the epidemiology of hepatocellular carcinoma. In: Hollinger FB, Lemon SM, Margolis HS, eds. Viral hepatitis and liver disease: proceedings of the 1990 International Symposium on Viral Hepatitis and Liver Disease. Contemporary Issues and Future Prospects. Baltimore, MD: Williams & Wilkins, **1991**:532–5.
5. Beasley RP. Hepatitis B virus: the major etiology of hepatocellular carcinoma. Cancer **1988**; 61:1942–56.
6. Lolekha S, Warachit B, Hirunyachote A, Bowonkiratikachorn P, West DJ, Poerschke G. Protective efficacy of hepatitis B vaccine without HBIG in infants of HBeAg-positive carrier mothers in Thailand. Vaccine **2002**; 20:3739–43.
7. Mast EE, Ward JW. Hepatitis B vaccines. In: Plotkin SA, Orenstein WA, Offitt PA, eds. Vaccines. 5th ed. Philadelphia, PA: Elsevier, **2008**: 205–41.
8. Beasley RP, Trepo C, Stevens CE, et al. The e antigen and vertical transmission of HBsAg. Am J Epidemiol **1977**; 105:94–8.
9. Mast EE, Margolis HS, Fiore AE, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: immunization of infants, children, and adolescents. MMWR Recomm Rep **2005**; 54(RR-16):1–31.
10. Kane M. Global programme for control of hepatitis B infection. Vaccine **1995**; 13:S47–9.
11. World Health Organization. Expanded programme on immunization: Global Advisory Group—part 1. Wkly Epidemiol Rec **1992**; 67:11–5.
12. Poovorawan Y, Sanapavat S, Pongpunglert W, et al. Long-term efficacy of hepatitis B vaccine in infants born to hepatitis B e antigen positive mothers. Pediatr Infect Dis J **1992**; 11:816–21.
13. Poovorawan Y, Sanpavat S, Chumdermpadetsuk S, Safary A. Long-term hepatitis B vaccine in infants born to hepatitis B e antigen positive mothers. Arch Dis Child Fetal Neonatal Ed **1997**; 77:F47–51.
14. Poovorawan Y, Sanpavat S, Pongpunglert W, et al. Comparison of recombinant DNA hepatitis B vaccine alone or in combination with hepatitis B immune globulin for the prevention of perinatal acquisition of hepatitis B carriage. Vaccine **1990**; 8(Suppl):S56-9.
15. Poovorawan Y, Sanpavat S, Pongpunlert W, et al. Protective efficacy of a recombinant DNA hepatitis B vaccine in neonates of HBe antigen positive mothers. JAMA **1989**; 261:3278–81.
16. Poovorawan Y, Chongsrisawat V, Hutagalung Y, Srinavasa K, Hoet B. Long-term efficacy of hepatitis B vaccination of newborns born of hepatitis B surface antigen-positive mothers in Thailand [abstract P522]. In: Program and abstracts of the 12th International Symposium on Viral Hepatitis and Liver Disease, 1–5 July 2006 (Paris, France).
17. Pol S, Michel ML. Therapeutic vaccination in chronic hepatitis B virus carriers. Expert Rev Vaccines **2006**; 5:707–16.

18. Hou J, Wang Z, Cheng J, et al. Prevalence of naturally occurring surface gene variants of hepatitis B virus in nonimmunized surface antigen-negative Chinese carriers. Hepatology **2001**; 34:1027–34.
19. Hu KQ. Occult hepatitis B virus infection and its clinical implications. J Viral Hepat **2002**; 9:243–57.
20. Dentico P, Crovari P, Lai PL. Anamnestic response to administration of purified non-adsorbed hepatitis B surface antigen in healthy responders to hepatitis B vaccine with long-term protective antibody titers. Vaccine **2002**; 20:3725–30.
21. Banatvala J, Van Damme P, Oehen S. Lifelong protection against hepatitis B: the role of immunogenicity in immune memory. Vaccine **2000**; 19:877–85.
22. Dentinger CM, McMahon BJ, Butler JC, et al. Persistence of antibody to hepatitis B and protection from disease among Alaska natives immunized at birth. Pediatr Infect Dis J **2005**; 24:786–92.
23. Banatvala JE, Van Damme P. Hepatitis B vaccine– do we need boosters? J Viral Hepat **2003**; 10:1–6.
24. Zanetti AR, Marian A, Romano L, et al. Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. Lancet **2005**; 366:1379–84.