

# 20 Years into the Gambia Hepatitis Intervention Study: Assessment of Initial Hypotheses and Prospects for Evaluation of Protective Effectiveness Against Liver Cancer

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## Abstract

Primary hepatocellular carcinoma is the commonest cancer in The Gambia. The Gambia Hepatitis Intervention Study (GHIS) was established in 1986 to evaluate the protective effectiveness of infant hepatitis B immunization in the prevention of chronic liver disease, particularly, hepatocellular carcinoma and cirrhosis later in adult life. This program was designed based on a series of assumptions. Here, we used data from observational and epidemiologic studies developed since 1986 to examine the validity of these assumptions. We found that (a) hepatitis B vaccine coverage was 15% more than originally assumed, (b) protection against hepatitis B virus (HBV) infection was not dependent on

the number of vaccine doses received, (c) perinatal infection with HBV was of negligible importance, and (d) the HBV attributable risk of hepatocellular carcinoma at age <50 was 70% to 80%, lower than initially assumed. Based on these data, the final outcome of the GHIS should be measurable from 2017, sooner than originally assumed. The GHIS strategy takes into account-specific patterns of virus epidemiology and natural history of hepatocellular carcinoma in Africa and provides a model for integrating and evaluating new vaccines into the Expanded Programme of Immunization of sub-Saharan African countries. (Cancer Epidemiol Biomarkers Prev 2008;17(11):3216–23)

## Introduction

Hepatocellular carcinoma is the most frequent form of primary liver cancer and is a major cause of death in sub-Saharan Africa and eastern Asia (1). The main etiologic factor in these regions is chronic infection with hepatitis B virus (HBV). Other factors that contribute to the

etiology of primary liver cancer include hepatitis C virus (HCV) and dietary exposure to aflatoxins, a group of mycotoxins that are natural contaminants of the staple diet. The latter has a multiplicative effect in conjunction with HBV infection on the risk of developing hepatocellular carcinoma (2–4).

Several vaccination programs aimed at evaluating the efficacy of hepatitis B vaccination against primary liver cancer have been developed worldwide. An ecological study in Taiwan following the introduction of nationwide hepatitis B immunization has shown a sharp decrease of childhood primary liver cancer among children vaccinated with hepatitis B (5, 6). However, only two randomized field trials have been implemented to test the protective efficacy of childhood hepatitis B vaccination against primary liver cancer, in the Qidong Province, China (7), and in The Gambia (8).

The Gambian Hepatitis Intervention Study (GHIS) is a collaborative undertaking by the IARC, The Government of the Republic of The Gambia, and the Medical Research Council of the United Kingdom. This program was launched in 1986 with the objective of evaluating the effectiveness of hepatitis B vaccination in childhood for the prevention of HBV infection, chronic liver

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This manuscript is dedicated to the memory of Lorenzo Tomatis (deceased in September 2007), whose insights and leadership as director of IARC (1982–1994) made this project possible.

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disease, and hepatocellular carcinoma in adulthood in a population at high risk (8). The implementation of GHIS involves three overlapping phases. During phase I (1986-1990), hepatitis B vaccine, approved by the WHO, was phased into the Gambian Expanded Programme of Immunization over a 4-year period from July 1986 to February 1990. Two groups of children were recruited, one of about 60,000 children who received all Expanded Programme of Immunization vaccines (Bacillus Calmette-Guerin, diphtheria-pertussis-tetanus, polio, measles, yellow fever) and the other of a similar number of children who received all vaccines plus hepatitis B. Since February 1990, hepatitis B vaccination has been offered to all newborns as part of the Expanded Programme of Immunization in The Gambia. During Phase II (1991-1997), the efficacy of hepatitis B vaccine against infection and chronic carriage has been estimated through longitudinal and cross-sectional surveys in selected groups of hepatitis B-vaccinated and -unvaccinated children. Phase III (initiated in 1998) consists of long-term follow-up through cancer registration, using hepatocellular carcinoma as the primary endpoint.

The original GHIS design was based on a series of assumptions about age-specific cancer incidence, HBV chronic carriage, vaccine coverage, vaccine efficacy against HBV carriage, risk for hepatocellular carcinoma attributable to HBV, and attrition in the cohort over the 35 to 40 years of follow-up. Midway through this program, a wealth of data has been generated on all these aspects. Additional sources of data to test these assumptions are the observational study on vaccine effectiveness in the two Gambian villages of Keneba and Manduar, a sentinel project initiated in 1984, before the inception of GHIS (9), as well as case-control studies on the role of HBV infection in the natural history of hepatocellular carcinoma in The Gambia (4, 10). Based on these data, we present here a critical assessment of the assumptions of GHIS design and of the accomplishment of the program, and we provide an updated estimate of the duration of GHIS follow-up.

### **GHIS Design, Implementation, and Main Results**

The Gambia is the smallest country in Africa and has a population of 1.4 million. Hepatitis B vaccine was introduced into the Gambian Expanded Programme of Immunization using a "stepped-wedge" design (8) in which the vaccine was progressively phased in the Expanded Programme of Immunization over a period of 4 years (the study design available as supplementary material). Recruitment started in July 1986. A four-dose vaccine schedule was used with the first dose given as soon as possible after birth (during the child's first attendance at an infant welfare clinic) and subsequent doses scheduled at the ages of 2, 4, and 9 months. The unit of randomization was the Expanded Programme of Immunization team, stratified according to four ecological zones. This design was deemed to have a statistical power of >70% of that of a conventional individually randomized design (8).

By February 1990, a cohort of 124,577 children was recruited, of whom 61,065 received hepatitis B vaccine. Three methods were set-up for the long-term identifica-

tion of subjects. Firstly, at recruitment, personal details of children were recorded, such as name, parent's name, birth date, sex, health district, health centre of birth, and ethnic group. Secondly, at the age of 4 months or older, dermatoglyphic prints of a hand and foot of each child were taken for confirmation of identity later in life. Thirdly, the usual site of the Bacillus Calmette-Guerin vaccination and the resulting scar were altered for children in the study (left forearm for those who received hepatitis B vaccine, right forearm for those who did not). The site of this vaccination reverted to the left upper arm on completion of recruitment.

Two subgroups of the GHIS cohort have been studied in detail with the aim of evaluating immunogenicity of the vaccine and its efficacy in preventing infection and chronic carriage. Group 1 was a cohort of 1,041 children, including approximately the first 250 hepatitis B-vaccinated children in each of the four ecological zones. These children have been followed up annually to the age of 9 years (except for the 6th and 8th year). Group 2 consisted of two cross-sectional surveys, each including 800 unvaccinated subjects who were 4 and 9 years old, which acted as controls for Group 1. Vaccine efficacy did not significantly change over time and was 84% against infection and 94% against chronic carriage at 9 years of age. No difference in efficacy was observed between the four ecological zones (11, 12).

In July 1986, a population-based National Cancer Registry (NCR) was set up to identify and record data on cancers of all types occurring in The Gambia (13, 14). The cases are ascertained in both public health facilities and private clinics. Confirmation of clinical diagnosis is supported by the histopathology unit of the National Health Laboratory Services program of the country. For hepatocellular carcinoma diagnosis, clinical criteria, ultrasonography, and  $\alpha$ -fetoprotein measurement are used in combination. This criterion was validated against histology in a study on liver cancer patients in Senegal and was shown to be >90% specific for liver cancer diagnosis in the Sahel region of Africa (15). Since 1986, through the network established by the NCR, a register of chronic liver diseases (Chronic Liver Disease register) diagnosed in the Gambia was established and maintained. Any chronic liver disease diagnosis is eligible for inclusion into this register if the diagnosis was done by a medically qualified doctor. However, the final outcome of GHIS will be evaluated through record linkage between hepatocellular carcinoma cases in the NCR and the GHIS database of vaccinated and unvaccinated children. The CLD register will be used for describing the epidemiologic patterns of chronic liver disease in The Gambia.

### **Evaluation of the Initial Assumptions of GHIS**

The GHIS program was designed on the basis of assumptions about cohort attrition, vaccine coverage, hepatitis B vaccine efficacy against carriage, impact of perinatal acquisition of the chronic carrier status, and proportion of hepatocellular carcinoma attributable to HBV. Assuming 75% vaccine coverage and 50% attrition in the cohort (due to death, migration, or incomplete record linkage), it was estimated that slightly >35 years would be needed to obtain unequivocal results. Based on

the data generated since the inception of the program, we have assessed the validity of each of the initial assumptions. In the following section, we present these assumptions as quoted in the original study design and we provide a critical assessment.

### Assumption 1: Vaccine Coverage

**Initial Assumption.** Of the eligible children, 85% would present themselves for at least 1 injection in the hepatitis B vaccination series, 80% would present themselves for at least 2, and 75% for  $\geq 3$  injections.

**Critical Assessment.** Of the estimated number of eligible children (approximately half of the total GHIS cohort of 124,577 children), 98% ( $n = 61,065$ ) received at least 1 dose, 92% ( $n = 55,985$ ) received at least 2 doses, and 81% ( $n = 49,558$ ) received  $\geq 3$  doses. Thus, a greater hepatitis B vaccine coverage than originally expected was achieved in the GHIS cohort vaccinated in infancy with hepatitis B vaccine.

### Assumption 2: Hepatitis B Vaccine Efficacy

**Initial Assumption.** Hepatitis B vaccine efficacy depends on the number of doses received. Among those who are vaccinated and are not infected perinatally, 20% respond after 1 or 2 injections, 80% respond after 1 or 2 injections plus a booster, and 95% would respond after  $\geq 3$  injections. Response to hepatitis B vaccine would be durable due to continuing viral challenge in the environment.

**Critical Assessment.** A level of anti-hepatitis B surface antigen (HBsAg) of  $\geq 10$  IU/mL was found to be a marker of protection against chronic carriage (16). Of the vaccinated children, 98% achieved that level of protection (Table 1). In contrast to the initial assumption, protective responses did not depend on the number of vaccine doses received because  $>95\%$  of children that received at least 1 dose responded to vaccination with anti-HBsAg titers  $\geq 10$  IU/mL and were protected against chronic carriage early in life (11, 12, 16-18).

Several studies have confirmed that there was continuing HBV circulation despite ongoing countrywide vaccination of infants (19) and that there was persisting exposure to HBV in the GHIS cohort (11, 12, 16, 18). In recent follow-ups, the efficacy of hepatitis B vaccination in preventing chronic carriage was consistently high. In a cross-sectional study on adolescents with the age of 15 years, the vaccine efficacy was 67.0% against anti-hepatitis B core positivity [95% confidence interval (95% CI), 58.2-74.6%] and 96.6% against HbsAg carriage (95% CI, 91.5-100%). Thus, vaccination in infants confer

**Table 1. Proportion of children at 1 year of age with anti-HBsAg antibody titers  $\geq 10$  IU/mL by number of doses received (17)**

Doses received	1	2	3	4
Children tested ( $n$ )	47	55	288	376
With titers $\geq 10$ IU/mL ( $n$ )	46	53	283	369
Proportion	98%	96%	98%	98%

an excellent protection against carriage at least until adolescence despite waning titers of anti-HBsAg antibodies at this age (20). A similar situation was observed in the pilot vaccination studies in the two villages of Keneba and Manduar (9).

### Assumption 3: Impact of Perinatal Acquisition of HBV Carrier Status

**Initial Assumption.** Among children who will become HBV carriers, 10% acquire their status perinatally, only 50% of whom would be protected by vaccination.

**Critical Assessment.** Group 1 surveys indicated that only 0.6% to 0.7% of vaccinated children became chronic carriers after vaccination. Of the 4 children who were chronic carriers by the age of 4 years, 3 had HBsAg-positive mothers, out of whom 2 were also hepatitis B surface antigen positive. These children of these two hepatitis B surface antigen mothers had already acquired carrier status by the age of 1 year (17). In addition, they did not respond to the vaccine (anti-HBsAg titer,  $<10$  IU/mL) despite receiving 3 injections plus a booster dose. This observation is consistent with the assumption that 50% of the vaccinated children who become carrier may have acquired their HBsAg carrier status perinatally (11, 12, 18, 21, 22). Thus, based on the evidence derived from the available empirical data, we consider the proportion of subjects infected perinatally as unlikely to influence the estimate of vaccine efficacy against chronic HBV carriage and primary liver cancer.

### Assumption 4: Attributable Risk for HBV

**Initial Assumption.** Under the age of 50 years, 80% to 90% of liver cancer is attributable to HBV.

**Critical Assessment.** The proportion of hepatocellular carcinoma attributable to HBV and HCV infections was assessed in three case-control studies (10, 23) and Hall et al.<sup>9</sup> (but reviewed in ref. 4). Comparison of the earliest to most recent study indicates that this proportion has remained stable over the past 25 years. In the most recent study (10), 197 incident cases of hepatocellular carcinoma and 405 matched hospital-based controls were recruited from the 3 main tertiary referral centers in the country. HBV carriage was present in 63% (124 of 196) of hepatocellular carcinoma cases and 16% (64 of 402) of controls, whereas 19% (33 of 174) of hepatocellular carcinoma cases were HCV seropositive compared with 3% (11 of 382) of controls. Increased hepatocellular carcinoma risk was strongly associated with chronic HBV (odds ratio, 18; 95% CI, 10-32), HCV (odds ratio, 21; 95% CI, 8-54), and dual infection (odds ratio, 35; 95% CI, 4-350). Different patterns of infection were observed according to age at diagnosis. HBsAg positivity decreased with increasing age. The proportion of HbsAg-positive cases was  $>90\%$  in patients  $<45$  years old but decreased to 65% in patients with the age of 45 to 54 years and to 20% to 25% in patients  $>55$  years.

<sup>9</sup> Unpublished observation.

**Table 2. Vaccine efficacy against carriage at 1 year according anti-HBsAg cutoff level (16) and number of doses of hepatitis B vaccine received (17) and estimated vaccine efficacy against hepatocellular carcinoma in adulthood by HBV attributable risk**

Levels of anti-HBsAg Ab	Doses received	VE against carriage at 1 y	VE* against HCC	
			60% AR	70% AR
≥10 IU/mL	1	97%	58%	68%
	≥2	97%	58%	68%

Abbreviations: Ab, antibody; VE, vaccine efficacy; HCC, hepatocellular carcinoma; AR, attributable risk.

Conversely, anti-HCV was detected almost exclusively in older participants because only 3% (2 of 72) of hepatocellular carcinoma cases <45 years of age were anti-HCV positive. The median age at diagnosis was 60 years for HCV-related hepatocellular carcinoma compared with 40 years for HBV-related hepatocellular carcinoma. Taken together, these results suggest that at least between 70% and 80% of hepatocellular carcinoma under the age of 50 years were attributable to HBV, a proportion slightly lower than initially assumed. On the other hand, HCV infection seems relatively unimportant in people with the age of ≤50 years.

### Revised Evaluation of the Duration of Follow-Up

**Vaccine Effectiveness against Hepatocellular Carcinoma.** To critically re-evaluate vaccine effectiveness against hepatocellular carcinoma (Table 2), we assumed attributable risk for HBV to be 70% in The Gambia, which is less than initially assumed in the design of the GHIS project. In addition, we made use of the latest evidence from a cross-sectional study on adolescents in The Gambia, which suggested that the proportion of children protected against chronic carriage at 1 year of age is maintained at later ages (20). In fact, in The Gambia, it was shown that the risk of becoming a carrier from exposure to HBV decreases with age and is 1% to 2% between ages 1 to 14 years (4, 10, 19). Thus, it follows that, in later ages, the effect of acquisition of carriage on

the risk of hepatocellular carcinoma can be considered to be negligible. Furthermore, based on our hypothesis of attributable risk of 70%, the conservative estimate of the protective effectiveness of hepatitis B vaccination against hepatocellular carcinoma that we could reasonably use in this review was 68%. Table 3 shows the approximate number of hepatocellular carcinoma cases expected to occur to have a 95% chance of detecting a significant difference between the vaccinated and unvaccinated groups. It follows that a sample size of about 31 cases of hepatocellular carcinoma is required in the unvaccinated group.

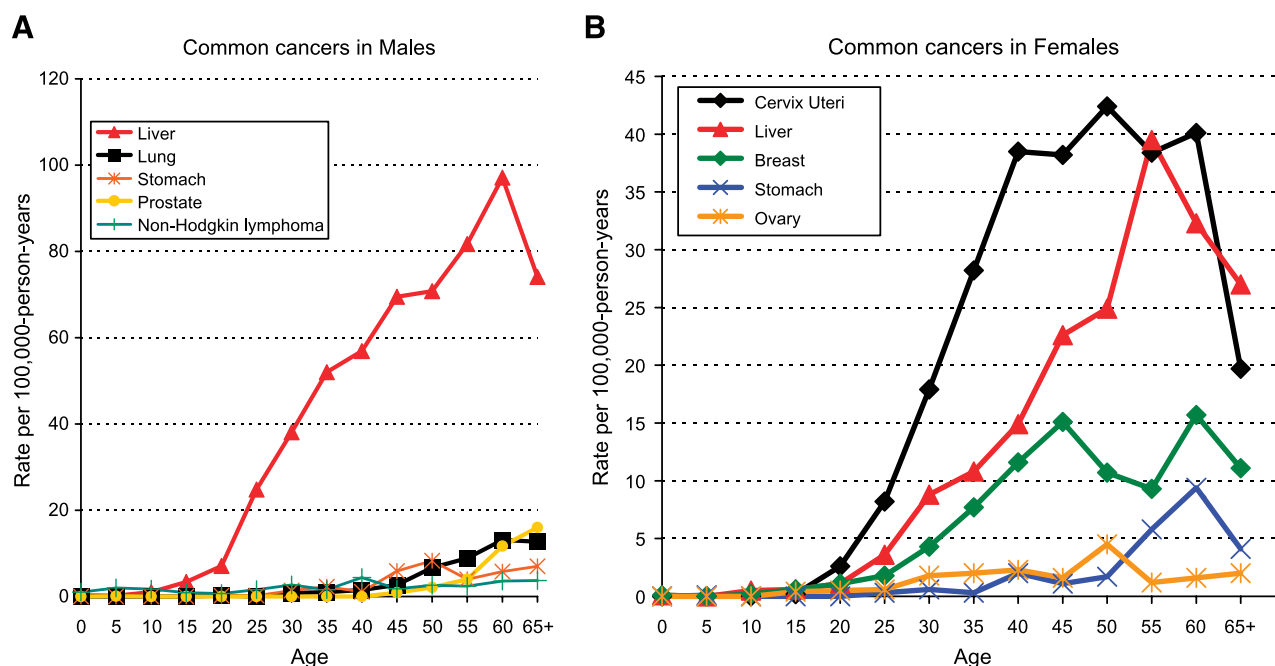
**Attrition in the GHIS Cohort.** Group 1 and Group 2 surveys have indicated that a loss to follow-up of 25% was observed during the 1st year of life due to death, migration, or refusals. After 9 years, 65% of the children were still available for follow-up, although no systematic attempt was made to trace subjects who may have changed residence because of in-country migration. In further studies, several methods of record linkage to the GHIS database were tested in cross-sectional studies on children and adolescents attending outpatient clinics. These studies showed correct linkage for a proportion of subjects varying between 66% and 73%, depending upon the available matching parameters.<sup>10</sup> A matching exercise with the GHIS database was conducted in 2004 to 2005 in the context of a study aimed to confirm long-term vaccine efficacy of infant vaccination in a large adolescent sample, representative of the general population (20). Over a period of 6 months, 3,709 potentially eligible participants were identified in the catchment areas of 5 health centers in which vaccination started in 1989. Matched recruits were defined as confirmed or probable matches. Confirmed matches by definition had the same combination of infant welfare card number and date of birth, whereas parental and recruit names and place of birth were identical, allowing for differences in spelling and abbreviations. Most of the probable matches lacked a birth date for definite confirmation. For 2,147 recruits (57.9%), a probable match was found in the GHIS database, and an absolute match was confirmed for 1,414 subjects (38%). On the other hand, demographic data indicate that in-country migration explains the movement of up to 30% of the overall population with ages between 24 to 34 years (24). These subjects would remain available for linkage through the NCR. Thus, a hypothesis of 50% attrition in the GHIS cohort due to demographic or logistic factors is sustainable 20 years after the initial study design.

**Table 3. Number of hepatocellular carcinoma cases required in the unvaccinated and vaccinated cohorts to be 95% sure of detecting a difference if the vaccine is truly effective**

Protective efficacy	Expected number of cases required*	
	Unvaccinated cohort	Vaccinated cohort
95	13	1
90	15	2
80	21	4
70	29	9
60	42	17
50	65	33
40	109	65
30	205	143
20	487	390
10	2,057	1,851
5	8,443	8,021

\*Based on the approximation formula:  $(Z_a + Z_b)^2(2 - e)/e^2$  and  $Z_a = Z_b = 1.645$ , where  $e = \text{protective efficacy} = 1 - \text{incidence of HCC in vaccinated}/\text{incidence of HCC in unvaccinated}$ .

<sup>10</sup> E. Bah et al., unpublished observation.



**Figure 1.** A. Age-specific incidence rates of the most common cancers in males in The Gambia: 1987 to 2002. B. Age-specific incidence rates of the most common cancers in females in The Gambia: 1987 to 2002.

### Cumulative Number of Cases and Duration of Follow-Up

Figure 1A and B shows that liver cancer is the most common form of cancer in men and the second in women. By age-adjusted incidence rates (using the World Standard population), these rates are comparable with those reported elsewhere in West Africa (1, 25). These rates are similar to earlier reports (13, 14), and we consider them as reasonable estimates and representative of the unvaccinated Gambian population. Thus, in this review, we used these rates to predict the cumulative number of cases ascertained in the unvaccinated cohort, taking into account the attrition rate of 50% (Table 4). By interpolating data in Tables 3 and 4, the number of cases

needed to detect a significant difference between vaccinated and unvaccinated groups will be reached when GHIS subjects are about 30 years old (5% level with 95% power). The stepped-wedge design has been estimated to have an efficiency of >70% of that of an individually randomized trial. Thus, the required number of cases in the control group to achieve statistical significance with adequate power is 40% more than the above figures. However, given the rate of case accrual between 30 and 39 years of age, the impact of this factor on the duration of follow-up is minimal. Overall, a conservative estimate is that between 30 and 35 years of total follow-up will be necessary to obtain unequivocal results. Therefore, the final outcome of GHIS should be measurable between 2017 and 2020.

**Table 4.** Expected cumulative number of hepatocellular carcinoma cases in GHIS unvaccinated subjects under the hypothesis of 50% attrition, 70% attributable risk (that is, 68% vaccine efficacy against hepatocellular carcinoma)

Age group	M		F		Total (M+F)
	Incidence rates*	Cumulative number of cases	Incidence rates*	Cumulative number of cases	
0-4	0.3	0	0.1	0	0
5-9	0.1	0	0.0	0	0
10-14	0.7	1	0.4	0	1
15-19	3.4	3	0.5	1	4
20-24	5.2	7	0.9	1	8
25-29	16.3	20	3.3	4	24
30-34	25.7	39	7.9	10	49
35-39	39.9	69	8.8	16	85
40-44	46.8	104	13.0	26	130
45-49	72.0	158	17.2	39	197

Abbreviations: M, males; F, females.

\*Age-specific incidence rates for HCC in The Gambia, 1987-2002.

## Discussion

**Revised Evaluation of Duration of GHIS.** Twenty years after it was conceived, the design of the GHIS seemed to have fulfilled most, if not all, of its initial expectations. On the basis of available evidence, we are in a position to determine that the major endpoint of the study, the evaluation of the protective efficacy of childhood hepatitis B vaccine against hepatocellular carcinoma, is reachable by 2017, sooner than the initial assumption of an overall follow-up of 35 to 40 years (8). When comparing the initial assumptions with the actual data acquired over the first 20 years of the study, only few divergences appear. Three assumptions were found to be more conservative than the results reported here. Firstly, a higher hepatitis B vaccine coverage was achieved than expected, and protection against chronic carriage was not as strongly dependent on the number of doses received as initially assumed. Secondly, the proportion of subjects that could have been infected perinatally is slightly lower than assumed. This proportion is unlikely to influence the estimate of vaccine efficacy against hepatocellular carcinoma. Thirdly, 20 years of observation of the unvaccinated Gambian population through nationwide cancer registration have shown that the age-specific incidence rates of liver cancer were actually higher than assumed in the original calculations, in which estimates of incidence based on a single year of observation were used. Conversely, case-control studies in The Gambia do not fully support the initial assumption that between 80% and 90% of hepatocellular carcinoma occurring below 50 years of age would be attributable to HBV infection. These studies show that the proportion of HbsAg-positive cases decreases with age. Whereas a proportion of 80% to 90% is likely to be attained in cases <35 years, this proportion drops rapidly in cases with the age of >45 years. Overall, we therefore consider that an attributable risk of 70% before the age of 50 years is an acceptable conservative estimate. It should be noted, however, that the current estimate is exclusively based on HBV serology and does not take into account the proportion of occult hepatitis B infections, which is not known in hepatocellular carcinoma cases from The Gambia (26). It remains to be determined whether this proportion is likely to have an impact on the overall risk of hepatocellular carcinoma attributable to HBV and whether occult carriage is effectively prevented by hepatitis B vaccination.

## Factors that May Affect GHIS Outcome

**Hepatocellular Carcinoma Registration.** Successful completion of GHIS objectives require comprehensive population coverage through a sustained long-term program of active surveillance for hepatocellular carcinoma, with regular monitoring of all available data sources in primary, secondary, and tertiary health centers. It also involves improving patient's management and clinical care to provide them with the best available options and thus encourage referral. The absence of satisfactory management strategies is a major obstacle that can deter hepatocellular carcinoma cases from seeking care in hospitals, leading them to seek treatment

from traditional healers who remain a popular option in The Gambia. To support the efforts of the Gambian health system and to achieve the best possible coverage, the NCR is operating according to an active case-searching strategy. Registry clerks are permanently posted in the main referral hospital where they assist medical staff in patient's triage at outpatient clinics and follow them up during diagnosis and admission. They facilitate the processing of specimens for laboratory analysis and the access of patients to ultrasonography. They contribute to providing information to patients and families on diagnosis, treatment, and palliation. Finally, the NCR works in close integration with hospital record offices and assists in keeping and maintaining clinical records and cancer patient's files in each hospital location. Thus, the operations of the NCR are fully integrated into the daily hospital routines. In addition, NCR clerks visit primary and secondary health centers in defined catchment areas on a regular basis, and a telephone hotline is in place for reporting suspected cases on a 7-days-a-week basis. The NCR also collects and maintains a similarly active surveillance register of chronic liver diseases, including cirrhosis, chronic hepatitis, and chronic inflammatory liver diseases.

**Hepatocellular Carcinoma Case Definition.** Detection and diagnosis of hepatocellular carcinoma in a low-resource context requires the implementation of a simple, robust, and specific case definition using clinical, imaging, and laboratory screening techniques designed for field use. Because of logistic and resource constraints, more sophisticated diagnostic technologies such as computerized tomography and magnetic resonance imaging are not available for use in low-resources countries. Because of the lack of therapeutic options and of appropriate structures to handle possible adverse effects, obtaining biopsies for histopathologic confirmation of hepatocellular carcinoma is not a realistic option for most of the patients. The current case definition uses a combination of a compatible clinical assessment,  $\alpha$ -fetoprotein testing (levels  $\geq 100$  ng/mL), and positive ultrasonographic findings, assessed against histopathology using a limited number of good quality biopsies obtained in the course of a recent case-control study (10, 27). This definition has a high specificity of >95% for detection of hepatocellular carcinoma. However, about 40% of clinically and ultrasonography diagnosed hepatocellular carcinoma have levels of AFP <100 ng/mL. Further research using additional robust biomarkers is required to improve the sensitivity of current case definitions.

**Waning Hepatitis B Immunity.** The waning hepatitis B immunity and increased prevalence of breakthrough infections in the GHIS cohort needs to be monitored on a regular basis. Although vaccine efficacy against chronic carriage remained remarkably high during adolescence, antibody response decayed with time, with undetectable antibody levels (<10 mIU/mL) in 37% of vaccinated subjects at a median age of 16.2 years (9, 20). Hepatitis B core antibody conversions were detected in 49% of subjects with undetectable antibody titers (although very few resulted in carriage and none in clinical hepatitis). Furthermore, the role, if any, of sexual exposure to HBV in boosting immunity or in increasing carriage remains to be determined. In the context of reduced virus exposure

as a result of countrywide vaccination since 1990, teenagers vaccinated in infancy may lose immunity because of the lack of exposure and a booster dose may become necessary to maintain protection into adulthood. A subset of GHIS vaccinated adolescents who were given a booster dose of HBV vaccine showed an excellent response that persisted for at least 1 year (20). Further cross-sectional studies in the GHIS cohort are required to evaluate the maintenance of immunity at older ages, as well as possible early effects of vaccination on mild chronic liver diseases than may be prevalent in the nonvaccinated population.

**Impact of Other Risk Factors.** The impact of several other risk factors for hepatocellular carcinoma need to be further considered. Firstly, HCV infection, a factor that was not detectable at the time of GHIS design, need to be carefully monitored. Current data show a population prevalence of 2.9% and of 18.9% among hepatocellular carcinoma cases. However, the age distribution of HCV in hepatocellular carcinoma cases is such that the attributable risk to HCV before the age of 50 years can be estimated at a maximum of 5% to 10% at the most (10). The age distribution of HCV, in the normal population and in hepatocellular carcinoma patients, is consistent with a cohort effect. The mechanisms of HCV transmission in The Gambia are not well understood. The hypothesis of a historical contamination due to the reuse of contaminated equipment, in routine medical and traditional health care settings, has been proposed and discussed in a recent review (4). However, there is little data to identify the specific events that may have caused this contamination. Another matter of concern is the potential impact of HIV infections on the immunologic status of hepatitis B vaccinated subjects. The availability of HIV treatment will increase the likelihood of interactions with HBV and/or HCV infections, as well as effects on immune responses, that will require further investigation. Currently, HIV seroprevalence in The Gambia is considered to be low (1-2%). However, this prevalence will need to be specifically assessed in GHIS cohort subjects, who are now reaching adulthood.

The carcinogenic effects of exposure to aflatoxin represent another factor that may affect GHIS outcome. Its combined effect with chronic hepatitis B carriage is more than multiplicative, with a relative risk of about 60 (reviewed in ref. 28). The risk associated with aflatoxin alone (in HbsAg-negative subjects) has been evaluated in four cohort studies in Southeast Asia and has been found to vary from 0.3 to 17.4. However, there is only limited data on the risk of aflatoxin alone in populations from Western Africa. Aflatoxin contamination of staple diet is widespread in The Gambia (29, 30) and recent economic data indicate that groundnuts, the main source of aflatoxin exposure, represents a major crop and an essential source of income in rural Gambia. Changes in dietary patterns in relation to changes in lifestyle and migration from rural area to the urban or semi-urban areas are being observed in The Gambia, affecting in particular young adults. Such changes may potentially produce a downward trend in levels of exposure to aflatoxin in this population. Furthermore, simple and effective methods to improve crop triage, storage, and packaging have been shown to significantly reduce aflatoxin exposure in Guinea (31), a neighboring country.

In effect, it is very likely that further efforts to reduce aflatoxin contamination can increase the preventive effectiveness of hepatitis B vaccination in The Gambia.

**GHIS Cohort Attrition.** Although several strategies have been set up for linkage between the NCR and the GHIS vaccination database, massive demographic changes due to unpredictable social, political, and economic factors are still possible. Although the country has enjoyed political stability since 1994, such changes may preclude the final evaluation. Maintaining a record linkage success rate of 50% is critical for the conclusion of GHIS within the expected time frame. In this context, the long-term feasibility of linkage using the database and dermatoglyphic prints need to be further explored. As new matching techniques are being developed, it may become possible to adopt a computerized iterative protocol for linking subject identifiers in the NCR and in the GHIS database.

### Global Public Health Impact of GHIS

GHIS differs from hepatitis B vaccine interventions elsewhere in the world by its design, which takes into account the specific patterns of HBV epidemiology and natural history of hepatocellular carcinoma in Africa, and by its public health impact, advocating the generalization of hepatitis B vaccination in sub-Saharan Africa (4, 6). The final success of this unique long-term endeavor will depend upon the continuing development of a sustainable infrastructure for cancer detection, diagnosis, and registration, for monitoring viral infections, and for doing record linkage. This infrastructure is contributing to capacity building in the Gambian health system. The strategy adopted for the development of GHIS provides a model for the evaluation of the introduction of new vaccines in the Expanded Programme of Immunization of sub-Saharan African countries. The model developed in The Gambia shows that population-based cancer registration can work as an integral part of routine medical and hospital practice to assess the long-term effect of countrywide hepatitis B vaccination to prevent liver cancer in a low-resource setting. This model provides a blueprint for other population-based interventions by vaccination or other means to reduce cancer burden in Africa.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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