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THE GAMBIA HEPATITIS INTERVENTION STUDY (GHIS): FUTURE PLANS

1. INTRODUCTION

As a leading cause of liver cancer and liver disease, Hepatitis B virus (HBV) infection is an important threat to global health. Worldwide, an estimated two billion people have been infected with HBV, while over 360 million people have chronic liver infections and are at risk of developing life-threatening liver diseases. More than 780 000 people die every year due to the acute or chronic consequences of HBV.

Many HBV infections are acquired early in life: perinatally, or horizontally during early childhood, particularly in highly endemic regions where these routes are the most common modes of transmission (1). The global burden of disease and injury study (2) estimated that out of 235 causes of death, liver cirrhosis ranked 12th and liver cancer ranked 16th in 2010. Therefore even though HBV vaccination has been taken up by many countries, individuals infected before the introduction of the vaccine continue to suffer from the consequences of chronic carriage, namely liver cirrhosis and hepatocellular carcinoma (HCC) (3). It is projected that there will be increasing numbers of HCC cases over the next five to six decades in countries where HBV is endemic (4).

HCC is the commonest cancer in males and the second commonest in females in The Gambia. It is a major cause of death in sub-Saharan Africa and eastern Asia (5). There is strong evidence that some 70% of HCC in sub-Saharan Africa is due to chronic infection with HBV (6). During the early 1980s a vaccine became available that protected against both acute and chronic infection with HBV as judged by serological markers. The Gambia Hepatitis Intervention study (GHIS) was established because it was then unknown how long that protection might last and whether it would prevent HCC. Other factors that contribute to the etiology of HCC include hepatitis C virus (HCV) and dietary exposure to aflatoxins, a group of mycotoxins that are natural contaminants of maize and groundnuts, which form the staple diet in this region. Aflatoxin exposure has a multiplicative effect in conjunction with HBV infection on the risk of developing HCC (7-9).

GHIS is a long-term, collaborative project by IARC, The Government of the Republic of The Gambia (GG), and the Medical Research Council of the United Kingdom (MRC), Gambia Unit. This programme was launched in 1986 with the objective of evaluating the effectiveness of HBV vaccination in childhood for the prevention of HBV infection, chronic liver disease, and HCC in adulthood in a population at high risk (10).

Study Design and implementation

The design and implementation of GHIS involved three overlapping phases.

Phase I (1986–1990)

Vaccination phase

A plasma-derived HBV vaccine, approved by the WHO, was phased into the Gambian Expanded Programme of Immunization (EPI) in a "stepped wedge" design over a four-year period from July 1986 to February 1990. The vaccine was gradually introduced to each immunization team on a team-by-team basis. This resulted in half the children born over these four years receiving HBV vaccine and half only receiving the other routine vaccinations (BCG, DPT, polio, measles and yellow fever). A total of 124 577 children were recruited, of whom 61 065 received HBV vaccine.

Three methods were set-up for the long-term identification of individuals. First, at recruitment, personal details of children were recorded, such as name, parents' name, birth date, sex, health district, health centre of birth, and ethnic group. Second, at the age of four months or older, dermatoglyphic prints of a hand and foot of each child were taken for confirmation of identity later in life. Third, the usual site of the BCG vaccination and the resulting scar were altered for children in the study (left forearm for those who received HBV vaccine, right forearm for those who did not). The site of this vaccination reverted to the left upper arm on completion of recruitment. Since February 1990, HBV vaccination has been offered to all newborns as part of EPI in The Gambia.

Establishing a National Cancer Registry (NCR)

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 (11, 12). The NCR database uses the CANREG system developed at IARC. It collects personal information including names, usual residence, age, sex, ethnic and marital status as well as details on the tumour including primary site, morphology, basis and date of diagnosis and hospital/clinic of initial diagnosis and registration. All patients diagnosed by a medically qualified doctor are eligible for inclusion into the registry database. Tumour site and morphology are coded according to the International Classification of Diseases for Oncology, third edition (ICDO-3).

Cancer registration is carried out by trained tumour registration officers (TRO) who visit all health institutions, both public and private, to collect data. The TRO are posted at the major tertiary care facilities, namely, Edward Francis Small Teaching Hospital (EFSTH) in Banjul, (formerly, Royal Victoria Teaching Hospital), MRC in Fajara, AFPRC General Hospital in Farafenni and Bansang Hospital in Bansang, with responsibility to collect data on all cancer diagnoses within a specified geographical area of the country (see Figure 1). They not only liaise with clinicians but also check all the identified data sources for cancer diagnoses (patient's medical records, death certificates, histopathology department reports).

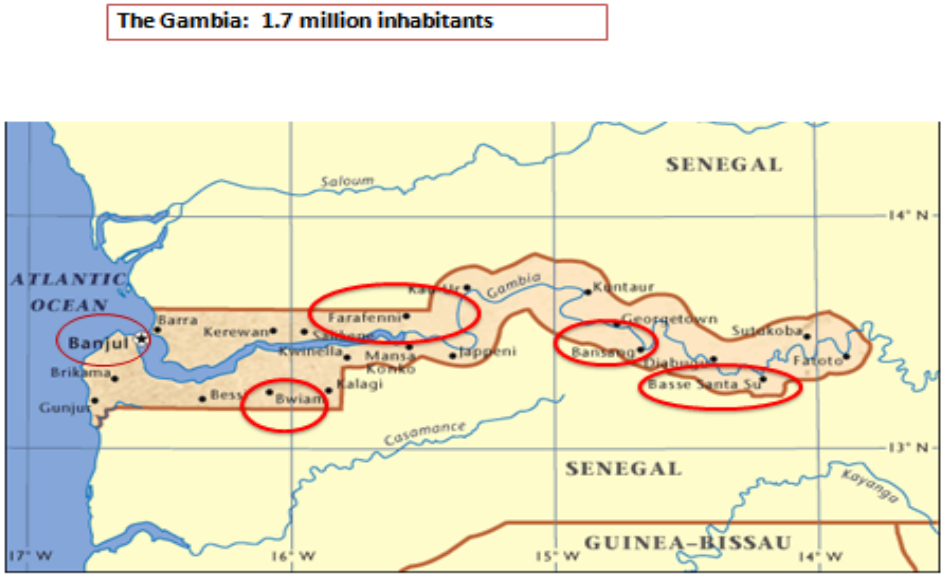

Figure 1: Outreach clinics are marked in red

The histopathology unit of the EFSTH supports confirmation of clinical diagnoses. For HCC diagnosis, clinical criteria, ultrasonography, and alpha-fetoprotein measurement were used in combination. Although this criterion was validated against histology in a study of liver cancer patients in Senegal and was shown to be >90% specific for liver cancer diagnosis in that region (13), it has become evident with the appointment by IARC of a hepatologist and Head of the GHIS project (see below), that the quality of diagnosis and registration hitherto was not optimal to ensure a successful outcome to the study.

With the commencement of ancillary studies such as PROLIFICA (see below), a second hepatologist joined the Group. This coincided with the availability of a highly sensitive, quantitative alpha-fetoprotein assay, advanced imaging in the form of fibro-elastography, and computed tomography (CT) scans in two urban centres; when combined with liver biopsy in selected cases, nearly a fifth of cases that would have been entered into the registry as HCC turned out to have alternative diagnoses (Dr Ramou Njie, personal observation). It therefore became necessary to refine the diagnosis of HCC and chronic liver disease in line with international standards (see Appendix 1 for case definitions). Additional histopathology support is provided by IARC and Imperial College London.

Phase II (1991–1997)

Vaccine Efficacy Studies

A second phase of assessment of the effect of vaccination on rates of infection with HBV began in 1986. 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 HBsAg carriage (15-19). Group 1 was a cohort of 1041 children, including approximately the first

250 HBV–vaccinated children in each of the four ecological zones. These children were 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 with its cohort of 1000 vaccinated children. Two further cross sectional surveys of both vaccinated and unvaccinated individuals were made at the age of 15 years and at the age of 20 years (20). See below for detailed results.

Phase III (1998–to date)

Intensive Surveillance for HCC and chronic liver disease

The third phase of the study consists of the identification of cases of HCC and chronic liver disease in the study groups and linkage to their vaccination status. This work is ongoing. Between the end of phase II and the start of intensive follow-up, the NCR continued, albeit with incomplete coverage of the rural areas and the elderly population (21). Multiple ancillary studies were conducted during this period, and included, but were not limited to: studies of dietary intake of aflatoxins and albumin-bound aflatoxin in serum; longitudinal studies on aflatoxin-contaminated foodstuffs and individual and seasonal variations in exposure; studies addressing TP53 R249S mutations in serum DNA with aflatoxin exposure, as well as a liver cancer case-control study.

These studies showed that dietary exposure to aflatoxin is widespread, primarily through ingestion of contaminated groundnuts, that HCC is associated with aflatoxin exposure, and that the 249ser TP53 mutation, a biomarker of aflatoxin mutation is detectable in tumours and plasma DNA from HCC patients and a proportion of asymptomatic individuals. Striking age differences were observed in the patterns of HBV and HCV infections and in the burden of HCC associated with each infection (6, 14, 22, 23).

The final outcome of GHIS will be evaluated through record linkage between HCC cases in the NCR and the GHIS database of vaccinated and unvaccinated children. A hepatologist (Dr Ramou Njie) was recruited in 2011 to lead the GHIS project in The Gambia and to enhance diagnoses and registration over the subsequent 5 to 10 years. Given the study participants are now in their mid-to-late twenties, it is estimated that a clear quantitative estimate of the protective efficacy of vaccination against cancer will be possible in this time period. To be able to achieve this and improve the quality of registration, a new cancer registrar and two new TRO have been recruited to improve coverage of the country, especially the rural parts of the north bank region. In addition, extensive training in CANREG-5 and improvements in diagnostic and clinic facilities (see below) have been undertaken. There is only one other study globally addressing this question which is in China and used a similar cluster randomized design but on the background of a very different epidemiology of HBV infection (24).

Evaluation of initial assumptions

The GHIS programme was designed on the basis of assumptions about vaccine coverage, HBV vaccine efficacy against carriage, impact of perinatal acquisition of the chronic carrier status, proportion of HCC attributable to HBV, and cohort attrition. Based on the data generated twenty years into the programme, the validity of each of the initial assumptions was critically assessed in a review by Viviani et al. in 2008 (25).

HBV Vaccine Coverage

It was initially assumed that of the eligible children, 85% would present themselves for at least one injection at the HBV vaccination sites, 80% for at least two, and 75% for ≥3 injections. Of the estimated number of eligible children (approximately half of the total GHIS cohort of 124 577), 98% (n = 61 065) received at least one dose, 92% (n = 55 985) received at least two doses, and 81% (n = 49 558) received ≥3 doses. Thus, a greater HBV vaccine coverage than originally expected was achieved.

HBV Vaccine Efficacy

Because HBV vaccine efficacy usually depends on the number of doses received, it was assumed that among those who were vaccinated, 20% would respond after one or two injections, 80% would respond after one or two injections plus a booster, and 95% would respond after ≥3 injections. Response to HBV vaccine would be durable due to continuing viral challenge in the environment. A level of anti–hepatitis B surface antigen (HBsAg) of ≥10 IU/mL was found to be a marker of protection against chronic carriage (17).

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 one dose responded to vaccination with anti-HBsAg titers ≥ 10 IU/mL and were protected against chronic carriage early in life (15, 16, 17-20). Several studies have confirmed that there was continuing HBV transmission despite ongoing countrywide vaccination of infants and that there was persisting exposure to HBV in the GHIS cohort (18).

In subsequent follow-ups, the efficacy of HBV vaccination in preventing chronic carriage was consistently high. In a cross-sectional study on adolescents at the age of 15 years, the vaccine efficacy was 67.0% against anti-hepatitis B core positivity (a marker of past infection) and 96.6% against HBsAg carriage (26). Thus, vaccination in infants conferred excellent protection against carriage until adolescence, despite waning titers of anti-HBsAg antibodies at this age.

A similar situation was observed in the pilot vaccination studies in the two villages of Keneba and Manduar (27). Mendy et al., in the most recent survey showed that 24 years after vaccination, vaccine efficacy against chronic infection with HBV was 95.1%, which did not vary significantly between age groups or villages. Efficacy against infection was 85.4%, falling significantly with age. Concentrations of HBs antibody fell exponentially with age varying according to peak response, and 20 years after vaccination only 17.8% of persons with a low peak response (10-99mIU/ml) had detectable HBs antibody compared to 27% of those with a high peak response (>999mIU/ml). Time since vaccination and a low peak response were the strongest risk factors for HBV infections; males were more susceptible and marriage was not a

significant risk for females. Overall the study demonstrated that adolescents and young adults vaccinated in infancy were at increased risk of HBV infection, but not chronic HBsAg carriage. From this survey, it was concluded there is no compelling evidence for the use of a booster dose of HBV vaccine in The Gambia. (20). Nevertheless it was noted that further research was required to determine if breakthrough viral infections, signified by anti-HBc seroconversion, may persist in the liver and cause harm or whether they are beneficial by boosting immunity. Practices to improve coverage of the HBV birth dose vaccine to prevent early HBV infection were advocated, in line with WHO policy (28).

Impact of perinatal HBV acquisition carrier status

It was assumed that among children who will become HBV carriers, 10% acquire their HBV status perinatally, 50% of whom would be protected by vaccination. However, Group 1 surveys indicated that only 0.6% to 0.7% of vaccinated children became chronic carriers after vaccination. Of the four children who were chronic carriers by the age of 4 years, 3 had HBsAg-positive mothers, out of whom 2 were also HBe-antigen positive. These children of these two HBsAg-positive mothers had already acquired carrier status by the age of one year (27). In addition, they did not respond to the vaccine (anti–HBsAg titer, <10 IU/mL) despite receiving three injections plus a booster dose. This observation is consistent with the assumption that 50% of the vaccinated children who become carriers may have acquired their HBsAg carrier status perinatally (15, 16, 19, 29, 30). Thus, based on the evidence derived from the available empirical data, the proportion of subjects infected perinatally was thought to be unlikely to influence the estimate of vaccine efficacy against chronic HBV carriage and HCC.

Attributable risk for HBV

It was initially assumed that under the age of 50 years, 80% to 90% of HCC would be attributable to HBV. Subsequently the proportion of HCC attributable to HBV and HCV infections was assessed in three case-control studies (6, 7, 31). Comparison of the earliest to most recent study indicates that this proportion has remained stable over the past 25 years. The data from the three studies suggested that between at least 70% and 80% of HCC under the age of 50 years were attributable to HBV, a proportion slightly lower than initially assumed. On the other hand, HCV infection appears to be relatively unimportant in people aged less than 50 years.

Attrition in the GHIS cohort

Assuming 75% vaccine coverage and 50% attrition in the cohort (due to death, migration, or incomplete record linkage), it was estimated that more than 35 years would be needed to obtain unequivocal results on HCC prevention. However, Group 1 and Group 2 surveys have indicated that a loss to follow-up of 25% was observed during the first year of life due to death, migration, or refusals.

On the basis of available evidence, it would appear that the evaluation of the protective efficacy of childhood HBV vaccine against HCC is reachable by 2017, sooner than the initial assumption of an overall follow-up of 35 to 40 years (7). Table 1 shows the approximate number of HCC cases expected to occur to have a 95% chance of detecting a significant difference between the vaccinated and unvaccinated groups. By this calculation, a sample size of 35 cases of HCC is

required in the unvaccinated group. Table 2 shows the cumulative number of HCC cases in GHIS unvaccinated individuals under the hypothesis of 50% attrition and 70% attributable risk based on previous estimates.

Table 1 **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

Table 1: number of cases of HCC required to be 95% sure of detecting if vaccination is truly effective, based on the approximation formula: $(Z_a + Z_b)^2(2 - e)/e^2$ and $Z_a=Z_b=1.645$, where e = protective efficacy = 1 – incidence of HCC in vaccinated/incidence of HCC in unvaccinated.

Table 2 **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.

2. RESULTS ACHIEVED TO DATE/CURRENT STATUS

2.1. Long-term vaccine efficacy/vaccine coverage

As discussed above, long-term vaccine efficacy has been demonstrated (20, 32, 33, 34). This cohort of individuals given HBV vaccine in infancy is the largest to date in sub-Saharan Africa and has the longest follow-up in the world; it has also served to monitor the impact of vaccination on the epidemiology of HBV infection in a highly endemic area in sub-Saharan Africa. Thanks to support from UNICEF, the WHO country office and the Global Alliance for Vaccines and Immunization (GAVI), HBV vaccine coverage rates in The Gambia as a whole have been consistently at 90% or higher since 2001 (WHO country report, 2008).

Despite different vaccination regimes, vaccine efficacy against chronic infection remains high in this population, around 94–96%. Infections, defined by the presence of anti-HBc antibody (anti-HBc), have occurred in vaccinated individuals. These infections increased with age and time since vaccination ranging from 2–3% in young children to 20–30% in persons 20 years old (26, 27). In some cases the infections were transient, but in others in whom anti-HBc persists it is not known if the virus is present in occult form, and hence further research in this area is warranted.

2.2. NCR and Cancer Registration

Since the inception of the NCR in 1986, the Gambian national boundaries have remained unchanged. From the most recent census of 2003 by the Gambia Bureau of Statistics (GBOS), there has been a significant population increase in the urban areas of the country, due to an increasing number of people looking for work and for better standards of living. These population factors may influence incidence rates of cancer in these areas. Another factor, which may influence recorded incidence rates of cancer, is the availability of cancer diagnostic services, which are improving, particularly in the urban areas. PSA screening and mammography are done in tertiary centres. CT scans with contrast are available in two of the urban centres. The diagnostic criteria for HCC and cirrhosis have been strengthened with quantitative alpha-fetoprotein assays and the availability of fibroelastrography. Histopathology support from Imperial College London adds an additional layer of validation.

The GNCR is one of the few nationwide population-based cancer registries in Africa; most registries in the region are hospital based and cover populations of large cities (36). A recent evaluation by Shimakawa et al. (21) found that the system used for classification and coding of neoplasms at the GNCR followed international standards and that despite the difficulty of working under circumstances of low histological confirmation and death certificate proportions in this context, the data in the GNCR provided valuable information on cancer incidence in sub-Saharan Africa. However, under-reporting was identified, especially in the elderly and rural populations. Incidence rates in rural regions were lower than in the urban part of the country, except amongst young male adults. To address the issue of under-reporting, a new TRO was recruited in 2012 and stationed at Farafenni to cover the rural areas of the north bank region of the country.

Figures 2 and 3 show the number of cases as well as age-specific incidence rates (ASR) of common cancers in males in The Gambia from 2010–2014, while Figures 4 and 5 show similar data for females. HCC remains by far the commonest cancer in males, followed by prostate and lung cancer. In females, cancer of the cervix is the commonest followed by HCC and breast. The age-adjusted incidence rates are comparable with those reported elsewhere in West Africa (5, 38). These rates are similar to previous reports from The Gambia (11, 12), and are considered to be reasonable estimates and representative of the unvaccinated Gambian population.

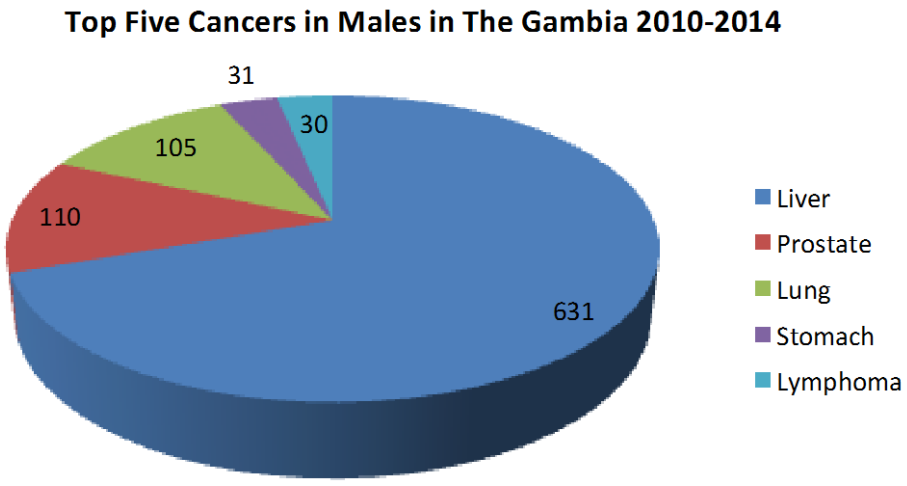

Figure 2: Top Five common male cancers in The Gambia 2010–2014 showing number of cases recorded for each cancer type.
Note that for 2014, data is for the first 6 months only

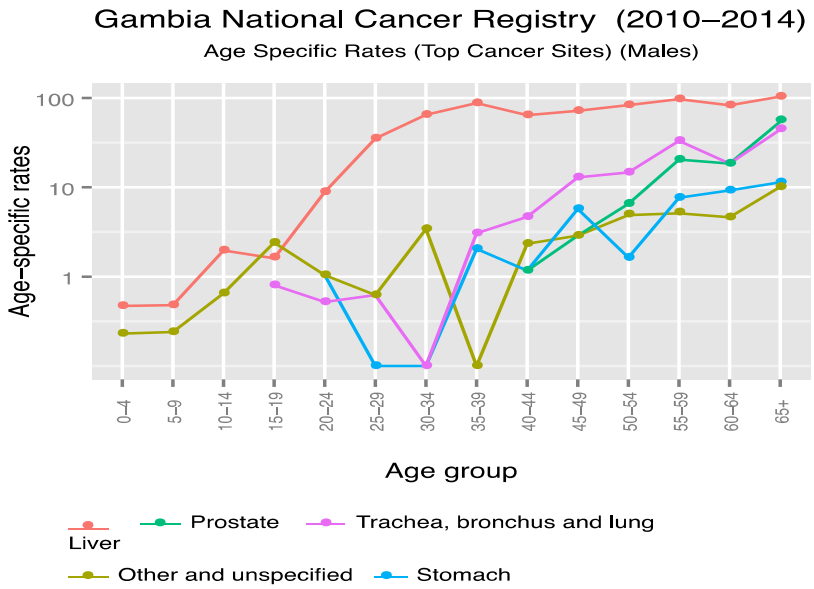

Figure 3: Age-Specific rates of the top five most common male cancers in The Gambia 2010–2014.
Note that for 2014 data is for the first 6 months only.

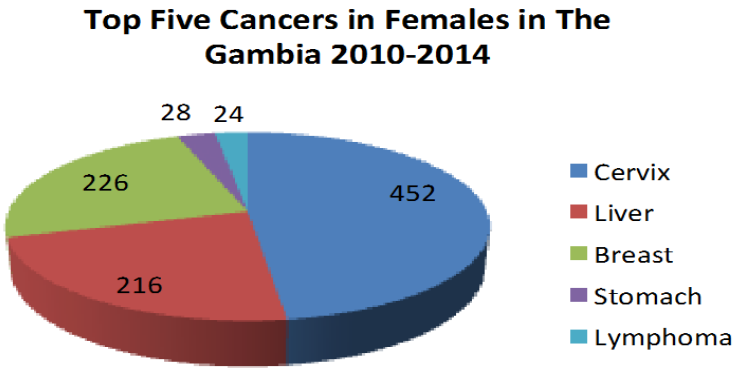

Figure 4: Top five female cancers in The Gambia 2010–2014, showing the number of cases recorded for each cancer type

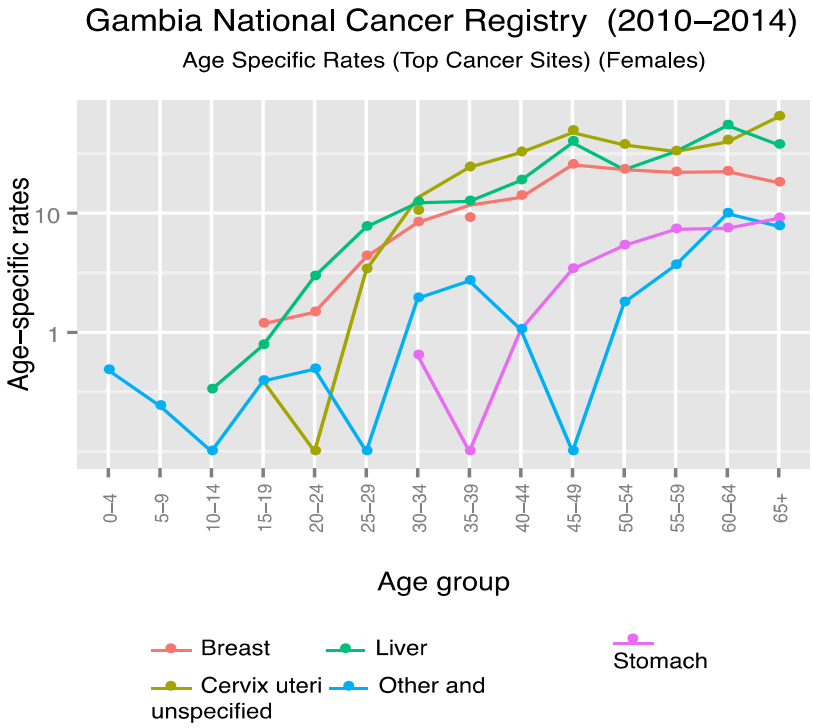

Figure 5: Age-specific incidence rates of top five cancers in females in The Gambia 2009–2014.
Note that for 2014 data is for the first 6 months only

Similar HCC incidence rates were used to predict the cumulative number of cases ascertained in the unvaccinated cohort, taking into account the attrition rate of 50%. (Table 2). By interpolating data in Tables 1 and 2, the number of cases needed to detect a significant difference between vaccinated and unvaccinated groups will be reached when GHIS participants are about 30 years old (5% level with 95% power), which is within the next five years. The stepped-wedge design is estimated to have an efficiency of >70% of that of an individually randomized trial. Thus, the required number of HCC 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, therefore, it was estimated that between 30 and 35 years of total follow-up would be necessary to obtain unequivocal results with the final outcome of GHIS measurable between 2017 and 2020 (25).

2.3. Record Linkage

Maintaining a record linkage success rate of 50% is critical for the conclusion of GHIS within the expected time frame. Previous attempts have been made to test record linkage to the GHIS database in cross-sectional studies on children and adolescents attending outpatient clinics; these studies showed correct linkage for a proportion of individuals varying between 66% and 73%, depending upon the available matching parameters (6). The matching parameters used are: name, sex, date of birth, birthplace, name of parents, site of BCG scar and dermatoglyphic palm and footprints.

A matching exercise with the GHIS database was conducted in 2004 to 2005 in the context of a study aimed at confirmation of long-term vaccine efficacy of infant vaccination in a large adolescent sample, representative of the general population (19). Over a period of six months, 3709 potentially eligible participants were identified in the catchment areas of five health centres 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, where parental and recruit names and place of birth were identical, allowing for differences in spelling and abbreviations. Most of the probable matches lacked a date of birth for definite confirmation.

For 2147 recruits (57.9%) a probable match was found in the GHIS database, and an absolute match was confirmed for 1414 individuals (38%). Demographic data also indicated that in-country migration explained the movement of up to 30% of the overall population with ages between 24 to 34 years (34). These individuals would remain available for linkage through the NCR. Thus, a projected attrition of 50% in the GHIS cohort due to demographic or logistic factors remains a reasonable assumption over 20 years after the initial study design (25). In this context, the long-term feasibility of linkage using the GHIS database and dermatoglyphic palm and footprints is being actively explored. A computerized algorithm for linking individual identifiers in the NCR and in the GHIS database has also been developed at IARC, after the names database was checked for spellings and abbreviations, and is ready to be tested.

As mentioned above, the NCR has recently been strengthened with additional staff and training. The diagnostic accuracy of HCC has been enhanced and is now as robust as it can be under the circumstances, using the criteria detailed in Appendix 1. Figure 6 below shows the number of HCC cases registered in the GNCR from its inception to date, in males and females. Although the number of HCC cases appear to be falling, it is highly likely, from the experience in PROLIFICA (see below), that a significant number of cases recorded in the past as HCC were in fact, not primary liver cancer but secondary malignancies to the liver, decompensated macronodular cirrhosis or other intra-abdominal pathology affecting the liver. With the availability of contrast CT scans in the past two years, and, in some cases, histology, many patients referred to the

liver clinic with a diagnosis of HCC turned out to have alternative diagnoses. These patients by and large did not meet the more stringent criteria detailed in Appendix 1 and the diagnoses recorded included, among others: liver metastases from pancreatic, gastric, colonic and ovarian cancer, macronodular liver cirrhosis, intrahepatic cholangiocarcinoma, abdominal tuberculosis, lymphoma and schistosomiasis. This was true for nearly a fifth of the cases referred by primary care doctors from the peripheral health centres.

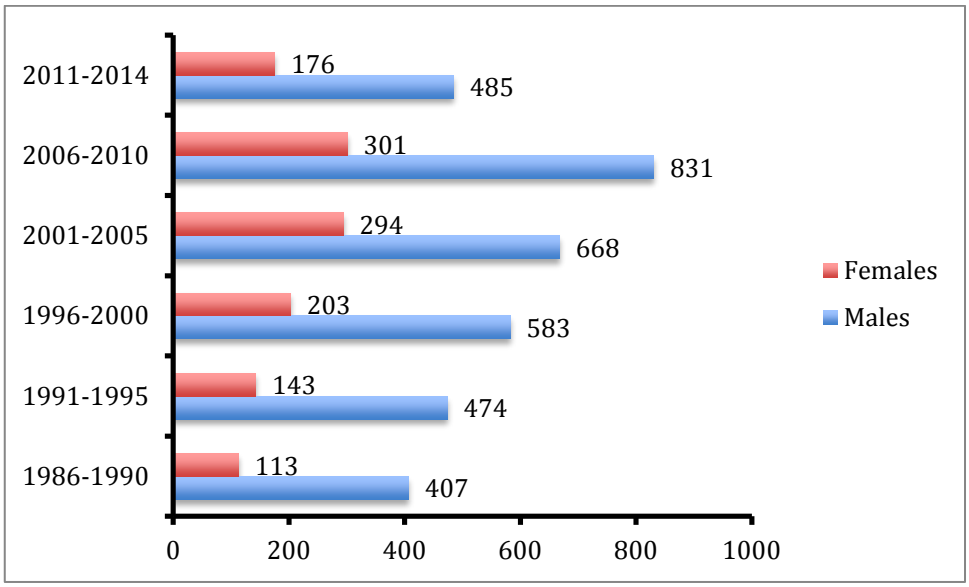

Figure 6: Number of liver cancers diagnosed from the inception of the GNCR in 1986 to date in males and females in The Gambia. The diagnostic criteria have been refined since 2012 with the availability of improved diagnostic facilities. NB: 2014 data only for 6 months

At the same time, from the PROLIFICA HC4 data (see below) the number of cases of chronic liver disease (significant fibrosis and established cirrhosis which may or may not be decompensated) appear to be on the increase due to improved diagnostic facilities, quantification of the alpha-fetoprotein level and better care provided for these patients who make up a significant proportion of the outpatient workload. The increase in the number of cases of chronic liver disease has been observed even after eliminating cases of cardiac disease, renal disease and abdominal TB, which often cause diagnostic confusion in this setting.

3. ANCILLARY STUDIES

While HBV vaccination will eventually lead to major decreases in incidence of chronic liver disease and HCC, there remain a large number of individuals with chronic HBV infection who are at high risk of HCC, particularly in aflatoxin-endemic areas such as The Gambia. The existence of the GHIS has made possible important ancillary studies, which can address this significant scientific and public health question; these are either ongoing or planned and will be briefly described here.

3.1. PROLIFICA (Prevention Of Liver Fibrosis and Cancer in Africa)

The Prevention of Liver Fibrosis and Cancer in Africa (PROLIFICA) study is a five-year multi-centre project funded by the European Union involving The Gambia, Senegal and Nigeria. It consists of two arms: The West African Treatment Cohort for Hepatitis B (WATCH) and the Hepatocellular Cancer Case Control Study (HC4).

The primary objective of WATCH is to assess whether screening for HBsAg in adult patients aged ≥30 years and suppression of HBV replication with a nucleotide analogue (Tenofovir) can reduce the incidence of HCC in West Africa. Identified HBsAg-positive individuals are invited to the liver clinic for assessment, and offered treatment if they meet European Association for the Study of the Liver (EASL) criteria. (See Appendix 2). HBsAg-positive individuals who do not meet the EASL criteria are enrolled into an observation cohort. Both treatment and observation cohorts will be followed-up long-term (≥5 years). The incidence rates of HCC in the treatment and observation groups will be compared with historical data from the GNCR. Figure 7 below shows a schematic diagram of the different aspects of WATCH and the number of individuals recruited into each group.

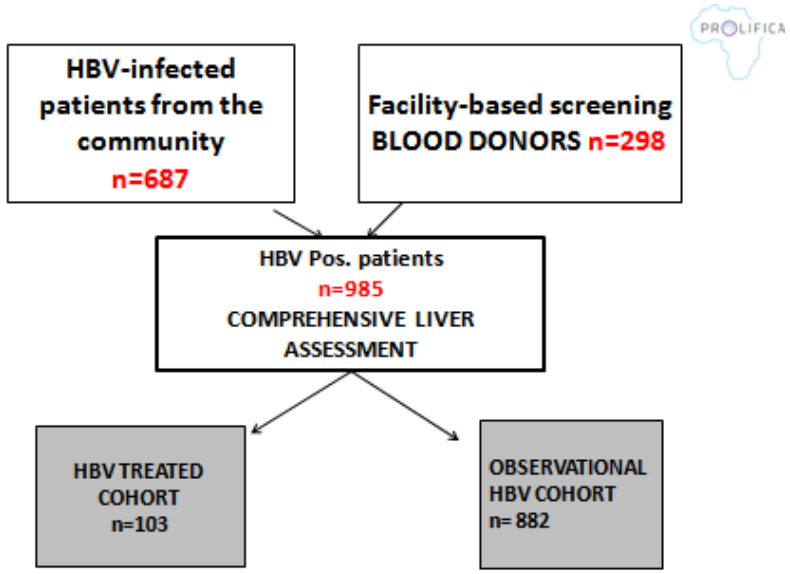

Figure 7: PROLIFICA WATCH cohort November 2014

Over eight thousand people from the Western Region of The Gambia were screened for HBsAg (5980 from the community and over 2000 from EFSTH blood donors) and a total of 985 HBsAg-positive individuals were identified. 342 HBsAg-negative individuals from the screening exercise were randomly selected to be negative controls, bringing to a total of 1327 participants being followed-up in the WATCH cohort.

The future plan is to apply the lessons learned in WATCH to whole population screening, and subsequent integration of HBV treatment into the already existing HIV treatment clinics and infrastructure. This will allow critically important questions to be addressed pertaining to treatment outcomes, appropriate treatment criteria for patients in Africa and other resource-limited settings, optimal duration of therapy, and the feasibility of a population-based approach to tackling the significant and devastating HBV disease burden.

The aim of HC4 is to develop a research platform for studies of proteomics, metabolomics, molecular diagnostics, host genetics, HCV and HBV viral genetics and environmental factors such as aflatoxin, in HCC in West Africa. HCC case finding began in the first half of 2012 and the aim is to recruit at least 800 cases. The diagnostic criteria for HCC and cirrhosis are as in Appendix 1. Figure 8 shows the numbers recruited so far, while Figure 9 shows the numbers in the various diagnostic categories. Recruitment is ongoing and several ancillary studies are currently in progress or planned for the future. These include studies looking into the role of NK cell immunosurveillance in hepatocarcinogenesis in the context of HBV (on-going); discovery and assay development of potential biomarkers for HCC/liver cirrhosis (on-going); circulating free tumour DNA detection in plasma as a diagnostic biomarker for HBV-related HCC (planned); identification of specific miRNA expressions in serum/plasma associated with the severity of chronic HBV by evaluating and comparing the miRNA expression pattern in three patient groups in West Africa – Group 1: chronic carriers, Group 2: patients with cirrhosis and Group 3: HBV-infected patients with HCC (planned).

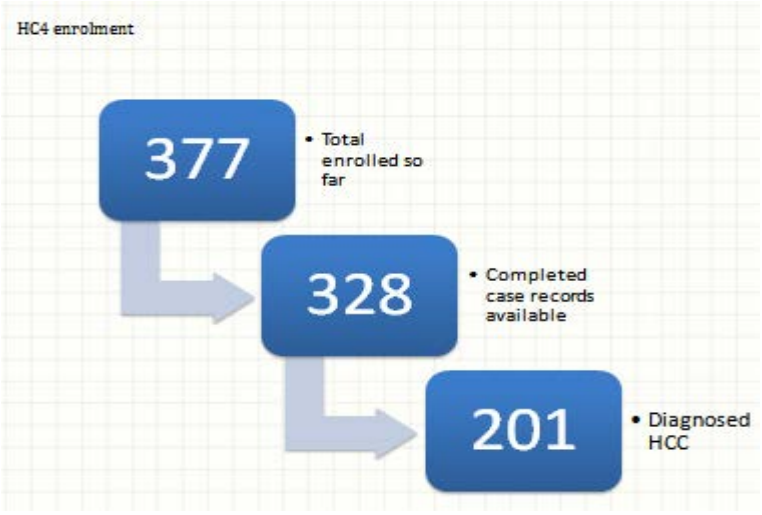

Figure 8: HC4 (Gambia) enrolment June 2012–October 2014

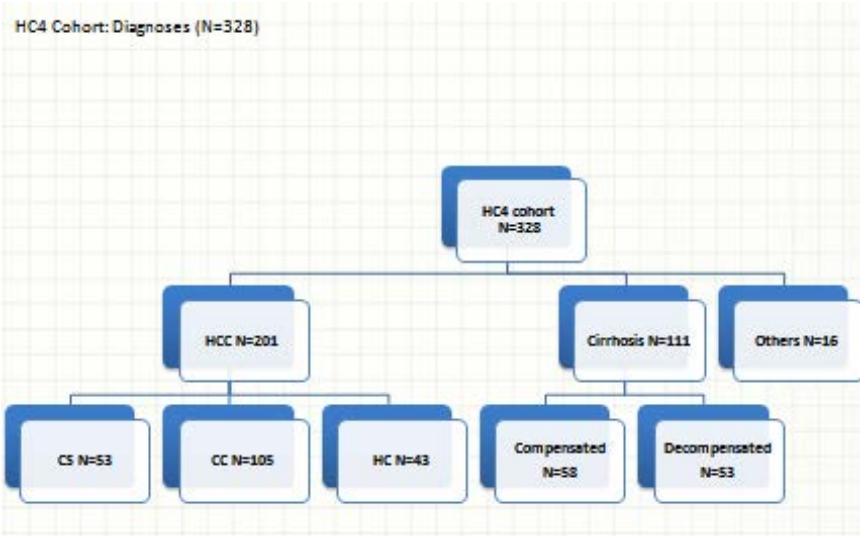

Figure 9: HC4 cohort diagnoses: CS=clinically suspected; CC=clinically confirmed; HC=histologically confirmed

3.2. The effect of Aflasafe intervention on human aflatoxin exposure

Aflatoxins are secondary metabolites of *Aspergillus* fungi and are a common contaminant of the Gambian diet. Previous biomarker data have shown ubiquitous exposure across the country in people of all ages (22, 23). Aflatoxins are a known cause of HCC but also of acute aflatoxicosis and have also been linked to child stunting following early life exposures. Aflatoxins will continue to pose an HCC risk even in an HBV vaccinated population, but particularly also among those individuals with existing chronic HBV infection.

Various approaches to reduce aflatoxin exposure have been explored, including improved post-harvest processing and storage of groundnuts and maize, two of the major sources of exposure (36, 38). A novel approach is through biocontrol whereby non-aflatoxigenic strains (Aflasafe SN01®) of *Aspergillus* are sprayed on fields to compete with those strains producing aflatoxins. While early results in carefully controlled field trials are promising, Aflasafe has not been shown to be effective in reducing aflatoxin exposure in populations consuming crops from fields that have been sprayed.

The International Institute for Tropical Agriculture (IITA) based in Nigeria is working with several African countries to apply Aflasafe on fields in demonstration projects and one of these is The Gambia. Many donors are supporting the scale-up of this initiative including the Bill and Melinda Gates Foundation, the World Bank etc. The interest from the agricultural partners as well as the long history of aflatoxin research at IARC means the GHIS is well-placed to study effects of Aflasafe on aflatoxin exposure using biomarkers. The current study is being proposed in partnership with the National Agriculture Research Institute (NARI), and the plan is to conduct it in 2015, subject to ethical agreement and funding.

The plan is to design the application of Aflasafe in The Gambia in such a way that blood samples could be collected from index individuals in the villages receiving Aflasafe or not on their fields, measuring blood levels of toxin after harvest and storage as well as levels in groundnuts. This would be the first test of the impact of this intervention on human exposure. Figure 10 shows the areas of the country sampled for aflatoxin levels in crops in pilot studies, while Figures 11 and 12 show a summary of the incidence and severity of aflatoxin contamination of crops respectively.

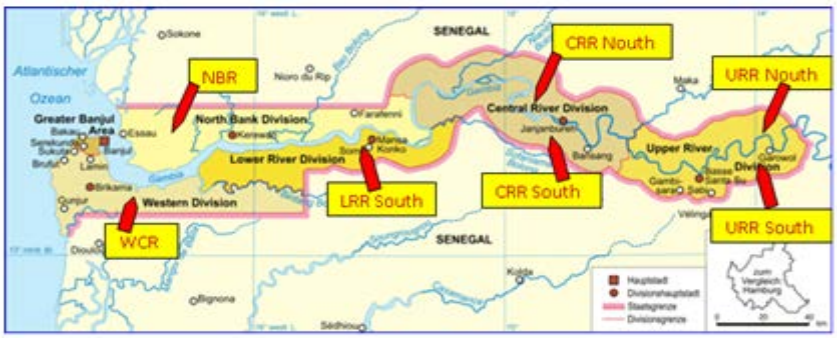

Figure 10: Groundnut sampling in The Gambia 2013. WCR-West Coast Region, NBR-North Bank Region, LRR-Lower River Region, CRR-Central River Region, URR-Upper River Region

- Number of samples/region = 20 (4 villages per region; 5 samples per village) = 140 samples
- Samples plated on semi-selective medium; 12 Aspergillus isolates / sample (except 6)
- Isolates characterized into species and strains; 72% L; 27% SBG and 1% *parasiticus*
- All L-strain isolates interrogated for presence of the four Aflasafe SN01 VCGs

Incidence

Regions*	Total Aflatoxins (ppb)			
	Mean	Median	Minimum	Maximum
West Coast	268	19	ND	1,845
Lower River	3	ND	ND	21
Upper River South	17	1	ND	208
Upper River North	5	1	ND	44
Central River South	39	7	ND	253
Central River North	102	13	ND	1,157
North Bank	102	23	ND	526

*Number of samples/region = 20 (4 villages per region; 5 samples per village)

Figure 11: Aflatoxin contamination in groundnuts in The Gambia 2013

Severity

Regions*	Samples (%) with Total Aflatoxins (ppb)			
	ND → 4	>4 → 20	>20 → 100	>100
West Coast	40	10	10	40
Lower River	85	10	5	0
Upper River South	60	25	10	5
Upper River North	85	5	10	0
Central River South	45	30	10	15
Central River North	35	25	20	20
North Bank	40	10	20	30

*Number of samples/region = 20 (4 villages per region; 5 samples per village)

Figure 12: Severity of aflatoxin contamination of groundnuts in The Gambia 2013 ND=Not Detected

4. NEXT STEPS

The GHIS is an important long-term study, one of only two such studies worldwide and the only one of its kind in Africa. Important lessons have been learned so far: through population surveys, field trials and a series of case-control studies, the patterns and natural history of HBV, HCV and aflatoxin exposures have been defined within the Gambian population. These investigations have paralleled and informed the development of molecular markers of these etiologic agents and contributed to the understanding of the complex mechanisms involved in hepatocarcinogenesis.

The GHIS has been and will continue to be a model for partnering etiologic and mechanistic investigations with applied research. As discussed in this report, it is expected that the next five to ten years should give a clear quantitative estimate of the effect of infant HBV vaccination on HCC incidence rates.

The successful completion of GHIS requires comprehensive population coverage of the NCR through a sustained long-term programme of active surveillance for HCC, with regular monitoring of all available data sources in primary, secondary, and tertiary health centres. This is being done through regular weekly clinics in the main tertiary centres together with monthly outreach clinics in the more peripheral health centres from which regular referrals are made of patients with suspected liver disease. Ward patients are seen at the main EFSTH in Banjul and the MRC unit wards, as well as in other Government hospitals. The clinics are accompanied by regular training sessions for medical, nursing and registry staff in the investigation and management of liver disease as well as in cancer registration. Sustained improvements in the NCR require continuing staff training, technical support in monitoring of data quality and a move away from paper-based to electronic data capture. From experience in PROLIFICA, the latter leads to more efficient capture of data with the elimination of transcription errors.

An important aspect of successful completion of the GHIS involves improvements in patients' management and clinical care to provide them with the best available options and thus encourage referral. To this end the GHIS has partnered with the Gambia National Palliative Care Association in drafting, disseminating and teaching staff about the WHO pain control ladder and principles of palliative care. We offer therapeutic paracentesis for massive ascites, prompt intravenous antibiotic treatment for spontaneous bacterial peritonitis, in-patient treatment for hepatic encephalopathy, endoscopy and band ligation of bleeding oesophageal varices as well as symptom control for HCC. These services were previously not available and provision has greatly improved referrals to the liver clinic.

Continuous reinforcement of the accurate diagnosis of HCC and inclusion in the NCR remains a priority going forward. This has entailed capacity building with the recruitment and training of three Gambian doctors in ultrasonography, fibroelastrography, diagnosis and management of liver disease, including liver biopsies. Added to this are regular lectures and tutorials on diagnosis and management of chronic liver disease and HCC, the importance of cancer registration and referral pathways in the main government hospitals.

Establishment of the record linkage approach is an important next step in the GHIS. The components of the linkage approach include data quality assessment with inbuilt programs to eliminate duplicate records in CANREG-5, identity resolution which deals with the very many similar Gambian names with lack of standard spelling, and data matching. The latter utilizes a computer algorithm to determine linkage based on the number of individual identifiers (name, surname, mother's name, father's name, date of birth, village of birth, sex, HBV vaccination status). Recent clinical experience suggests that the site of the BCG scar may not be a reliable identifier because the vaccination scars seem to have faded in many of these individuals. An additional identifier may be the use of palm and footprints. These were taken well before new, standard digital, and more recently, contactless, 3-dimensional fingerprint scanners became available. Although the actual photos of the palm and footprints of the GHIS cohort taken in

infancy have been digitally scanned into a database, it remains to be seen if these will be useful in the linkage process.

Another factor that might affect the outcome of GHIS entails changes in aflatoxin exposure: either increased exposure through greater consumption of aflatoxin-contaminated crops, or reduced exposure due to changes in dietary patterns in relation to changes in lifestyle and migration from rural areas to the urban or semi-urban areas especially in young adults, or as a consequence of widespread preventive interventions.

Aflatoxin contamination of staple diet is widespread in The Gambia and groundnuts, the main source of aflatoxin exposure, represents a major cash crop and an essential source of income in rural Gambia. Unlike in a similar study in China, there are no nationwide primary aflatoxin exposure interventions as yet in The Gambia. The recently formed partnership for control of aflatoxin in Africa (PACA) based at the African Union secretariat organized a partnership platform meeting in November 2014 that brought together different stakeholders engaged in aflatoxin control programmes. It is likely that a multi-disciplinary approach involving the Departments of Agriculture, Food Safety, Health and Trade will be needed to tackle the issue of aflatoxin contamination in The Gambia and elsewhere in Africa utilizing proven, simple and effective methods to improve crop triage, storage, and packaging; develop regional laboratory capacity for measuring aflatoxin levels not only in crops and foodstuffs but also in human urine and serum to quantify exposure; and engage in widespread sensitization and education of farmers, medical personnel, public health and food safety officers and the general public on the adverse health effects of aflatoxin.

The planned aflatoxin control programme using biological methods has important potential impact on the outcome of GHIS if it successfully and sustainably reduces aflatoxin exposure. If aflatoxin is a late promoter then this may reduce the power of GHIS and potentially extend the period until an endpoint is reached. It remains to be seen if field treatment of crops has an impact on human exposure.

Despite the uptake of HBV vaccination in many countries as recommended by WHO, the large number of people who had been infected prior to the introduction of the vaccination programmes continue to suffer from chronic HBV carriage and HCC. The predicted rise in the number of HCC patients over the next five to six decades in countries where HBV is endemic is a ticking time bomb of preventable human suffering and misery, not to mention the devastating economic consequences on low-resource countries that can least afford such a disease burden. This can potentially be averted by the identification and treatment of chronically HBV infected individuals.

The availability of safe, effective, orally administered nucleotide analogue (NA) drugs at affordable prices, portable non-invasive tools for measuring liver fibrosis and point-of-care diagnostics make this a realistic approach. The insights we have gained from PROLIFICA so far show that not only is this population-based approach feasible and cost-effective, but that it is also acceptable to the general population with high uptake of and adherence to treatment. New, cost-effective, point-of-care technologies being developed to measure HBV load in the field should make population-based programmes available to more resource-limited countries.

The Gambia, a small country with the infrastructures of GHIS, the NCR and The Global fund for HIV treatment already in place, can serve as a model for such a population-based approach. In this regard, several important unresolved issues can be addressed. These include: (1) improving knowledge of the natural history, response to treatment, and prognosis especially in HBeAg-negative patients with serum HBV DNA levels below 20 000 IU/ml., a common scenario in Africa; (2) assessment of the role of non-invasive markers (serum and biophysical) for the evaluation of the severity of liver disease and for the follow-up of treated and untreated patients; (3) further clarify the role of serum HBsAg levels in the evaluation of the natural history, prediction of therapeutic responses and treatment individualization; (4) assess host genetic and viral markers to determine prognosis and optimize patients' management; (5) assess the impact of early diagnosis and early treatment intervention; (6) assess long-term safety and resistance to the current first line NAs (Entecavir and Tenofovir); (7) identify markers that predict successful NA discontinuation; (8) assessment of new drugs and therapeutic approaches, particularly immune-modulatory therapies, to enhance loss of HBeAg and HBsAg and subsequent seroconversion; and finally (9) assess long-term impact of therapy on the prevention of cirrhosis and its complications and HCC. This approach will allow for further important scientific investigations into the mechanisms of hepatocarcinogenesis. Planned studies to identify specific miRNA expressions in serum/plasma associated with the severity of chronic HBsAg carriage in West Africa have the potential to yield biomarkers for early prediction of HBV-related liver disease. The recent acquisition of a mass spectrometer in The Gambia has made it possible to participate in studies aimed at discovering potential urinary biomarkers that out-perform alpha-fetoprotein and that can be used in the surveillance and early diagnosis of HCC. This raises the prospect of a urine dipstick test for HCC that can be used in the field.

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APPENDIX 1. PROLIFICA HC4 CASE DEFINITIONS/ DIAGNOSTIC CRITERIA 2012–2014

- **Significant liver fibrosis**: LSM≥7.5 Kpa by Fibroscan
- **Liver cirrhosis**: LSM≥9 Kpa by Fibroscan or USS diagnosis
- **Decompensated cirrhosis**: presence of haematemesis, ascites or hepatic encephalopathy in a cirrhotic patient
- **Clinically suspected (CS) HCC**: 2/3 of (i) focal liver lesion ≥2cm; (ii) AFP≥400ng/ml; (iii) presence of cirrhosis
- **Clinically confirmed (CC) HCC**: Focal liver lesion ≥2cm consistent with HCC **and** presence of liver cirrhosis **and** AFP≥400ng/ml
- **Histologically confirmed (HC) HCC**: HCC with biopsy & histological confirmation

Castera et al, 2005, Gastroenterology; Bruix J and Sherman M, 2005, Hepatology

APPENDIX 2: EASL CRITERIA FOR TREATMENT OF HBV

HBV DNA	ALT	Liver fibrosis (Either/or)		Action
		Fibroscan (kPa)	Liver biopsy	
> 2000 iu/ml	Any	>5	F > 1	Treat
Detectable	Any	>10	F > 3	Treat
Detectable	> 80	> 5	F > 1	Treat