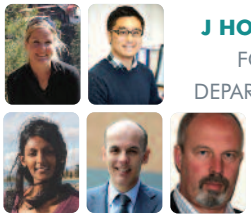
PREVENTION OF HEPATITIS B-RELATED LIVER FIBROSIS AND LIVER CANCER IN AFRICA: THE PROLIFICA PROJECT



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Hepatitis B virus (HBV) infection is endemic in sub-Saharan Africa (SSA), yet access to HBV screening and treatment is limited. The Prevention of Liver Fibrosis and Cancer in Africa (PROLIFICA) study is a large multicentre study in The Gambia, Nigeria and Senegal, which will evaluate the burden of HBV-related liver disease and establish feasibility and cost-effectiveness of community-based HBV screening and treatment for prevention of advanced liver disease and liver cancer in West Africa.

Hepatitis B virus (HBV) infection is endemic in sub-Saharan Africa (SSA). However, the full impact of HBV-related liver disease and liver cancer remains poorly characterized in SSA. Access to screening and treatment programmes is severely limited and HBV treatment guidelines tailored to the African setting do not currently exist. The Prevention of Liver Fibrosis and Cancer in Africa (PROLIFICA) study seeks to evaluate the burden of liver disease and liver cancer from HBV infection in West Africa and demonstrate that community-based screening and treatment of HBV is feasible, efficacious and cost-effective. In this review, the PROLIFICA programme is outlined in detail and preliminary results of the study are highlighted.

The global impact of hepatitis B infection

Hepatitis B virus (HBV) infection causes both acute and chronic liver disease, with chronic infection ranging from inactive chronic carrier status to progressive liver inflammation and damage, culminating in end-stage cirrhosis and liver cancer *(1)*. Globally, over one third of the world's population has been or is currently infected with HBV and 350–400 million people remain chronic HBV surface antigen (HBsAg) carriers *(1)*. There are over 500–750,000 deaths annually due to HBV-related cirrhosis and liver cancer worldwide and this figure underestimates the true burden of HBV disease in all likelihood, due to inadequate disease and cancer surveillance in many resource-poor countries where HBV is endemic (WHO. Hepatitis B. WHO [online], http://www.who.int/csr/disease/hepatitis/HepatitisB_whocdscsrlyo2002_2.pdf accessed 21 April 2014). The sheer scale of HBV prevalence worldwide, coupled with the over-representation of severe HBV-related liver disease burden in resource-poor countries, such as those within SSA, the Pacific Islands and Territories and South Asia, combine to make the HBV epidemic of critical importance to global public health.

Hepatitis B and hepatocellular carcinoma in sub-Saharan Africa

In SSA, HBV infection is endemic and the HBV-related liver disease and liver cancer burden is high. The lifetime risk of HBV infection is over 60% and over 8% of the population remain chronic HBV carriers who are at risk of progressive liver damage and HBV-related hepatocellular carcinoma (HCC)(http://www.who.int/csr/disease/hepatitis/global_report/en/; accessed 21 November 2014). SSA has one of the highest HBV-related liver cancer rates in the world *(2)*, and HBV-related HCC is the most common cancer among males and the third most common cancer among females in the

region *(3, 4)*. To place these figures in context, according to Globocan data for The Gambia, HCC accounts for more than half of all cancer in men (176 of 292 cases per year in 2012) and 62 of 265 cases per year among women in 2012. In comparison cervical cancer, another cancer associated with viral infection, accounted for an estimated 98 of the 265 reported cases in 2012, whilst breast cancer accounted for an estimated 40 cases *(5)*. Aflatoxin is a powerful mycotoxin; exposure through *Aspergillus flavus*-contaminated groundnut consumption in many regions of SSA works synergistically with HBV infection to increase cancer risk *(6, 7)*. Unfortunately, the natural history of HCC usually follows a highly aggressive course and limited treatment options are available, particularly in resource-poor settings such as SSA *(8)*. Furthermore, HBV-related HCC affects African patients in their working and reproductive years *(8)*, maximising the negative socioeconomic effects of this disease. HBV therefore represents a critical threat to health in the African continent.

Early detection and treatment of HBV infection reduces HCC incidence and mortality (primary prevention) *(9, 10)*. Furthermore, HCC survival is improved by early detection of potentially treatable HCC by screening of at-risk patients (secondary prevention) *(11)*. However, limited access to affordable medical care is a major limiting factor in hepatitis and liver cancer management in SSA. Routine HBV and HCC screening and surveillance programmes for the general population are virtually non-existent in SSA and there is a lack of infrastructure to support channelling of screened patients into long-term treatment programmes (http://www.who.int/csr/disease/hepatitis/global_report/en/; accessed 21 November 2014). Safe and effective treatments for HBV exist, but treatment access is severely limited in SSA due to cost. Despite highly effective nucleoside analogues such as tenofovir being available in most countries in SSA, at no cost through the Global Fund, or at generic price for the treatment of HIV, very few African countries offer publicly-funded HBV treatment (http://www.who.int/csr/disease/hepatitis/global_report/en/; accessed 21 November 2014).

Treatment options for HCC are severely limited in SSA, with percutaneous ethanol injection being the only modality available at a small number of centres. Furthermore, this modality is only effective for early stage HCC *(8)* and limitations in access to screening and diagnostic services in SSA mean most HCC cases are diagnosed when they are already at an advanced stage and no longer amenable to therapy.

Vaccination is the cornerstone of HBV prevention and is most effective when given within 24 hours of birth (12, 13). Multiple studies from SSA have demonstrated that HBV vaccination of infants is both feasible and highly effective for preventing chronic HBV carriage *(14–16)*. Despite WHO guidelines recommending HBV vaccination be commenced within 24 hours of birth, the vaccine schedule of 6, 10 and 14 weeks has been adopted in most African countries to allow the use of combination vaccines and to minimise costs and logistic expenses by streamlining vaccination schedules *(17)*. There is limited data on the relative effectiveness of this approach compared to birth dose vaccination in sub-Saharan Africa. Data from The Gambia Expanded Programme on Immunization, where complete delivery of the intended intervention of birth dose vaccination was not always achieved due to logistical constraints, suggested efficacy of 95% in preventing chronic HBV infection overall *(18, 19)*. However, the number of women in these studies who were HBeAg positive with high viral load was small and vaccine failure appeared much higher in this group *(18)*. Furthermore, in Africa there is incomplete HBV vaccine coverage, widely ranging from 10% in Chad to 99% in The Gambia, with the average less than 70% for Africa as a whole) *(20, 21)* (http://www.who.int/csr/disease/hepatitis/global_report/en/; accessed 21 Nov 2014; http://www.afro.who.int/; accessed 21 November 2014).

Control of HBV prevalence is a major goal for the World Health Organization (WHO) worldwide, with a key focus on HBV prevention in African countries. In 2010, the World Health Assembly (WHA) passed a resolution calling for public health intervention to prevent and control viral hepatitis. There is also a forthcoming WHA resolution requesting the Global Health Fund to provide antiviral medications for HBV mono-infected patients. However, until greater global action for equitable treatment access is achieved, there remains a significant burden from HBV-related liver disease and HCC in SSA, with little access to life-saving screening and treatment. Importantly, there is a significant lack of Africa-specific guidelines for HBV and HCC screening and treatment.

Prevention of hepatitis B-related liver fibrosis and cancer in Africa: The PROLIFICA study

Against the sombre backdrop of HBV-related liver disease in SSA described above, the Prevention of Liver Fibrosis and Cancer in Africa (PROLIFICA) project was established in 2011 to address some of the urgent HBV research needs in three West African countries: Senegal, The Gambia and Nigeria. This is an on-going five-year translational research project funded by the European Union Framework 7 (www.prolifica.eu; EU-FP7 #265994) and represents a

collaboration between Imperial College, London; MRC Unit, The Gambia in Fajara, The Gambia; IARC, Lyon France; Le Dantec University Hospital, Dakar and University of Thiès, Sénégal; and Jos University Teaching Hospital, Jos, Nigeria.

The overall aim of the project is to reduce the incidence of HBV-related HCC in West Africa. Specific aims of the project include:

- Investigating the natural history and epidemiology of HBV-related liver disease and HCC in West Africa, including overall burden of disease;
- Demonstrating the feasibility of mass screening for HBV infection and subsequent enrolment in an antiviral treatment programme;
- Establishing whether treatment of HBV with nucleoside analogues is a cost-effective method to reduce the burden of HBV-related liver disease and liver cancer in West Africa;
- Developing novel biomarkers to predict and diagnose HBV-related liver fibrosis and HCC has also been a key research goal for the project.

Ultimately, data provided by the study will be used to develop screening and treatment guidelines for both HBV and HCC for the African context, which will then inform WHO HBV guideline development for resource-poor settings.

PROLIFICA study design

The PROLIFICA study consists of two platforms: West African Treatment Cohort for HBV (WATCH) and hepatocellular carcinoma case-control study (HC4). The WATCH study has been conducted in two parts.

The first part is a population-based study, with 13,500 people screened for HBV infection using a point-of-care test in Senegal and The Gambia. Subjects were invited for screening from community, workplace, health service and voluntary blood donor sites. In The Gambia, most participants were recruited from community screening through stratified selection from urban and rural areas representative of western Gambia and also voluntary blood donors; whereas in Senegal screening has been predominantly through health facilities and workplaces. All HBsAg positive participants and a proportion of HBsAg negative control subjects are then invited to participate in a full liver assessment. This includes clinical evaluation by a trained hepatologist, ultrasound, transient elastography (FibroScan™, Echosens, France), liver biopsy where required and blood tests. HBsAg negative controls provide a single blood and urine sample and HBsAg positive participants provide blood and urine samples at regular intervals, when they also attend for liver disease assessment and follow-up.

The second study is a non-randomised, open label study of tenofovir therapy for HBsAg positive patients who fulfilled EASL 2012 treatment guidelines for nucleoside analogue therapy *(1)*. These include HBsAg positive patients with 1) advanced liver fibrosis or cirrhosis (Metavir F3 or greater fibrosis stage on biopsy or equivalent on Fibroscan) and any detectable HBV viral load; or 2) HBV DNA level > 2000IU/mL, ALT >40IU/mL and moderate fibrosis (as evidenced on elastography or liver biopsy) and/or significant inflammation on liver biopsy; or 3) HBV DNA level >20,000IU/mL and ALT >80IU/mL; or 4) viral load >20,000IU/mL and a family history of hepatocellular carcinoma, or age over 30 years and any ALT level.

In parallel, HBsAg positive patients who did not fulfil EASL criteria for nucleoside analogue therapy were followed up over time and offered six-monthly HCC screening by ultrasound as per EASL guidelines *(1, 8)*. Once on therapy, participants undergo close monitoring for side effects from tenofovir therapy and to assess virological and clinical response to therapy. Study participants are then followed for five years and the effect of treatment on HBV disease outcome will be compared to historical control data of matched patients available for each country. The outcome of those who did not fulfil EASL treatment criteria will also be assessed, to gauge whether current international guidelines for HBV therapy are appropriate for the African setting.

The HC4 study was designed to develop a research platform for discovering and validating biological markers of HCC in Nigeria, The Gambia and Senegal. HCC cases as well as controls have been recruited in tertiary hospitals in these countries.

Achievements of the PROLIFICA research platform so far

Screening and recruitment is now complete in The Gambia and almost complete in Senegal. In The Gambia, 5,980 inhabitants in randomly selected communities have been screened for HBsAg and clinical information and samples have been collected. To date, the PROLIFICA study has provided important insights into HBV-related HCC in West Africa, including identification of novel metabonomic biomarkers which may prove useful for HCC diagnosis in resource-poor settings *(22, 23)*, validation of simple, inexpensive tests more appropriate for Africa to guide HBV therapy *(24, 25)* and epidemiological insights into risks for more severe HBV-related liver disease *(26–28)*. These are now outlined in more detail.

Epidemiology and natural history of HBV infection and HBV-related liver disease

The PROLIFICA study will provide accurate HBV infection prevalence data for regions within The Gambia and Senegal, as well as data on risk factors for HBsAg positivity. Important data on vaccination coverage will also be provided. The PROLIFICA study also represents the first large-scale study describing the prevalence and magnitude of HBV-related liver disease and incidence of cirrhosis and HCC in SSA, obtained through comprehensive liver assessment of people identified as HBsAg positive at screening. Risk factors for HCC and its predictors will also be examined: of these, mode of HBV transmission was suggested to be associated with prolonged hepatitis B e antigenaemia in The Gambia *(2)*. The symptoms and end-of-life issues in HBV-related chronic liver disease have been also described *(29)*.

Implementation of HBV screening, liver assessment and treatment

A key-aim of the study is to demonstrate that both community and facility-based screening for HBV infection and assessment of HBV-related liver disease is achievable and beneficial for improving health in West Africa. Data for the effectiveness of HBV screening and liver disease management in the African context are lacking and this information is crucial to inform government health policy-makers. The project has also provided data to inform strategies to improve HBV screening uptake and medical follow-up within West African countries *(27)*. Importantly, our data to date have shown excellent uptake of HBV screening and generally high attendance to medical clinics by patients diagnosed with HBV infection, albeit within the setting of a clinical trial *(28)*.

Effectiveness of nucleoside analogue therapy for improving HBV-related outcomes

Tenofovir therapy is widely established as an effective drug with a high barrier to resistance for the treatment of HBV infection *(1, 31)*. However, studies have not been conducted specifically within the African context, particularly examining safety and effectiveness in African populations. Data from PROLIFICA will therefore be critical to inform local ministries of health and government health policy developers and economists in Africa, as well as the World Health Organization, on the feasibility, effectiveness and costs of implementing tenofovir therapy in Africa.

Cost-effectiveness of HBV screening and treatment

Economic analyses use information on the costs, as well as the effectiveness of interventions, to provide a framework for guiding public health interventions. Although this research is arguably even more crucial in resource-poor settings, where health-care budgets are severely limited and burden of disease is high, previous economic models of HBV treatment have been limited to high-income and Asian settings or specific to targeted screening of high-risk groups only.

One of the barriers to universal implementation of screening and treatment for hepatitis B has historically been the cost associated with delivering such an intervention. However, the major progress in price reduction of antiviral therapy including tenofovir, which is now available at a generic price of US$ 48 per year of treatment, has made this a more feasible strategy in low-middle income countries (Source: http://www.msfaccess.org/content/untangling-web-antiretroviral-price-reductions-16th-edition; accessed 12 December 2014). Within PROLIFICA, community based screening for hepatitis B has also been shown to be cheap (£6.47/ per person screened) *(30)* and cheaper alternative methods of assessing liver fibrosis have been compared to the current gold standard of liver biopsy *(25)*. Further models evaluating cost-effectiveness of screening and treatment in terms of cost per life year saved are being developed in collaboration with Imperial College London (Department of Infectious Disease Epidemiology). This research will be of utmost importance in informing international hepatitis guidelines by WHO, as well as in helping local health ministries prioritize implementation of screening and treatment of HBV, in places where it poses a significant health care burden.

Virological determinants in HBV infection

PROLIFICA has provided a unique opportunity for research on HBV genotype E, which is the dominant species in West Africa. Next generation sequencing has allowed investigation of novel associations between the viral genome and clinical disease. Development of cheaper, efficient in-house assays to guide clinical management and investigation of new technologies and biomarkers of virological activity are also being investigated within the PROLIFICA platform.

Development of novel point-of-care tests and non-invasive tests for HBV diagnosis and management

A requirement of the EU grant was developing a research collaboration with a small-sized commercial partner. Partnership with commercial diagnostics company Xeptagen has fast-tracked development of point-of-care tests for HBV

for resource-poor settings, including "Apps" for mobile phones and other devices to facilitate easy result verification in the field. Validation studies of several non-invasive and inexpensive blood-based diagnostic models such as the Aspartate to Platelet Ratio Index (APRI) and Fib-4 *(25, 34)* have also been conducted in the African context. Accuracy of transient elastography has also been assessed in African patients *(25)*. Results of these studies will be paramount for implementation of liver disease assessment in resource-poor settings.

New diagnostic markers for HCC – genomics, epigenomics, proteomics and metabonomics

Several groups within the PROLIFICA team have been investigating genetic, proteomic and metabonomic markers for HCC diagnosis as current standard diagnostic modalities such as CT scan and MRI scan are not available throughout most of Africa. Key urinary metabolytes have been identified which have excellent discriminative ability for HCC, even in patients with background liver disease and cirrhosis *(22, 33)*, offering the exciting potential of a urinary dipstick test for HCC diagnosis.

Overall benefits of the platform

Importantly, the PROLIFICA platform has resulted in substantial capacity building in each of the three countries involved in the study. Local health infrastructure has benefitted from new technologies, such as Fibroscan™ to assess liver fibrosis using ultra-sound-based transient elastography a mass spectroscopy system in The Gambia for local biomarker research and the development of in-house laboratory assays, as well as skills transference to build capacity for improved liver cancer health care in West Africa.

The data provided by PROLIFICA will be crucial for the development of local guidelines for the effective management of HBV and HCC, including screening and treatment. These data will also be of paramount importance for the development of WHO HBV treatment guidelines, which are currently underway.

Local nursing, medical and laboratory staff have benefitted from training, education and employment opportunities. Procedures and training in effective and secure data management and ethical research practices has also been a central part of the PROLIFICA platform. Local researchers have availed themselves of specialized academic mentorship at each of the three sites involved in the study to obtain higher research degrees and highly-regarded academic publications. Finally, community education on HBV infection, mode of transmission and prevention is also likely to have had a positive impact on HBV awareness, both at the community and political level.

Above all, the PROLIFICA platform has facilitated the development of strong relationships for future research collaborations between major academic centres and a deeper understanding of the barriers to improved health-care delivery in Africa. All centres have equally benefitted from the experience and knowledge shared between the investigating teams. These are arguably the greatest achievements yielded from this project to date. International collaborative projects between academic institutions in Africa and the rest of the world are a fantastic opportunity to share ideas, specialized expertise and new technologies and foster a united approach to solving some of the greatest public health challenges in Africa today. Collaborations also provide a stronger international voice to raise global awareness of the importance of liver disease in Africa to governments, the pharmaceutical industry and the international community.

Conclusion

HBV screening and subsequent treatment that is accessible and affordable to all is a pressing requirement in SSA and greater support from the international medical community is critical to engender support from the pharmaceutical industry for equitable drug availability. The crucial importance of viral hepatitis research cannot be overstated in achieving this goal, particularly research demonstrating that screening and treatment for HBV not only saves lives, but is cost-effective. Furthermore, the strong collaborative relationships formed by international projects like PROLIFICA between local and international partners enable a strong, united international voice to campaign for equitable access to HBV treatment and prevention and improved lives for people living with HBV infection in Africa.

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References

1. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol*. 2012;57(1):167-85.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;55(2):74-108.
3. Parkin DM, Sitas F, Chirenje M, Stein L, Abratt R, Wabinga H. Part I: Cancer in Indigenous Africans--burden, distribution, and trends. *Lancet Oncol*. 2008;9(7):683-92.
4. Kirk GD, Lesi OA, Mendy M, Akano AO, Sam O, Goedert JJ, et al. The Gambia Liver Cancer Study: Infection with hepatitis B and C and the risk of hepatocellular carcinoma in West Africa. *Hepatology*. 2004;39(1):211-9.
5. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136(5): E359-86.
6. Kirk GD, Lesi OA, Mendy M, Szymanska K, Whittle H, Goedert JJ, et al. 249(ser) TP53 mutation in plasma DNA, hepatitis B viral infection, and risk of hepatocellular carcinoma. *Oncogene*. 2005;24(38):5858-67.
7. Kuniholm MH, Lesi OA, Mendy M, Akano AO, Sam O, Hall AJ, et al. Aflatoxin exposure and viral hepatitis in the etiology of liver cirrhosis in the Gambia, West Africa. *Environ Health Perspect*. 2008;116(11):1553-7.
8. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56(4):908-43.9.. Robotin MC, Kansil MQ, George J, Howard K, Tipper S, Levy M, et al. Using a population-based approach to prevent hepatocellular cancer in New South Wales, Australia: effects on health services utilisation. *BMC health services research*. 2010;10:215.
10. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *The New England journal of medicine*. 2004;351(15):1521-31.
11. Lopez PM, Villanueva A, Llovet JM. Systematic review: evidence-based management of hepatocellular carcinoma--an updated analysis of randomized controlled trials. *Alimentary pharmacology & therapeutics*. 2006;23(11):1535-47.
12. Poovorawan Y, Chongsrisawat V, Theamboonlers A, Srinivasa K, Hutagalung Y, Bock HL, et al. 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. *J Infect Dis*. 2009;200(1):33-8.
13. Poovorawan Y, Chongsrisawat V, Theamboonlers A, Leroux-Roels G, Kuriyakose S, Leyssen M, et al. Evidence of protection against clinical and chronic hepatitis B infection 20 years after infant vaccination in a high endemicity region. *J Viral Hepat*. 2011;18(5):369-75.
14. Mendy M, Peterson I, Hossin S, Peto T, Jobarteh ML, Jeng-Barry A, et al. Observational study of vaccine efficacy 24 years after the start of hepatitis B vaccination in two Gambian villages: no need for a booster dose. *PLoS One*. 2013;8(3):e58029.
15. Montesano R. Hepatitis B immunization and hepatocellular carcinoma: The Gambia Hepatitis Intervention Study. *J Med Virol*. 2002;67(3):444-6.
16. Chiang CJ, Yang YW, You SL, Lai MS, Chen CJ. Thirty-year outcomes of the national hepatitis B immunization program in Taiwan. *JAMA: the journal of the American Medical Association*. 2013;310(9):974-6.
17. Ekra D, Herbinger KH, Konate S, Leblond A, Fretz C, Cilote V, et al. A non-randomized vaccine effectiveness trial of accelerated infant hepatitis B immunization schedules with a first dose at birth or age 6 weeks in Cote d'Ivoire. *Vaccine*. 2008;26(22):2753-61.
18. Inskip HM, Hall AJ, Chotard J, Loik F, Whittle H. Hepatitis B vaccine in the Gambian Expanded Programme on Immunisation: factors influencing antibody response. *Int J Epidemiol* 1991; 20(3): 764-9.
19. Mendy M, Peterson I, Hossin S, Peto T, Jobarteh ML, Jeng-Barry A, et al. Observational study of vaccine efficacy 24 years after the start of hepatitis B vaccination in two Gambian villages: no need for a booster dose. *PLoS One* 2013; 8(3): e58029.
20. Jemal A, Bray F, Forman D, O'Brien M, Ferlay J, Center M, et al. Cancer burden in Africa and opportunities for prevention. *Cancer*. 2012.
21. Howell J, Lemoine M, Thursz M. Prevention of materno-foetal transmission of hepatitis B in sub-Saharan Africa: the evidence, current practice and future challenges. *J Viral Hepat*. 2014;21(6):381-96.
22. Shariff MI, Ladep NG, Cox IJ, Williams HR, Okeke E, Malu A, et al. Characterization of urinary biomarkers of hepatocellular carcinoma using magnetic resonance spectroscopy in a Nigerian population. *J Proteome Res*. 2010;9(2):1096-103.
23. Ladep NG, Dona AC, Lewis MR, Crossey MM, Lemoine M, Okeke E, et al. Discovery and validation of urinary metabotypes for the diagnosis of hepatocellular carcinoma (HCC) in West Africans. *Hepatology*. 2014.
24. Lemoine M, Shimakawa Y, Njie R, Njai HF, Nayagam S, Khalil M, et al. Food intake increases liver stiffness measurements and hampers reliable values in patients with chronic hepatitis B and healthy controls: the PROLIFICA experience in The Gambia. *Aliment Pharmacol Ther*. 2014;39(2):188-96.
25. Lemoine M, Shimakawa Y, Goldin R, Khalil M, Lloyd J, Suso P,

Nayagam S, Freeya Njai H, Taal M, Ndow G, D'Alessandro U, Njie R, Thursz M. Validation and comparison of non-invasive markers of liver fibrosis in West African patients with chronic hepatitis B living in The Gambia. *J Hepatol* 2014; 60(suppl 1): S414

26. Shimakawa Y, Bottomley C, Njie R, Mendy M. The association between maternal hepatitis B e antigen status, as a proxy for perinatal transmission, and the risk of hepatitis B e antigenaemia in Gambian children. *BMC Public Health*. 2014;14:532.Y. Shimakawa,
27. Lemoine M, Freeya Njai H, Jatta A, Sanneh B, Taal M, Corrah MT,

D'Alessandro U, Njie R, Thursz M. Community-based screening for hepatitis B virus infection in The Gambia, West Africa: prevalence of infection and factors affecting the screening attendance. *J Hepatol* 2013; 58(Supp1): S21.

28. Lemoine M, Njai H, Shimakawa Y, Taal M, Corrah T,

D'Alessandro U, Njie R, Thursz M. Rural and urban community-based hepatitis B screening in The Gambia: Assessment of liver disease reveals a significant proportion of eligible patients for antiviral therapy. *J Hepatol* 2013; 58(suppl 1): S402

29. Shimakawa Y, Takao Y, Anderson ST, Taal M, Yamaguchi T, Giana L, et al. The prevalence and burden of symptoms in patients with chronic liver diseases in The Gambia, West Africa. *Palliat Med*. 2014.
30. Shimakawa Y, Yan HJ, Tsuchiya N, Bottomley C, Hall AJ. Association of early age at establishment of chronic hepatitis B infection with persistent viral replication, liver cirrhosis and hepatocellular carcinoma: a systematic review. *PLoS One*. 2013;8(7):e69430.
31. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology*. 2009;50(3):661-2.
32. Nayagam S, Shimakawa Y, Lemoine M, Njie R, Tamba S, et al. Feasibility and cost analysis of community based hepatitis B screening programme in Sub-Saharan Africa. *J Hepatol* 2014; 60: S36.
33. Ladep NG, Dona AC, Lewis MR, Crossey MM, Lemoine M, Okeke E, et al. Discovery and validation of urinary metabotypes for the diagnosis of hepatocellular carcinoma in West Africans. *Hepatology*. 2014;60(4):1291-301.
34. Xiao G, Yang J, Yan L. Comparison of Diagnostic accuracy of APRI and FIB-4 for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: A systemic review and meta-analysis. *Hepatology*. 2014.