

**Characterization of “occult” HBV yield cases identified by
NAT (ID or small pool) or anti-HBc screening**

Reported by Jean-Pierre Allain on behalf of the ISBT TTI working party “HBV
safety” study-group

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Abstract

Background and objectives. The availability of commercial assays to detect Hepatitis B virus (HBV) DNA with high sensitivity in individual plasmas or in plasma pools has uncovered in blood donors a new category of HBV infection called 'occult'. Occult HBV infection is characterized by the presence of HBV DNA without detectable HBsAg outside the window period. The objective of the present article is to provide the global transfusion community with the basis of a collaborative study on HBV blood safety coordinated by the International Society of Blood Transfusion Transfusion-Transmitted Infection Working party (ISBT TTI WP).

Methods. This group proposes to collect samples from HBsAg negative blood donors initially screened with either anti-HBc or HBV NAT. Confirmed anti-HBc positive samples will then be tested for HBV DNA by NAT. Samples confirmed as occult HBV will be characterized with a large array of serological, cellular and molecular testing intended to determine whether they originate from recovered or chronic infection and which virus- or host-related mechanisms are involved.

Participating blood centers are encouraged to collect follow-up samples as well as archived samples from previous donations, to conduct look-back studies whenever possible and to determine whether or not donors were vaccinated to HBV. Recalled donors will be submitted to detailed clinical evaluation.

Expected outcome. It is anticipated that a sufficient number of donors carrying occult HBV infection of all six major genotypes (A-F) will be studied to answer

essential questions such as infectivity by transfusion and clinical relevance of this newly identified condition.

Introduction

Hepatitis B and its causal agent hepatitis B virus (HBV) have been studied for many years both clinically and biologically. Yet a number of important questions remain unanswered. Individuals carrying the occult form of HBV infection defined as HBV DNA positive but HBsAg undetectable represent a missing link between clinically identified infection, recovery and the severe complications such as liver cirrhosis and hepatocellular carcinoma (HCC) (1). Although classically these severe complications develop in individuals with biochemical evidence of inflammation and/or fibrosis and of hepatocyte lysis such as elevated ALT, chronic sequelae may also develop in individuals who have escaped detection for many years and are considered carriers of occult infection.

Extremely sensitive nucleic acid detection and quantification systems systematically applied to large numbers of apparently healthy individuals such as candidate blood donors have identified a group of HBV infected individuals that would have otherwise escaped medical scrutiny. These assays, alone or combined with the long used anti-HBc assay, are being applied increasingly to individual donation screening in areas with different epidemiological patterns in terms of HBV prevalence and genotype. Applied to a previously unscreened population, HBV DNA screening will identify a new cohort of viremic but HBsAg negative donors (2, 3) providing a unique opportunity to collect a large number of cases of occult HBV infections for scientific and clinical investigation of a nearly unknown aspect of HBV infection natural history. The proposal below will address issues not only of blood safety but also of clinical and basic science. The transfusion community has a unique opportunity to explore the risks for donors who are detected with occult HBV infection and patients previously exposed to components prepared from occult carriers of a virus affecting globally close to a billion individuals.

This document was drafted for the purposes of the International Society of Blood Transfusion (ISBT) working party (WP) for Transfusion Transmitted Infections (TTI). This WP includes several subgroups such as Transmissible Spongiform Encephalopathies (TSE), Bacteriology, Parasites and Hepatitis B virus which the subject of the present document. It is intended to inform

interested parties on the HBV safety study initiated as a result of the recent worldwide interest for low level HBV DNA uncovered by the dissemination of HBV DNA amplification methods.

Background

Blood screening

HBV screening in blood donations consists of HBsAg serological testing with assays varying in sensitivity from less than 0.1 to several nanograms/ml according to test platforms ranging from the fully automated highly sensitive PRISM to insensitive rapid tests such as particle agglutination. This screening is applied in areas with HBsAg prevalence ranging from 0.05 to 25% in populations where exposure to HBV as measured by the presence of anti-HBc ranges from 0.2 to 100%, respectively. Although HBsAg screening assays were developed against HBV genotype A (prevalent in North America and North West Europe) most infections are with genotype B and C in the Far East and South East Asia, genotype D in India, the Middle East, Eastern Europe, the Mediterranean basin and part of Africa, genotype E in Sub-Saharan Africa and genotype F in native populations from Latin America (4, 5).

Anti-HBc was introduced in the USA as a surrogate marker for non-A, non-B hepatitis but, even following the introduction of anti-HCV screening, anti-HBc testing was maintained to enhance HBV transfusion safety although its exact efficacy remains under study (2, 6-9). The use of anti-HBc to improve HBV safety is however limited to areas where HBV seroprevalence is relatively low (<2% anti-HBc reactivity) otherwise the impact on donor deferral is considered unsustainable, particularly in Southern Europe, Asia and Africa. The efficacy of the test is also limited by two main factors: its notorious lack of specificity and the very high proportion of samples containing high levels of anti-HBs (70->90% of units positive for anti-HBc) making these blood units essentially safe for transfusion but, according to current procedures, excluded from the blood supply (6). Moreover this approach on its own omits two sources of potentially infectious blood: the pre-seroconversion window period

and the tail-end cases of chronic infection that are anti-HBc negative but DNA positive (10).

HBV NAT has been employed for several years in some blood centers in Germany, Austria and Luxembourg, but only in Japan is it mandatory (11-13). In these countries and in specific studies conducted in the USA and West Africa, HBV NAT was applied to plasma pools varying in size from 10 to 500 individual plasmas (2, 3, 13 -15). From these studies and more recent unpublished data, it is clear that the yield of occult HBV infection was 3-10 times lower when tested in pools compared to individual donation samples (ID) (2, 3). In the context of this proposal, ID screening with highly sensitive NAT is critical to study anti-HBc yield samples where anti-HBc screening is in use and everywhere else where NAT is the only feasible screening measure to supplement or replace HBsAg assays.

A slightly different, but connected issue is the discrepancies observed between HBsAg and HBV DNA when the former is confirmed reactive and the latter is negative. In most such cases, HBsAg reactivity was confirmed by neutralization and the presence of anti-HBc reactivity. In many cases where routine HBV NAT was negative, HBV DNA could be detected subsequently by using highly sensitive research tests. Nevertheless, some HBsAg positive samples have tested negative even by the most sensitive HBV NAT. HBsAg positive/HBV DNA negative samples have been identified in several recent studies (2, 16, 17), further highlighting the lack of correlation between the HBsAg and HBV after the window period in chronically infected individuals. This is a second type of occult HBV infection, except that it is HBV DNA that is negative, and HBsAg positive. No systematic study of these cases has been undertaken, particularly in terms of infectivity and clinical significance.

Occult HBV and transfusion safety

At the present time, there is little data on two critical issues relevant to transfusion: i) What is the infectivity of blood units or components containing occult HBV DNA with or without anti-HBc or anti-HBs outside the window period. In the window period, as little as 10 viral particles may be infectious. In contrast, in occult HBV carriers, the Dane particles are often immune-complexed with neutralizing anti-HBs or other antibodies not necessarily

detectable, and are presumably less infectious (18, 19). ii) What is the significance to the donor deferred on the basis of occult HBV both in terms of possibility of transmitting the infection sexually or through other contacts and the impact on their own health status (1). The former is directly linked with general issues related to the natural history of the infection discussed later. Methodological approaches to answer these questions are limited and all are to some extent unsatisfactory since prospective studies are not ethically feasible and animal or in vitro experiments are not necessarily applicable to humans. The main approach is look-back exercises, which are not representative of the index sample because of the temporal difference, unless archived donor samples are available (6, 18). Such look-backs also have the major drawback of being limited by recipient susceptibility to HBV infection, survival post-transfusion or unavailability, and by considerable cost. The clinical significance could be addressed by long-term clinical prospective studies. Such studies, however would be expensive and a number of biological examinations can be performed that would provide essential information, including liver biopsies.

Another factor regarding the susceptibility of blood transfusion recipients is the influence of their health and general immune status at the time of contact with HBV. In England, for instance, over 50% of recipients of blood components have some degree of immunodeficiency that can affect their susceptibility to infection by lowering the threshold of infectivity and increasing the clinical severity of the infection once established. Evidence of this considerable problem has been reported for HBV in severely immunosuppressed recipients of livers from donors carrying anti-HBc only, anti-HBs and anti-HBc or only HBV DNA (20-22).

The place of occult HBV in the natural history of HBV infection

Occult HBV may have three main origins (1):

- Tail end of chronic carriage (anti-HBc reactive but anti-HBs negative) following years in the non-replicative phase.

- Persistence of low-level replication in the liver or PBMCs after recovery from infection as characterized by the development and persistence of neutralizing anti-HBs (23-26).
- Occurrence of escape mutants in vaccinated (breakthrough infection) or unvaccinated individuals that evade detection by current HBsAg capture assays (26, 27).

The presence or absence of anti-HBs is the main parameter that generally distinguishes between chronic and recovered infection and high viral load escape mutants. A majority of HBV NAT yield cases have no detectable anti-HBs because of its relatively short lifespan and present as “anti-HBc only” with low viral load (<500IU/ml) making it difficult to distinguish between recovered and chronic infection (2, 3), since it cannot be completely excluded that some tail end carriers produce low levels of non-neutralizing anti-HBs. Such distinction may be important since recovered donors are assumed to be at reduced risk of liver disease and infectivity while chronic cases are at higher risk of progressive complications and more likely infectious by transfusion.

There are several possible approaches to discriminate between these two forms of occult HBV infection, including molecular biology, cellular immunology and clinical investigation.

Molecular biology

Viral load needs to be determined since it is generally related to infectivity. This can be undertaken with sensitive real time PCR (QPCR) (28) or endpoint titration using more sensitive qualitative NAT systems. Quantitative NAT might not always be reliable with a viral load below 100 copies/ml. While confirmation of a DNA positive test result can be inferred by the presence of serological HBV markers (anti-HBc and/or anti-HBs), whereas confirmation of samples with DNA as the only marker of infection requires additional strategies including concordance with alternative ultra-sensitive screening assays; repetition of testing; using larger volumes or multiple archived aliquots retained from the plasma bag; or testing look-back or prospective samples from the index individual. Preliminary observations indicate a particular infectivity of platelet concentrates possibly related to the

preferential binding of HBV to the platelet surface (MP Busch, personal communication). Low level HBV replication in leucocytes may also warrant investigation.

Sequencing the 'a' region of the S gene, as most people have done to identify mutants that might escape HBsAg capture antibody, should be supplemented by sequencing the regions important for viral replication (26, 27, 29). There may be specific genetic features of occult HBV that explain the maintenance of long-term low-level replication. To that end, obtaining the complete genome sequence would be required to determine whether or not the pre-S, X, Basic core promoter (BCP) and pre-core regions as well as the polymerase reading frame are involved. The little data currently available suggests that recovered infections tend to be of wild type in the pre-core and BCP regions, while strains found in chronic infections often carry the pre-core 1896 stop codon and the 1762-1764 mutations or deletions of BCP (30).

Preliminary evidence indicated that the natural history of the infection might be influenced by genotype (10, 31-34). This is well known for genotypes B and C but data obtained for genotypes A, D and E seem also to indicate differences, in particular in the prevalence of occult HBV (3). In large parts of the world, with standardized and sensitive HBsAg and NAT screening, there is an opportunity to compare the rate of occult HBV infection in previously anti-HBc unscreened donor populations and to investigate if there is a relationship with the prevalent genotypes in the donor population. Genotyping of the HBV strains might therefore be important. Furthermore the sensitivity of the amplification system that enables sequencing of a fragment of the S gene is critical for discovering new escape mutations in very low viral load carriers (Koppelman et al, personal communication).

Finally, there are suggestions that HBV DNA in the absence of HBsAg might originate from incomplete virions or integrated viral DNA released into the circulation (35). This can be investigated by obtaining evidence of complete genome sequences or capturing viral particles with antibodies to surface antigen and detecting viral DNA (immuno-NAT) or treatment of plasma samples not containing EDTA with DNase to examine if non particle bound HBV-DNA replication intermediates circulate free in the circulation.

Cellular and humoral immunology

In this situation of near tolerance of the virus by the host, investigating the host immune response is critical. When anti-HBc is the only serologic marker, looking for memory B- and T-cells will be useful. It is expected that lymphocytes from individuals who have recovered from HBV infection will respond to stimulation with both core and surface antigen whereas lymphocytes from chronic infections will respond only with core. Some unexpected specific stimulating epitopes might be discovered (36, 37). Preliminary data indicates that although anti-HBs is not found in the circulation in chronic infection, other antibodies might bind to the virion surface and play a role in limiting infectivity (38). Alternatively, some individuals might carry anti-HBs at the virion surface that need dissociation procedures to reveal the seroreactivity.

The outcome of HBV infection was shown related to specific host genetic determinants, particularly HLA –A, B- or DR- (39, 40). There might be merit in investigating this, however the small number of predicted cases will probably defeat that purpose due to limited power to detect correlation with HLA haplotypes.

Clinical features

At present, no information is available regarding the potential association of occult HBV with liver disease. Studies of donors or patients who carry occult HBV indicate that ALT levels are normal (24, 25). Two studies examined cases of acute HBV infection in adults who recovered and were re-investigated after one or multiple decades, and found viral DNA in the liver as well as evidence of liver disease in a significant proportion of cases (24, 25). No histopathological data on the transplanted livers from anti-HBc positive donors that transmitted HBV has been published. However, there might be sufficient clinical evidence to justify performing liver biopsies in selected individuals. If such specimens were obtained, evidence of liver disease could be collected, viral DNA can be quantified, and the presence of integrated DNA in hepatocytes explored. This type of investigation might provide important data regarding the possibility of developing cirrhosis or HCC (41, 42).

Results of such investigations should provide the basic knowledge necessary to appropriately counsel donors deferred on the grounds of occult HBV.

This background provides the scientific basis for the wide range of investigations which should be undertaken are described in the paragraphs below.

Objectives of the study

1. To collect specimens (component units) and archived repository samples from donations found to be HBV DNA positive and HBsAg negative after NAT or anti-HBc screening, and from donors who test HBsAg positive and HBV DNA negative,
2. To characterize these samples molecularly and immunologically,
3. To conduct look-back studies on these selected donors: identify archived samples from previous donations, trace recipients and collect samples to determine HBV transmission from index donors,
4. To follow up donors and collect samples for molecular confirmation, immunological, genetic and clinical evaluation, including liver biopsy when possible,
5. To fully characterize occult HBV infections with respect to both virus and host parameters and provide the transfusion community with evidence on which to base testing/safety strategies as well as donor notification and counselling guidelines.
6. It is hoped to fully document 20 cases of occult HBV from each of the 7 main HBV genotypes.

Organization of the study (Figure 1)

The TTI WP subgroup will undertake to generate and communicate data to the ISBT at international or regional congresses. The active arm of the project will be a collaborative study group composed of individuals procuring samples, or participating clinically or scientifically to the achievement of the objectives of the study. Consistency in the study will be achieved by the use of a small number of designated laboratories with expertise in one or more of the specialised investigations recommended in this protocol. It is recognized that regional collaborations between blood centers and reference laboratories may already exist which will generate valuable data outside the designated

laboratories. Data will be centrally assembled and analyzed to allow for global implications to be drawn. The outcome of the study group will lead to major publications.

Proposed investigations

Investigations undertaken will depend on the origin of samples i.e. HBV NAT or anti-HBc as the primary screening test. Where HBV NAT is implemented, the presence of HBV DNA will be confirmed and the donor recalled for additional NAT or serologic markers (anti-HBc, anti-HBs titre) testing. All confirmed samples (HBsAg-/HBV DNA+) and corresponding donors will be eligible to enter the study. Where anti-HBc is the primary screening assay, HBV NAT will be part of the characterization process of these samples that will include confirmation of anti-HBc, detection and quantification of HBV DNA and anti-HBs. Serological markers will then be part of the NAT confirmation process. In addition, anti-HBc reactive donors with low anti-HBs titre (<100 mIU/ml) with or without detectable HBV DNA will be eligible for further study. Anti-HBc positive donors without detectable HBV-DNA with anti-HBs titre above 100 mIU/ml are considered safe for transfusion and in some countries eligible for re-entry in the blood supply for either labile components or manufacturing of plasma products only (the ultimate decision depending on national or regional regulatory requirements).

The various facets of the investigation described below will be applicable to all samples and donors or recipients considered confirmed positive for HBV DNA without HBsAg.

Collection of Index samples and corresponding data

Plasma and platelet concentrate or plasma separated from red cell when whole blood is used from donors whose screening sample is identified as containing HBV DNA but lacking HBsAg will be kept frozen at -30°C or lower. Such samples will constitute **Index samples** for any other donor sample retrieved from archive or prospectively collected. Index samples will need to meet certain criteria before being considered eligible to the study. These criteria include: 1. Confirmation of the presence and quantification of HBV

DNA by other assays than the screening assay; 2. Collection of at least one follow-up donor sample. Such follow up sample should contain HBV DNA in order to eliminate concern over contamination of the index sample and, in centres utilizing NAT for primary screening, to identify window period cases (converting to HBsAg) and secondary window period cases (converting to anti-HBs). These last two types of samples are not the primary concern of the study group but may constitute useful controls. It is recommended to collect further follow-up samples to identify individuals with fluctuating levels of DNA at the limit of detection of the screening assays (Figure 1). Look back recipient samples will be linked to the corresponding archived samples from the donor and, if unavailable, to the Index sample. Part of each sample and the laboratory data will be available to the study group. Published or unpublished donor and recipient data will be made available to the study group and will be entered in the study group global database.

Collection of other relevant samples

Blood centres identifying a yield case of occult HBV DNA will make effort to further investigate by searching for archived samples from the donor as first step of the look back process and by contacting the donor for prospective collection of samples (see above). In addition, whenever possible, the tracing of recipients of previous donations will be initiated and, if successful, recipient samples will be collected.

All samples from the Index donor or linked recipients will be tested for HBV markers including DNA analyses and investigated similarly to the Index sample.

Additional clinical studies

The clinical aspects of occult hepatitis B have not been well studied for lack of sufficient number or representative cases. Either they were identified in liver disease/hepatology clinics and presented with clinical symptoms or from occasional discoveries. There is therefore an opportunity to draw the bigger picture based on clinical assessment and follow-up of donors with occult HBV infection.

HBsAg without detectable DNA

In order to decrease the burden of blood centres on the subject, only samples with HBsAg below a sample to cut-off of 5 or a concentration of <100ng/ml would be eligible for further investigation. Investigation will aim at determining whether or not low levels of HBV DNA can be detected in such samples and secondly whether or not they are clinically infectious. Results of such an investigation are clearly critical in the debate regarding the potential role of NAT as replacement of both anti-HBc and HBsAg screening. A specific protocol is not available presently but will be developed at a later stage.

Impact of data collected

It is anticipated that the data collected by the study group will provide evidence on which health authorities can base specific strategies to address HBV blood safety. This information will be comprehensive including testing, epidemiology, efficacy of screening, risk of transmission by transfusion and clinical significance. The goal of this ISBT initiative is to standardize investigation and collect global blood screening yield data in a central database as a pre-requisite to understand the value of the laboratory parameters for the infectivity of blood donations. Ultimately this will be a unique tool for regulators to establish meaningful requirements to ensure the safety of the blood supply.

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Details of the planned investigations can be obtained by consulting the ISBT TTI WP website.

References

1. Allain JP. Occult hepatitis B virus infection: implications in transfusion. *Vox Sanguinis* 2004; 86: 83-91.
2. Kleinman SH, Strong DM, Tegtmeier GGE, Holland PV, Gorlin JB, Cousins CR, Chiaccherrini RP, Pietrelli LA. Hepatitis B virus (HBV) DNA screening of blood donations in minipools with COBAS ampliscreen HBV test. *Transfusion* 2005; 45: 1247-57.
3. Owusu-Ofori S, Temple J, Sarkodie F, Anokwa M, Candotti D, Allain JP. Pre-donation screening of blood donors with rapid tests: implementation and efficacy of a novel approach to blood safety in resource-poor settings. *Transfusion*, 2005; 45: 133-40.
4. Kidd-Ljunggren K, Miyakawa Y, Kidd AH. Genetic variability in hepatitis B viruses. *J Gen Virol* 2002; 83: 1267-80.
5. Schaefer S. Hepatitis B virus: significance of genotypes. *J Viral Hepatitis* 2005; 12: 11-124.
6. Allain JP, Hewitt PE, Tedder RS, Williamson LM. Evidence that anti-HBc but not HBV DNA testing may prevent some HBV transmission by transfusion. *Br J Haematol* 1999; 107: 186-95.
7. Blajchman MA, Bull SB, Feinman SV. Post-transfusion hepatitis: impact of non-A, non-B surrogate tests. *Lancet* 1995; 345: 21-5.
8. Hennig H, Puchta I, Luhm J, Schlenke P, Goerg S, Kirchner H. Frequency and load of hepatitis B virus DNA in first-time blood donors with antibodies to hepatitis B core antigen. *Blood* 2002; 100: 2637-2641.
9. Marusawa H, Uemoto S, Hijikata M, Ueda Y, Tanaka K, Shimotohno K, Chiba T. Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen. *Hepatology* 2000; 31: 488-95.
10. Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K. Hepatitis B NAT virus-positive blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. *Vox Sanguinis* 2005; 88: 77-86.
11. Roth WK, Weber M, Petersen D, Drosten C, Buhr S, Sireis W, Weichert W, Hedges D, Seifried E. NAT for HBV and anti-HBc testing increase blood safety. *Transfusion* 2002; 42: 869-75.
12. Murokawa H, Yoshikawa A, Ohnuma H, Iwata A, Katoh N, Miyamoto M, Mine H, Emura H, Todokoro K. Epidemiology of blood donors in Japan, positive for hepatitis B virus and hepatitis C virus by nucleic acid amplification testing. *Vox Sanguinis* 2005; 88: 10-16.

13. Mine H, Emura H, Miyamoto M, Tomono T, Minegoshi K, Murokawa H, Yamanaka R, Yoshikawa A, Nishioka K. High throughput screening of 16 million serologically negative blood donors for hepatitis B virus, hepatitis C virus and human immunodeficiency virus type-1 by nucleic acid amplification testing with specific and sensitive multiplex reagent in Japan. *J Virol Methods* 2003; 112: 145-151.

14. Roth WK, Weber M, Seifried E. Feasibility and efficacy of routine PCR screening of blood donations for hepatitis C virus, hepatitis B virus, and HIV-1 in a blood-bank setting. *Lancet* 1999; 353: 359-63.

15. Meng Q, Wong C, Rangachari A, Tamatsukuri S, Sasaki M, Fiss E, Cheng L, Ramankutty T, Clarke D, Yawata H, Sakakura Y, Hirose T, Impraim C. Automated multiplex assay system for simultaneous detection of hepatitis B virus DNA, hepatitis C virus RNA, and human immunodeficiency virus type 1 RNA. *J Clin Microbiol* 2001; 39, 2937-45.

16. Kuhns MC, Kleinman SH, McNamara AL, Rawal B, Glynn S, Busch MP. Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc: implications for future HBV screening policy. *Transfusion* 2004; 44: 1332-9.

17. Sato S, Ohhashi W, Ihara H, Sakaya S, Kato T, Ikeda H. Comparison of the sensitivity of NAT using pooled donor samples for HBV and that of a serologic HBsAg assay. *Transfusion* 2001; 41: 1107. 1113.

18. Satake M, Taira R, Yugi H, Tadokoro K. Lookback study for transfusion-related HBV infection in Japan. *Transfusion* 2005 ; 45 : Suppl abstract S25.

19. Brummelhuis HG, Over J. Virus inactivation by addition of neutralizing antibodies. *Curr Stud Hematol Blood Transfus* 1989; 56: 128-37.

20. Dickson RC, Everhart JE, Lake JR, Wei Y, Seaberg EC, Wieasner RH, Zetterman RK, Pruett TL, Ihitani MB, Hoofnagle JH. Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. *Gastroenterology* 1997; 113: 1668-74.

21. Uemoto S, Sugiyama K, Marusawa H, Inomata Y, Asonuma K, Egawa H, Kiuchi T, Miyake Y, Tanaka K, Chiba T. Transmission of hepatitis B virus from hepatitis B core antibody-positive donors in living related liver transplants. *Transplantation* 1998; 65: 494-9.

22. Roche B, Samuel D, Gigou M, Feray C, Viroit V, Schmets L, David MF, Arulnaden JL, Bismuth A, Reynes M, Bismuth H. De novo and apparent de novo hepatitis B virus infection after liver transplantation. *J Hepatol* 1997; 26: 517-26.

23. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat Med* 1996; 2: 1104-8.
24. Michalak TI, Pasquinelli C, Guilhot S, Chisari FV. Hepatitis B virus persistence after recovery from acute viral hepatitis. *J Clin Invest* 1994; 93: 230-9.
25. Yotsuyanagi H, Yasuda K, Iino S, Moriya K, Shintani Y, Fujie H, Tsutsumi T, Kimura S, Koike, -K. Persistent viremia after recovery from self-limited acute hepatitis B. *Hepatology* 1998; 27: 1377-82.
26. Yamamoto K, Horikita M, Tsuda F, Itoh K, Akahane Y, Yotsumoto S, Okamoto H, Miyakawa Y, Mayumi M. Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. *J Virol* 1994; 68: 2671-6.
27. Grethe S, Monazahian M, Bohme I, Thomssen R. Characterization of unusual escape variants of hepatitis B virus isolated from hepatitis B surface antigen-negative subjects. *J Virol* 1998; 72: 7692-6.
28. Allain JP, Candotti D, Soldan K, Sarkodie F, Phelps B, Giachetti C, Shyamala V, Yeboah F, Anokwa M, Owusu-Ofori S, Opare-Sem O. The risk of hepatitis B virus infection by transfusion in Kumasi, Ghana. *Blood* 2003; 101:2419-2425.
29. Weinberger KM, Bauer T, Bohm S, Jilg W. High genetic variability of the group-specific a-determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum. *J Gen Virol* 2000; 81: 1165-74.
30. Kao JH, Chen PJ, Lai MY, Chen DS. Sequence analysis of pre-S/surface and pre-core/core promoter genes of hepatitis B virus in chronic hepatitis C patients with occult HBV infection. *J Med Virol* 2002; 68: 216-20.
31. Chu CJ, Hussain M, Lok ASF. Hepatitis B virus genotype B is associated with earlier HBsAg seroconversion compared with hepatitis B virus genotype C. *Gastroenterology* 2002; 122: 1756-62.
32. Sanchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodes J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in Western patients. *Gastroenterology* 2002; 123: 1848-56.
33. Candotti D, Opare-Sem O, Rezvan H, Sarkodie F, Allain JP. Molecular and serological characterization of hepatitis B virus in deferred Ghanaian blood donors with and without elevated alanine aminotransferase. *J Viral Hepatitis* 2005; in press.
34. Chu CM, Hung SJ, Lin J, Tai DI, and Liaw YF. Natural history of hepatitis

B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. *Am J Med* 2004; 116:829-834.

35. Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely 'occult'? *Hepatology* 2001; 34: 194-203.

36. Cerny A, Chisari FV. Pathogenesis of chronic hepatitis C: immunological features of hepatic injury and viral persistence. *Hepatology* 1999; 30: 595-601.

37. Penna A, Artini M, Cavalli A, Levrero M, Bertolotti A, Pilli M, Chisari FV, Rehmann B, Del Prete G, Fiaccadori F, Ferrari C. Long-lasting memory T cell responses following self-limited acute hepatitis B. *J Clin Invest* 1996; 98: 1185-94.

38. Zahn A, Allain JP. Hepatitis C virus and hepatitis B virus bind to heparin: purification of largely IgG-free virions from infected plasma by heparin chromatography. *J Gen Virol* 2005; 86: 677-685.

39. Diepolder HM, Jung MC, Keller E, Schraut W, Gerlach JT, Gruner N, Zachoval R, Hoffmann RM, Schirren CA, Scholz S, Pape GR. A vigorous virus-specific CD4+ T cell response may contribute to the association of HLA-DR13 with viral clearance in hepatitis B. *Clin Exp Immunol* 1998; 113: 244-51.

40. Thursz MR, Kwiatkowski D, Allsopp CE, Greenwood BM, Thomas HC, Hill AV. Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. *N Engl J Med* 1995; 332: 1065-9.

41. Ohkubo K, Kato Y, Ichikawa T, Kajiya Y, Takeda Y, Higashi S, Hamasaki K, Nakao K, Nakata K, Eguchi K. Viral load is a significant prognostic factor for hepatitis B virus-associated hepatocellular carcinoma. *Cancer* 2002; 94: 2663-8.

42. Paterlini P, Gerken G, Nakajima E, Terre S, D'Errico A, Grigioni W, Nalpas B, Franco D, Wands J, Kew M. Polymerase chain reaction to detect hepatitis B virus DNA and RNA sequences in primary liver cancers from patients negative for hepatitis B surface antigen. *N Engl J Med* 1990; 323: 80-5.