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# HBs Antibody Seroprevalence of in HBs Antigen negative/total HBc Antibody positive blood donors at the Chad National Blood Transfusion Center in N'Djamena

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#### Abstract

The objective was to determine the level of anti-HBs Antibodies in order to define the immune status against hepatitis B of blood donors at the National Blood Transfusion Center (CNTS) in N'Djamena and to assess the risks of transmission. of hepatitis B by transfusion to help improve transfusion safety through optimal biological qualification.

From March 1 to May 31, 2020, a study of virological markers was conducted with blood donors (volunteers and first-time donors for the most part) at CNTS N'Djamena. The donors included were also tested negative for the markers (anti-HCV Ab, Ag/Ab HIV and syphilis) according to standard clinical microbiology methods.

Of the 278 donors included in this study, we determined a rate of 48.2% of anti-HBs Antibodies and 64% of anti-HBc Antibodies. The different profiles determined by combining the results of the three markers were: infected, cured and immune (42.81%); uninfected (30.94%); old infection (20.14%); vaccinated typical profile (6.12%) respectively. A significant difference was observed between the proportions of male (78%) and female (22%) donors with a probability of 0.001.

This study made it possible to determine the high level of anti-HBs Antibodies in blood donors with perfect immunity and their donation no longer constituted a danger for the recipients. In view of this result, we recommend screening for viral DNA B in blood donors with negative HBsAg and HBc Ab carriers, in order to better understand the physiognomy of hepatitis B virus infection in Chad.

Keywords: Hepatitis B, HBs Antibody, HBc Antibody, Blood donors, N'Djamena.

#### Introduction

Blood transfusions are an essential aspect of patient care and are sometimes the only lifesaving solution. When used correctly, they save lives and improve health, but they also pose potential risks of complications and infections. Unfortunately, many people still do not have timely access to safe blood, due to a lack of reliable blood donors. New data from the World Health Organization (WHO) reveals that an estimated 325 million people worldwide live with chronic hepatitis B virus (HBV) infection [1]. It is clearly accepted that blood transfusion is one of the modes of transmission and contamination of hepatitis B viruses [2]. In sub-Saharan Africa, factors that contribute to the transmission of infectious diseases through transfusion include: high rates of transfusion among certain patient groups, particularly women and children; a high prevalence of the hepatitis B virus in blood donor populations and a still low proportion of voluntary donors considered to be the safest group [5]. Even though previous work has shown that rapid screening tests have contributed to reducing the risk of transfusion, in the same way as fourth-generation ELISA serological tests. However, important parameters limit the effectiveness of this biological screening, including genetic diversity, the conditions of use of rapid and serological tests, the absence of a quality system in many centers blood transfusion, and above all the window of serological silence on which serological screening is ineffective [8]. Such contextual elements have a negative impact on blood safety.

In Chad, the search for HBs Antigen alone is used for the biological qualification of donations for hepatitis B, as well as for diagnosis. A negativity of this marker is considered as a harmlessness of the blood product, and/or an absence of hepatitis B infection in an individual. However, a recent study conducted in N'Djamena (2021) revealed a very high rate (41%) of anti-HBc Antibodies in HBsAg-negative blood donors (N= 452/1106) [2].

The objective of this study is to determine the frequency of HBs Antibodies in order to define the immune status against hepatitis B of blood donors at the CNTS of N'Djamena and to evaluate at the same time the risks of transmission. of hepatitis B by transfusion, hoping to contribute to the improvement of transfusion safety through optimal biological qualification.

# Material And Methods

### Study framework

This was a descriptive cross-sectional study of serum markers of hepatitis B, in particular the simultaneous detection of anti-HBc and anti-HBs Antibodies in blood donors previously labeled as HBs Antigen negative. The study took place over a period of 03 months, from March 1 to May 31, 2020. Blood samples were collected from blood donors who tested negative HBsAg at the CNTS laboratory in N'Djamena (Chad) to anti-HBs Antibodies and anti-HBc Antibodies research:

- At the Center for Study and Research in Applied Biology (CERBA) in Paris/France, within the framework of agreements for the execution of examinations impossible to carry out on site, and for quality control and evaluation of our results where all steps of electrochemiluminescence diagnosis have been performed.

### Inclusion and exclusion criteria

Included in this study are all blood donors (mostly volunteers and first-time donors) registered for a donation and having satisfied the selection criteria in force at the CNTS, and whose classic infectious markers (HBs, HCV, HIV, Syphilis) were negative.

We're not included, blood donors with clinical anemia, pregnant, postpartum or breastfeeding women, people with a chronic pathology and subjects tested positive for at least one of the markers: HBsAg, 1 HCV Ab, HIV Ag/Ab, Syphilis.

# Study population

Our study population was made up of all the people who came voluntarily to the CNTS to make a donation in favor of the soldiers injured in Lake Chad in the context of the terrorist attack, the sampling was exhaustive.

### Data processing

The data was collected from the donation collection forms in force at the CNTS. The variables retained were essentially: the age, sex and profession of the donors, these being of course all volunteers and first-time donors. The descriptive analysis was carried out using the calculations of the proportions for the qualitative variables and the different frequency comparisons were quantified using the Chi-square test. The statistical significance level was set at 5%.

## Microbiological Analysis Presentation of the tests.

The biologics we looked at are serum or plasma. These products have been tested in the laboratories with the various screening kits and we have taken as reference method the electrochemiluminescence carried out by the COBAS automaton of CERBA.

Serodiagnosis on each donation was carried out beforehand using the following rapid screening tests: SD Bioline for the HIV and hepatitis B and C test, and the ABON rapid test for the detection of IgG and IgM of syphilis.

# Immunochromatographic tests

One step SD Bioline VHB, anti-HCV and HIV Ab, and ABON (in cassettes and strips) immuno-chromatographic tests have been used for the rapid detection of HBV, HIV, anti-HCV Ab and syphilis following the manufacturer's instructions. All samples negative for HBs Antigen, HIV, hepatitis B and C and syphilis were collected, packaged and sent to the CERBA partner laboratory for the detection of anti-HBc Antibodies and anti-HBs Antibodies by the chemiluminescence technique. A total of 278 samples were retained and sent for analysis. No history of hepatitis B vaccination was determined during this study.

Qualitative determination of total anti-HBc Antibodies and anti-HBs Antibodies by the COBAS automaton (CERBA)

The immunological test for the qualitative determination of total anti-HBc Antibodies and anti-HBs Antibodies is carried out with human serum or plasma. This electrochemiluminescence test (ECLIA) is used on COBAS immunoassay systems (Roche, Germany).

COBAS systems have powerful software including programs for analysis and data management. A positive and negative quality control system at each run is available to validate a system test kit and an internal control for each sample. Single-use, barcoded, ready-to-use (no freezing or reconstitution) reagents were used.

The principle of COBAS is based on the pretreatment incubation of 40µL of sample with a reducing agent. Then the HBcAg and or anti-HBs Antibodies are added and incubated between 20 to 25 o C. An immune complex is formed with the HBc Antibodies and or anti-HBs Antibodies of the sample. Biotinylated antibodies, HBcAgantibodies or ruthenium-labeled anti-HBs specific antibodies and streptavidin-coated microparticles are added and incubated. The complex has just bound again on available sites of the HBc Antigens. The complex is attached to the solid phase by a biotin-streptavine bond. The reaction mixture is transferred into the measuring cell, the microparticles are held at the level of the electrode by a magnet. The elimination of the free fraction is carried out by the passage of ProCell or ProCell M. A potential difference applied to the electrode triggers the production of luminescence which is measured by a photomultiplier. The software automatically determines the results by comparing the luminescence electrochemistry signal generated by the reaction with the threshold value obtained during a calibration. The total analysis cycle time is 27 minutes.

The results of CERBA are returned to us within the framework of the collaboration of subcontractors between CERBA and the laboratory of the CHU-RN of N'Djamena.

#### Results

A total of 278 people were included in this study, including: 217 men (78%), 61 (22%) women ( $x^2 = 17.846>3.841$  ddl = 1, p = 0.001, significant difference in favor of men's participation in the 'study). The sex ratio was 3.56 (217/61). The average age was 32.45 years  $\pm$  9.63 years with extremes of 16-60 years. The majority of donors were students and civil servants with proportions of 24.1% and 23.38% respectively. HBc Antibody was present in 64% of cases (N = 178/278) and anti-HBs Antibody in 48.2% (N = 134/278). The different profiles determined by

combining the results of the three markers are as follows: infected, cured and immune (42.81%); uninfected (30.94%); old infection (20.14%); typical vaccinated profile (6.12%); with a protective anti-HBs Antibody threshold of 10 IU/L to more than 1000 IU/L at 48.2%.

#### Distribution of donors by sex

Figure 1 illustrates blood donor repair by gender. Participation of blood donors in the survey was predominantly male 217/278 (78%) (Figure 2).



Fig. 1: Distribution of donors by sex.

#### Distribution of donors by age group

Table 1 shows the distribution of blood donors by age group. The most represented age groups were those of 26-

30 years followed by 31-35 years and 36-40 years with the proportions of 21.6%, 29.1% and 12.9% respectively (table 1).

Age group (year)	Effective	Percentage (%)
16-20	24	8.6
21-25	51	18.3
26-30	60	21.6
31-35	56	20.1
36-40	36	12.9
41-45	24	8.6
46-50	7	2.5
51-55	13	4.7
56-60	7	2.5
Total	278	100

Table 1: Distribution of donors by age group.

# Distribution of donors according to the positivity rate of total anti-HBc Ab and anti-HBs Ab

Figure 2 illustrates the distribution of donors according to the positivity rate for total anti-HBc Ab and anti-HBs Ab  $\,$ 

according to age group. The highest proportions of anti-HBc Ab and anti-HBs Ab were found in the age groups of 31 to 35 years and 26 to 30 years (figure 2)



Fig. 2: Distribution of donors according to the positivity rates of total anti-HBc Ab and anti-HBs Ab.

**Distribution of the combined results of donors in positive or negative rates of HBsAg/ total anti-HBc Ab, anti-HBs Ab and the vaccination status of blood donors** Table 2 shows the distribution of donors in terms of positive or negative levels of total HBsAg anti-HBc Ab and anti-HBs Ab. The most represented blood donors were total anti-HBc Ab positive and anti-HBs Ab positive: 119 (42.81%) followed by total anti-HBc Ab negative and anti-HBs Ac negative: 86 (30.94%); total anti-HBc Ab positive and anti-HBs Ab negative: 56 (20.14%). A non-significant difference ( $x^2 = 3.816 < 3.841$  dof = 1, p < 0.05) was observed between the proportions of anti-HBs Ab negative blood donors: 134 (48.2%) and total anti-HBc Ab positive/anti-HBs Ac negative: 56 (20.14%) (Table 2).

Table 2: Distribution of donors in	positive or negative le	evels of total HBsAg anti-HBc Ab	and anti-HBs Ab.
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HBsAg	total anti-HBc Ab	Anti-HBs Ab	Effective	Percentage (%)	Interpretation
Negative	Positive	Positive	119	42.81	Old infection resolved or occult hepatitis possible
	Negative	Negative	86	30.94	Not infected or in window period
	Negative	Negative	56	20.14	Isolated HBc Ag infection
	Positive	Positive	17	6.12	Immunity following the vaccine
	Total		278	100	

# Discussion

The HBs Antigen is the most well-known marker of hepatitis B and practically the only one sought in all situations in Chad: screening for hepatitis B in a clinical context, in the field of epidemiological research and especially in terms of blood safety. Its negativity whatever the context is interpreted from the outset as the fact that an individual has never been in contact with the hepatitis B virus. including one of the most recent carried out at the National Blood Transfusion Center (CNTS) in N'Djamena, in particular the search for anti-HBc Antibodies in blood donors in whom the search for the HBs Antigen was negative, to realize that in reality many individuals have been in contact with the hepatitis B virus (41%) [7], which simply means that nearly half of the people hitherto considered suitable for donation are in potentially infectious for the hepatitis B virus (in this specific case: donors), which implies a certain threat as regards the biological qualification of blood products. Hence the importance of evaluating the immunity or even the immunocompetence of our donors in order to better assess the level of relative risk with regard to transfusion safety.

Of the 278 people who were part of our study, 217 were male (78%) and 61 (22%) female (p = 0.001). The sex ratio was 3.56 in favor of men among blood donors is in agreement on the one hand with the various annual activity reports of the CNTS of N'Djamena, and on the other hand, similar to other African studies which evoke as an argument, certain local ancestral beliefs still alive, which would suggest that: the man, more vigorous would be the more able than the woman to give blood [3 - 4]. Other authors mention the physiological constraints of women such as menstrual cycles, obstetrical factors, in particular multiple and close pregnancies and finally exclusive breastfeeding, which would be major determinants of the restriction of blood donation in women [5].

The average age was 32.45 years  $\pm$  9.63. This average age is close to that found by Goita et al in Mali (2019) who reported an average age of 31.57  $\pm$  9.050 [6], superimposable on the study by Kahamba et al 2013 in Kamina (DRC) who reported an average age of 28  $\pm$  6, and Kakisingui Ngama et al in 2016 in Lubumbashi (DRC) noted an average age of 31.59  $\pm$  8.2 years [10,11]. Indeed, the CNTS of N'Djamena recruits with predilection its donors at the level of schools and universities but also in religious communities as well as in the ranks of the Defense and Security Forces. This explains why in our study, as in the majority of African studies, we find an essentially young population of donors. The rate of total anti-HBc Antibodies of 64% noted is higher than that found in a recent study carried out at the CNTS of N'Djamena [12] which reported a frequency of 41%. This difference is probably attributable to the sampling, which in this case is very low. However, it confirms the fact that many people in Chad, and particularly the donors here, have been at one point in their lives in one way or another, once in contact with the Hepatitis B virus than what we did not believe.

Observation of the rate of the various markers reveals that in nearly 80% of cases, our donors are either uninfected, or either infected or not but immunized, respectively: uninfected (30.94%); post hepatic immunity (42.81%); post-vaccination immunity profile (6.12%). It being understood that no history of vaccination was noted during this study. From the foregoing, one might believe that approximately 80% of our blood products appeared to be safe for transmission by the hepatitis B virus, and that only the remaining 20% would be likely to carry the hepatitis B virus. hepatitis B. A study on the evaluation of the vaccination status among Beninese soldiers on mission in Côte d'Ivoire from November 2015 to April 2016, noted that of the 175 subjects who participated in the said study, a total 23.42% (N = 41) of soldiers were immunized against the hepatitis B virus. Of these, 14.3% (N = 25) were still immunized after viral contact (HBs Ag negative, positive total anti-HBc antibodies) and that only 9.1% (N = 16) had been vaccinated (3 doses of vaccine) [13].

The title of the various markers was determined, in particular that of the anti-HBs antibodies in order to better assess the immune profile of our donors and therefore the impact on the safety of their donation. Indeed, 136 subjects out of the 192 infected and immunized or not (69.79%) of people are carriers of anti-HBs, of which 134 (98.52%) had a protective titer (10UI/L to more than 1,000UI/ L). This finding, while far from ideal, nevertheless and very fortunately shows that many of our donors, even if they had been in contact with the hepatitis B virus, had perfect immunity and their donation no longer constituted a danger. for receivers.

This study despite some shortcomings related to the difficulties experienced by our transfusion system both in its organization and in the implementation of a quality approach. Nevertheless, it allows us to better understand the physiognomy of hepatitis B virus infection in our context. However, it would be useful to evaluate the frequency of BV DNA in order to appropriately define a better screening algorithm for hepatitis B.

#### Acknowledgments

We would like to thank the voluntary blood donors and first-time donors for the most part who agreed to donate their blood to save human life and participate in this study.

# **Conflict of Interest**

The authors declare that they have no conflict of interest.

### Conclusion

The detection of isolated HBsAg reassures the immune status against hepatitis B of blood donors, tested HBsAg negative/HBcAb positive but a certain high level of HBcAg persists, likely to infect the recipient. Transfusion safety requires, on the one hand, better selection and better retention of blood donors. The use of more sensitive and specific molecular techniques brings a significant decrease in the silent window and eliminates false positives, false negatives in the detection of bloodborne viruses.

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