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Interest of Confirmation Tests in the Diagnosis of Viral Hepatitis C to Blood Donors in Abidjan-Côte d'Ivoire

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Abstract

Introduction: The anti-HCV RIBA test verifies the presence of anti-HCV serum antibodies detected by the Elisa test. In Côte d'Ivoire, screening for hepatitis C is done exclusively by enzyme immunoassays. In order to reduce the number of HCV positive blood donor exclusions on ELISA, we conducted this study which aimed to demonstrate the value of the RIBA test in confirming diagnosis of viral hepatitis C to blood donors.

Methods: Our study, which took place from 02 to 23 February 2008 in the laboratory of Abidjan NBTC, focused on 200 sera of blood donors anti-HCV positive (Elisa test) selected according to the ratio. The DECISCAN HCV PLUS confirmation test of BIORAD was used.

Results: Among the 200 HCV samples positive by EIA, 49% (98/200) were confirmed positive. RIBA gave an indeterminate result in 40% of cases (80/200); and negative in 11% of cases (22/200) corresponding to false ELISA devices. In RIBA 96 samples had a low ELISA ratio of which 21% (20/96) were RIBA negative, and 79% (76/96) were indeterminate. RIBA positive samples (98/200) had a high ratio in 82% of cases (80/98). The presence of NS3 (C33) and NS4 (C100) was noted in 100% of cases (98/98, C2 in 37% (36/98) of cases and C1 in 18% of cases (18/98). RIBA indeterminate noted the presence of NS3 in 98% of cases (78/80) and NS4 in 30% of cases (24/80). Proteins C1, C2 and NS4 are essential for the diagnosis of confirmation of viral hepatitis C by RIBA.

Conclusion: These results attest to the lower specificity of enzyme immunoassays (ELISAs); hence the benefit of using RIBA confirmatory tests. A significant number of donors are excluded from blood donation in Côte d'Ivoire on the basis of false positive results obtained by the ELISA technique.

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Introduction

The transfusion of blood and its derivatives is essential for the treatment of various medical and surgical pathologies. However, it has certain undesirable effects, including immunological and infectious complications [1].

The role of transfusion in HIV transmission was demonstrated in the 1980s and led to the introduction of the transfusion safety concept for infectious agents.

Rigorous medical screening combined with systematic screening for the main infectious markers (HIV, Hepatitis B, Hepatitis C, syphilis) contributes to a reduction in the risk of transmission [1].

Viral hepatitis C is a public health problem. According to the World Health Organization (WHO), an estimated of 200 million people are infected with this virus, or about 3% of the world's population [2]. The developing countries of Southeast Asia and Sub-Saharan Africa are highly endemic areas with rates above 2.5%, while developed countries are areas of low endemicity with rates between 0 and 2.5% [2, 3, 4]. The various activity reports of the National Blood Transfusion Center of Côte d'Ivoire reported a prevalence of 4.2% among blood donors [5, 6].

Enzyme immunoassays are the most used in blood transfusion and are described as very sensitive, and unspecific generating false positives [7] which result in the reduction of the number of donors. It is recommended to use a specific confirmatory test including Recombinant Immunoblot Assay (RIBA) [8, 9]. RIBA is also an alternative to very expensive PCR for resource-limited countries.

The estimation of HCV seroprevalence among Côte d'Ivoire blood donors estimated (4.2%) based on the results of ELISA could be overestimated, due to false positives that they generate. It therefore seemed appropriate to carry out this study whose objective was to demonstrate the interest of the RIBA test in the confirmation diagnosis of viral hepatitis C to Cote d'Ivoire blood donors

Methodology

This is a cross-sectional prospective study that was conducted from February 2008 to August 2008 at the National Blood Transfusion Center (CNTS) laboratory located at Kilometer 4 Boulevard de Marseille, Treichville in Abidjan.

Our study focused on a panel of 200 positive anti-HCV ELISA samples from the CNTS serum bank.

HCV testing was performed with Abbott's Murex anti-HCV reagent (version 4.0) based on the enzyme-linked immunoassay (ELISA) technique.

In order to confirm the positive screening results we used BIORAD's DECISCAN HCV PLUS test. It is an online test that uses as a solid support, a membrane fixed on a plastic strip, where are successively coated (i) a control: Anti-human IgG, (ii) a fusion protein Glutathione S Transferase (GST) fused to the genes coding for the NS3 and NS4 proteins used as control (iii) HCV antigens of the nonstructural region: NS3 and NS4 and peptides of the C1 and C2 structural region.

All samples were thawed and homogenized prior to testing as recommended by the manufacturer.

The interpretation criteria are detailed in Table 1.

The donors from whom the indeterminate samples were obtained were serologically monitored at 3 months and 6 months. This follow-up consisted of the detection of anti-HCV antibodies by ELISA technique.

The Epi Info 6.04 software was used for data entry and statistical analysis.

Results

Prevalence of Anti HCV Positive Antibodies by Desiscan hcv plus BIORAD (Table 2)

Of the 200 samples tested, 98 were RIBA positive or 49% positive.

The analysis of the results of RIBA according to the ratios (OD / VS) obtained at the ELISA is summarized in Table 2. Samples with a ratio between 8.01 and 16 were all positive.

Listed Proteins and RIBA Results (Table 3)

The proteins found in RIBA according to the different results are listed in Table 3. We observed a reactivity of all the proteins tested when the RIBA was positive.

RIBA-Positive HCV Ab Relationship and Donor Status (Table 4)

Table 1. Criteria for interpreting the results.

LECTURE READING	INTERPRETATION
No HCV antigen band displayed	NEGATIF
1 HCV antigen band visualized or 2 HCV antigen bands visualized on the capsid gene (C1, C2)	INDETERMINE
2 visualized HCV antigen bands from 2 different genes (capsid gene, NS3 gene, NS4 gene) and with an intensity of 0.5+	POSITIVE PROBABLE
At least 2 visualized HCV antigen bands from 2 different genes (capsid gene, NS3 gene, NS4 gene) and with an intensity greater than 0.5+	POSITIVE

NB: the GST band is displayed, only the HCV C1 and C2 antigen bands (synthetic peptides) will be interpreted.

Table 2. Anti HCV Ab positivity rate with DESISCAN HCV plus BIORAD as a function of the ELISA ratio.

RATIO = DO/VS	Nomber (%) RIBA		
	NEG	IND	POS
1,01 – 5 (n=96)	20 (20.8)	76 (79.2)	0 (0)
5,01 – 8 (n=24)	2 (8.3)	4 (16.7)	18 (75)
8,01 – 16(n=80	0 (0)	0 (0)	80 (100)
TOTAL (n=200)	22 (11.0)	80 (40)	98 (49)

Table 3. Proteins listed and RIBA results

	PROTEINES			
RESULTS	**C1**	**C2**	**NS3**	**NS4**
POSITIF	8(18,18%)	16(36,36%)	90(100%)	90(100%)
INDETERMINE	0(0%)	0(0%)	35(97,22)	11(30,55%)
NEGATIVE	0(0%)	0(0%)	0(0%)	0(0%)

Table 4. RIBA positivity and status of the donor

Donor status	Frequency	Percentage (%)
New donors(n=110)	58	52,7
Regular donors(n=90)	40	44,4
Total	98	100

P=0,24

Table 5. Prevalence of anti HCV Ab after control of RIBA samples indetermined by the Elisa technique

Antibody anti-VHC	Frequency	Percentage (%)
Positive	10	**50**
doubtful	3	**15**
Negative	7	**35**
Total	**20**	**100**

Of the HCV positive RIBA samples, those from new donors accounted for 59%. The RIBA positivity rate was not statistically different for both types of donors (Table 4)

Prevalence of HCV Ab Ab after Control of Undetermined RIBA Specimens by ELISA (Table 5)

Only 40 of the 80 donors followed for an indeterminate RIBA result returned.

The positivity rate of the anti-HCV antibodies after the control performed in these undetermined RIBA donors was 50% (Table 5).

Discussion

The detection of antibodies against HCV is a systematic analysis performed during the biological qualification of blood donations in Côte d'Ivoire. This contributes to strengthening blood safety. However, the majority of enzyme immunoassays are responsible for a false positive with a high rate of rejection of blood products but also a postponement of donors [10].

The use of more specific confirmation tests is necessary. We therefore evaluated the interest of the DECISCAN HCV PLUS BIORAD immunoblot test on 200 ELISA-positive HCV sera.

We observed a RIBA positivity rate of 49%. This result is consistent with that of Mets et al [11] who found 48% in Rwanda. On the other hand, this rate contrasts with that of Maniez-Montreuil and Dubois [11] who found that only 17.6% of the positive signals observed with the enzyme immunoassays (EIA) were confirmed positive. This could be explained by the fact that African countries are areas of high endemicity [12].

RIBA gave an indeterminate result in 40% of cases. This rate is much higher than the 24.7% of indeterminate results reported by Maniez-Montreuil and Dubois [11].

In our study, we observed 11% negative RIBA. In this case, these are false positives observed with the EIA screening tests. This false positive rate is lower than the 57.7% obtained by Maniez-Montreuil and Dubois [12]. The proportion of negatives can be partly explained by the high sensitivity of the EIA screening tests on the one hand and the fact that the antibodies detected are residual despite the elimination of the virus by the immune system [13] .

Analysis of the RIBA results by ELISA ratio showed that 79.2% of the samples with a ratio between 1.01 and 5 were indeterminate. Our results are close to 73.7% reported by Pereira et al. [14] in Brazil.

On the other hand our results are superior to those of Othinger et al [7] who found 18% of indeterminate cases for ratios <5. The difference could be explained by the fact that their screening was carried out by chemiluminescence which seems more specific than the ELISA [15]

All positive samples had a ratio between 8.01 and 16. This result is in agreement with the findings of Othinger et al [7] who showed that the majority of RIBA positive samples had a high ratio.

Among the positive samples, the presence of NS3 (C33) and NS4 (C100) was noted in 100% of cases. C2 was present in 36.6% of cases and C1 in 18.3% of cases.

These results are superimposable to those reported in other studies including that of Othinger et al [7] and Powlotsky et al. [9] found a reactivity of 84.54% and 96%, and 85% respectively at NS4, NS3, C1 and C2.

Among the indeterminate RIBAs, NS3 was found

in 97.22% of cases. Our observations are similar to those of Pereira et al (Pereira et al., 2014), which report a reactivity rate of 86%). On the other hand, 50% of the indeterminate RIBA controlled cases gave a positive result by ELISA and 35% were negative. Thus, NS3 and NS4 detected with the undetermined RIBA, are sensitive but not specific because other viruses can possess them. C1 and C2 are very specific. NS4 is more specific than NS3. Therefore C1, C2 and NS4 proteins are essential for the diagnosis of confirmation of viral hepatitis C by RIBA.

No protein was found when RIBA is negative. This result agrees with that of Margret Othinger et al [7] who also did not demonstrate the presence of proteins.

New donors represent the highest proportion of HCV positive RIBA samples in our study. In fact, 59% of HCV positive for RIBA are new donors. This result is consistent with that of the 1998 study by Mets et al. [11] which showed that the majority of positives are found among new donors. This can be explained by the fact that over time the false positives are removed from the donation of blood.

Conclusion

This study shows that 51% of the ELISA-reactive samples are false positives.

It has shown that an ELISA ratio higher than 8 can be considered to define the true positives with the EIA technique.

RIBA is of particular interest in the confirmation of ELISA positive HCV serologies, in particular for a blood transfusion service when the ratios are less than 8.

It would therefore be desirable to include the RIBA test in the Hepatitis C screening algorithm at the biological qualification laboratory for blood donation in Côte d'Ivoire.

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