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Full Length Research Paper

Indirect diagnosis of HCV viremia in Malian women: Relevance of a Cost-effective Algorithm

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To diagnose an HCV infection is expensive because it is important to know to what extent seropositive patient is or not, viremic. The study aimed to define the best combination of serological tools to use in order to get the best prediction for HCV viremia at cheaper cost. The study questions focused on: usefulness of repeat tests, relevance of EIA test ratio (TR) values, use of a confirmatory test or a second screening test, costs of these alternatives, and choosing the best option giving an excellent probability of HCV-RNA detection. Costs were calculated using estimates for each EIA test, LIA-HCV, and PCR. Global data from two epidemiological surveys carried out between 2009-2010 in Mali were used. By using as an indicator, the magnitude of the signal-to-cutoff ratio measured with EIA, PPV (Positive Predictive value) of the presence of HCV RNA significantly increased from 41.2% to 82.4%. The highest PPV was obtained by performing two 4th generation EIA tests and exploitation of the threshold (half of the maximal ratio value measured). The cost of additional tests for virus detection ranged between 3550 USD and 1155 USD, and the algorithm presenting the highest PPV (87.5%) was not the most expensive (1551 USD).

Keywords: HCV Diagnosis, Cost, Effectiveness, Malian women, West Africa.

INTRODUCTION

Viral hepatitis is a result of liver inflammation caused by a virus, and there are five dominant hepatotropic viruses whose hepatitis C virus (HCV) (Trepo, 2006). Height HCV genotypes with 86 subtypes exist (Borgia, 2018; Hedskog, 2019) plus a subtype 6xg newly discovered from injection drug users (IDUs) (Ye, 2019).

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Although both genotypes one and two are reported in Mali, genotype 2 is predominant (easier to treat than genotype 1) (Bouare, 2012). Due to the disease prevalence, chronicity and related possible complications, viral hepatitis C is a significant public health concern worldwide. It is a high-risk viral hepatitis type because it can develop into a chronic disease with complicated outcomes such as liver cirrhosis and hepatocellular carcinoma (HCC). Globally it is reported that 71 million people have chronic hepatitis C infection, 399 000 people die each year among

them (mostly from cirrhosis and hepatocellular carcinoma) (WHO, 2018). The geographical distribution is variable with high prevalence regions such as Africa and Asia, where the prevalence exceeds 10%. In West Africa, WHO reported an HCV seroprevalence rate of 5.3% [95% CI: 2.9-9.1%] (WHO, 2016). Although, seemingly fewer cases in Mali, HCV epidemiological reports from recent literature, show both a prevalence and nosocomial transmission risk of hepatitis C virus infection (Bouare, 2013). We previously reported HCV prevalences of 0.2% and 6.5% respectively among pregnant women from six health centers and women aged over 50 years attending general practice in two hospitals in Bamako (Bouare, 2012). HCV seroprevalence rate of 10% was reported among diabetic patients from two health care facilities in Bamako (Diarra, 2013). In the absence of a vaccine, hygiene and safety precautions in hospitals and health centers, blood safety measures, systematic screening, treatment, treatment adherence, and its accessibility contribute to HCV infection eradication measures. Direct diagnosis of HCV by polymerase chain reaction (PCR) is expensive and requires considerable resources. The big question related to serology of hepatitis C is the identification of the extent to which a seropositive patient is viremic. Significant improvement in the positive/negative predictive value for the indirect diagnosis of viremia can be achieved by combining different serological tests, including HCV antibody (anti-HCV IgM) (Gerard, 1996). Furthermore, studies addressing HCV diagnostic accuracy were reported, but such investigations are relatively scarce in Mali. A high degree of accuracy between HCV viremia and test ratio value (TR, signal-to-cutoff ratio) was reported for the anti-HCV test (Seo, 2009; Cao, 2011; Bouare, 2012). We previously showed a 66.7% viremia prediction for samples with TR higher than 50% of the maximal signal value measured with an antibody test (anti-HCV) (Bouare, 2012). Therefore, undoubtedly this is an important study to generate new knowledge in the field of HCV diagnosis and therapeutic management in Mali, thus stimulating scientific interest to investigate the best predictive methods for evaluation of HCV-RNA presence at a cheaper cost.

MATERIALS AND METHODS

Study design

Two investigations were undertaken in Mali, successively in 2009 in pregnant women recruited from the six reference health centers in Bamako and in 2010, among women over 50 years old who frequented the general practice in two hospitals (CHU Gabriel Touré, and Hospital Mother and Child) from the same city (Bouare, 2012; Bouaré, 2013).

Initially, this was a cross-sectional study including 1000 pregnant women attending antenatal care (ANC).

In a second step, the study was extended to women over 50 years, for the higher risk of HCV infection in that age. In fact, 231 women > 50 years were enrolled.

Standard operating procedures were issued to control blood collection, preservation and transport. Physicians, researchers, midwives and laboratory technicians were trained in the application of procedures. Sample transport by drivers was supervised. The venous blood collected in vacutainer tubes. Serum or plasma were prepared, aliquoted, and stored frozen between -20 and -80°C before being transferred in good condition to Liege University Hospital for analysis. This work was conducted by following the guidelines of the World Medical Association Declaration of Helsinki (2000) and ethically approved under reference number 08-0006 / INRSP-EC (Ethical Committee of National Institute of Research in Public Health) in Mali. All women enrolled were randomized after a written informed and voluntary (opt in/opt out principle) consent (De Cock, 2003; Kiréré Mathe, 2008).

Diagnosis Methods

The laboratory tests were performed according to the manufacturer's instructions (used for testing) and according to the accredited procedures (standard EN15189) applied to the AIDS reference laboratory of the CHU of Liege. The samples were analyzed after a single thaw.

Serology: The mixed test MONOLISA HCV Ab/Ag Ultra 4th Generation (Bio-Rad Belgium) was used in both sets of women (young and old), while the antibody test (INNOTEST HCV Ab IV 4th Generation) (Innogenetics Belgium, currently called Fujirebio Europe) was used only in the young set. The antibody test (INNOTEST) was regarded as a second screening test. Both tests are based on the enzyme-linked immunosorbent assay (ELISA) principle. While MONOLISA performs dual detection of Ag HCV and Ab (anti-HCV IgG), INNOTEST detects Ab alone (anti-HCV IgG). The confirmatory test INNO-line immuno-assay (LIA) HCV, HCV score III v2 (incubation 16 hours), using a LiRAS scanner (Line Reader and Analysis Software), was used to confirm HCV seropositive results.

Molecular biology: Samples tested positive or indeterminate with the LIA confirmation test were analyzed by PCR (qualitative or quantitative). For the first part of the study, the molecular biology test (COBAS AMPLICOR hepatitis C. Roche Laboratory) was used for HCV-RNA detection. Samples with detectable HCV-RNA were analyzed by quantitative PCR (COBAS AMPLICOR HCV Monitor, Roche Laboratory). For the second part of the study conducted among older women, HCV m2000 real-time PCR kit (Abbott), was used to quantify HCV-RNA. All samples tested positive by the mixed test Monolisa but negative by the LIA, *a priori* indicative of very early infection, were re-analyzed by PCR to check the status of HCV active infection.

Search for the best algorithm

To test patients for HCV with better diagnostic efficacy, we tried to develop algorithms that enhance infectious disease management of HCV in scarce resources areas like Mali, based on the global epidemiological data from thesis works and our observations and experience of the tests (Bouare, 2012; Bouaré, 2013). We attempted to answer questions to identify methods that give the best positive predictive value for HCV-RNA detection. Our questions focused on the usefulness of repeat tests, relevance of test ratio values, use of a confirmatory test or a second screening EIA test, costs of methods, and choosing the best option with an excellent probability of HCV-RNA detection at a cheaper cost. The serology was even used to implement the estimated threshold 50% TRmax, and its analytical implications. Data generated using indirect HCV diagnosis methods from two epidemiological surveys were analyzed for viremia prediction.

The cost of additional tests was estimated for each diagnostic method implemented. For instance, when one considers an algorithm (Xi): Enzyme immunoassay (EIA) initial test positive + PCR; supplementary test to be done is equal to 0 for EIA + Nb PCR (i.e., the theoretical number of PCRs to run); Hence supplementary test cost = Nb PCR x (cost for one PCR test). Cost calculations are based on roughly estimates such as one EIA test 11 USD, LIA-HCV 27.5 USD, and PCR 55 USD. We derive supplementary test cost for (Xi) = 55 USD x Nb PCR. The choice of a suitable alternative depends on the probability of HCV-RNA detection at a lower cost.

Statistical Analysis

The results are presented as mean +/- standard deviation (SD) (range) or as median and quartiles for continuous variables, and frequencies (%) for categorical variables. The chi-square test was used to compare categorical variables between groups. PPV or probability of HCV-RNA detection was calculated for each algorithm or diagnosis method. We tested whether TR value allows for a qualitative characterization of samples i.e. answering the question of presence or absence of specific anti-HCV antibodies, its value can be also exploited quantitatively. TR values were compared between PCR results (+ or -) by the Kruskal-Wallis test. Results were considered significant at the 5% level ($p < 0.05$). The calculations were by using the Microsoft Excel software and SAS version 9.4 for Windows (SAS Institute, Cary, NC, United States).

RESULTS

For the first series of the study conducted in young pregnant women aged 14-50 years, mean age was 25.2 ± 6.3 years. As for the second series represented by women aged over 50 years, the average age was 62.1 ± 8.6 years (range 51-89 years).

HCV viremia prediction analysis

The results from the diagnostic methods implemented are summarized below.

Repeat the initial positive test: Out of the 50 positive or doubtful samples, 34 were re-analyzed by PCR, and 14 were positive and 20 negatives (PPV = 41.2%). Among the 35 positive results from samples re-analyzed three times, 34 were tested by PCR, and PPV remained the same.

The exploitation of TR values and 50% TRmax contribution: each test corresponds to a maximum value of the signal-to-cutoff Ratio (Test Ratio max or TRmax), which is the highest value of the signal measured in the positive samples. We tested that TR value can be exploited quantitatively. Indeed, we showed that the threshold 50% TRmax presents an interesting PPV as far as the presence of the virus itself is concerned. In other words, 50% of TRmax was able, with an interesting PPV, to discriminate between viremic and non-viremic patients. The following analysis focuses only on Monolisa test results since it was applied to both sets studied. Analyzing the sample data distribution (TR values according to PCR results) we demonstrated that TR values are higher for positive PCR than negative PCR (Median 5.66 Quartiles 5.42 – 5.91 vs. Median 1.90 Quartiles 1.42 – 2.77; $p<0.0001$ P value from Kruskal-Wallis test). Figure 1 represents the distribution of TR values

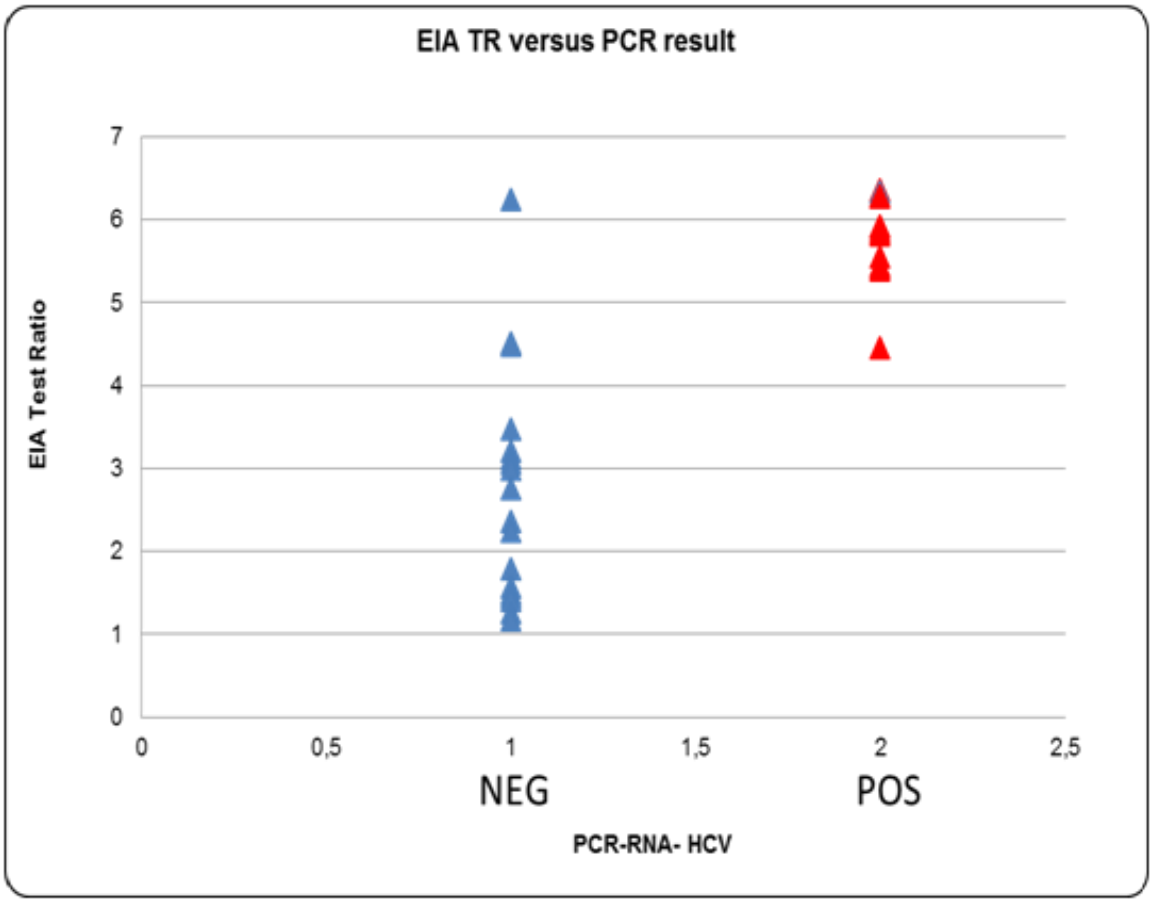

Figure 1. Association between HCV viremia and high value of the EIA signal-to-cutoff ratio (Test Ratio) in Malian women.

according to PCR results. In this study, the average values of TR (TR mean) were calculated from triplicate positive samples. TRmax (maximum signal value) was estimated as the maximum value of TR mean (TR mean max = TRmax = 6), and the half value (50%TRmax = 3) measured from the Monolisa test. In the hypothesis where the samples with TR ≥ 3 are tested in replicates, PPV reached 82.4% in the samples whose TR remains three times ≥ 3. Regarding the initial test (Table 1), four samples with initial TR > 3 tested in replicates do not reproduce the TR three times ≥ 3 so that the number of samples to be analyzed by PCR drops from 21 to 17, thereby increasing the PPV significantly (41.2% (14/34) vs. 82.4% (14/17); p<0.01).

Contribution of the confirmatory test INNO-LIA: In this alternative, the samples tested positive with the screening test were analyzed with the LIA confirmatory test. The PPV of the samples with LIA positive profiles was 81.2% (13/16). The PPV for indeterminate LIA profiles was 6.3% (1/16). Table 1 (see the alternative numbers 5, 7, and 8) shows, according to TR values, a regression of the LIA "indeterminate" rates. Indeed a high indeterminate rate 35% was obtained when the samples with TR ≥1 were tested with the LIA; a rate of 28.5% when samples with TR > 3 were tested; it drops down to 17.6% when the samples with TR three times higher or equal to 50% TRmax were tested. In the hypothesis where samples with TR three times >3 were tested by the LIA (Table 1 alternative 8), PPV for the PCR was equal to 82.4%.

The usefulness of a second screening test: In this alternative, we evaluated the contribution of an additional EIA test used as a second-line assay rather than the LIA. In this case, for the first test, EIA1 TR = TR1 and the second test EIA2 TR = TR2. The method to get higher PPV (Table 1; alternative number 10) is as follows:

Table 1: Comparison of diagnostic efficacy of different combinations of serological methods															
Alternatives			**EIA** Nb	LIA Nb	PCR Nb	**PPV** PCR+ (%)	**PCR-HCV-RNA**				**LIA-HCV**				
Single screening EIA (Monolisa)		N pos					pos	neg	nt	PCR	pos	neg	Ind	nt	LIA
1	TR ≥ 0.9 (±10%) tested 1x	50	0	0	50	41.2	14	20	16	34	0	0	0	0	0
2	TR ≥ 1 tested 3x	35	100	0	35	41.2	14	20	1	34	0	0	0	0	0
3	TR > 3 tested 1x	21	0	0	21	66.7	14	7	0	21	0	0	0	0	0
4	TR > 3 tested 3x	17	42	0	17	**82.4**	14	3	0	17	0	0	0	0	0
Monolisa + LIA															
5	TR ≥ 1 (±10%) tested 1x	44	0	44	29	41.2	14	20	10	34	15	9	14	6	29
6	TR ≥ 1 tested 3x	35	100	35	27	41.2	14	20	1	34	15	8	12	0	27
7	TR > 3 tested 1x	21	0	21	20	70.0	14	6	0	20	14	1	6	0	20
8	TR > 3 tested 3x	17	42	17	17	**82.4**	14	0	0	14	14	1	3	0	17
EIA1 (Monolisa) + EIA2 (Innotest)															
9	TR ≥ 0.9 tested 1x (for the 2 tests)	31	50	31	25	58.3	14	10	7	24	15	5	10	1	25
10	TR1 ≥ 3 for EIA1 1x and EIA2 1x: selection of results with TR2 ≥ 3	16	21	16	16	**87.5**	14	2	0	16	14	0	2	0	16

Perform a first test (EIA1); select samples with TR1 greater or equal to the threshold value 3 (i.e. 50% TRmax); test once (1x) with a second test (EIA2) and select samples whose TR2 is equally greater than or equal to 50% TR2max. In this study, TR2max was estimated equal to 6 with a threshold value of 3). Under these conditions, the PPV of this test combination reached 87.5% (14/16).

Algorithm cost estimation

HCV diagnostic accuracy with the economy of means is observed in three of the ten implemented alternatives (or algorithm) (Table 1 and 2). The corresponding number for these efficient alternatives is # 3, 4, and 10, which show viremia probabilities ranging between 66.7 and 87.5% for total testing costs between 1155-1551 USD.

Algorithm proposed for HCV Diagnosis

The proposed algorithm (Figure 2) comprises a first line screening EIA (4th generation, ELISA principle); selection of the samples (with TR1 ≥ 50% TR1max) for analysis using a second screening EIA (4th generation, ELISA principle). Under these conditions, for the samples whose TR2 ≥ TR2max 50%, the probability of viremia exceeds 85%.

Table 2: Comparison of cost-effectiveness of different methods of serological investigation

Alternatives			**EIA** Nb	**LIA** Nb	**PCR** Nb	**PPV** PCR+ (%)	**Cost estimation of algorithm** (considering: EIA 11 USD; LIA 27.5 USD; PCR 55 USD).			
Single screening EIA (Monolisa)		N pos					EIA	LIA	PCR	Total USD
1	TR ≥ 0.9 (±10%) tested 1x	50	0	0	50	41.2	0	0	2750	2750
2	TR ≥ 1 tested 3x	35	100	0	35	41.2	1100	0	1925	3025
3	TR > 3 tested 1x	21	0	0	21	**66.7**	0	0	1155	1155
4	TR > 3 tested 3x	17	42	0	17	**82.4**	462	0	935	1397
Monolisa + LIA										
5	TR ≥ 1 (±10%) tested 1x	44	0	44	29	41.2	0	1210	1595	2805
6	TR ≥ 1 tested 3x	35	100	35	27	41.2	1100	963	1485	3550
7	TR > 3 tested 1x	21	0	21	20	**70.0**	0	578	1100	1678
8	TR > 3 tested 3x	17	42	17	17	**82.4**	462	468	935	1865
EIA1 (Monolisa) + EIA2 (Innotest)										
9	TR ≥ 0.9 tested 1x (for the 2 tests)	31	50	31	25	58.3	550	853	1375	2778
10	TR1 ≥3 for EIA1 1x and EIA2 1x: select results with TR2≥3	16	21	16	16	**87.5**	231	440	880	1551

DISCUSSION

As for the main findings, the present study demonstrates no change in the PPV of the EIA (Monolisa) with the test repeat. However, it is interesting to note that test repeat reduces the number of PCR runs and increases the PPV, used as an indicator for the magnitude of the signal-to-cutoff ratio measured from a single screening test coupled to the test repeat. The rate of decrease in LIA indeterminate profiles did not change the PPV, and the EIA test combination reached the highest PPV indicating that a patient with this profile has a probability of more than 85% to be actively infected with HCV (EASL conference abstract) (Bouare, 2013). The remaining samples (20%) can be considered anti-HCV positive but not HCV viremic; it can be either old infections or cured (most likely) or false positives for HCV (unlikely). This test combination is cost-effective with the highest probability for HCV-RNA detection with a corresponding supplementary test cost of 1551 USD. Our findings address two major concerns related to HCV infection diagnosis wherein we implemented an accurate cost-saving method, based on both uses of two EIA-4 tests and considering the threshold 50% TRmax. This algorithm can be used for screening of infected patients likely to be treated and for HCV diagnosis in Mali, where a well-documented cost-effective screening program is lacking.

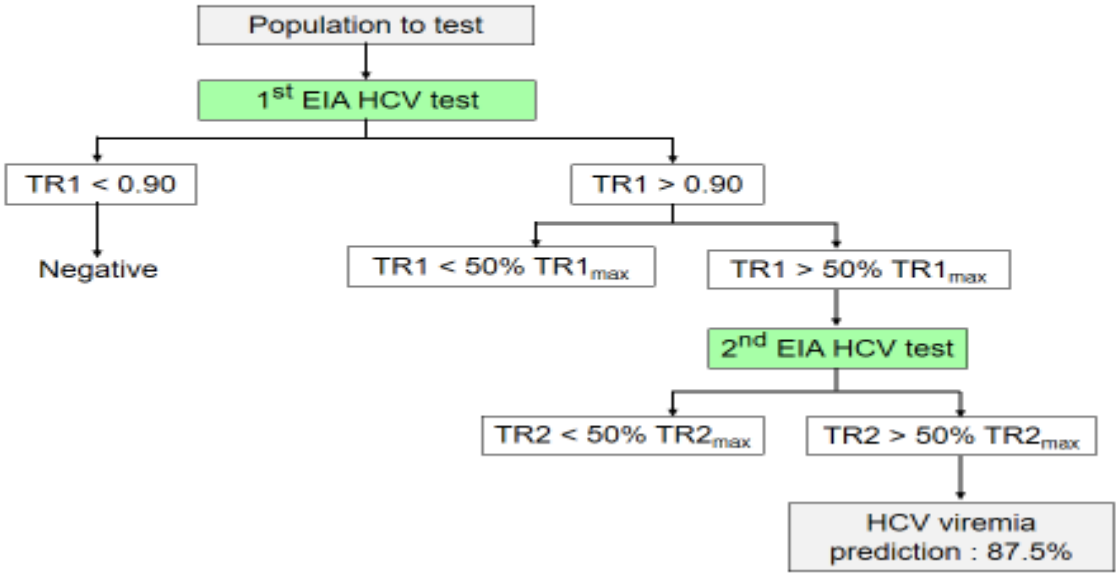

Figure 2. Algorithm based on two EIA serological tests that allows to predict a probability of viremia (PPV = 87.5%).

The weakness in our study is that PCR could not be tested on the entire series because of the high cost of this analysis. Therefore, it was not feasible to demonstrate the possible false negatives from screening EIA tests. That explains also why the negative predictive values (NPVs) were not calculated for indirect diagnosis of HCV viremia.

As for the confirmatory test, Bouare and co-workers estimated the PPV PCR+ from LIA that is not equal to 100% but 81.2% and 82.4% considering the threshold TRmax 50% (probably ± 20% of patients recover spontaneously according to the literature) (Makuria, 2012; Ciotti, 2013). Our results confirm the data showing that the confirmatory test is not necessary for the diagnosis of HCV (Ren, 2005; Pirillo, 2007; Vermeersch, 2008). Our study data also support the assumption for impertinence to repeat the test for positive samples since the current tests perform better (Ren, 2005; Pirillo, 2007). In addition, the choice of virus identification method should be left to the discretion of clinician depending on the eligibility criteria. Indeed, all viremic patients do not meet the criteria for treatment initiation (Delwaide, 2005). While genotyping may no longer be required in the pan-genotypic antiviral therapy context (Fourati, 2018), non-response to IFN-free regimens (5%) may be due to resistance associated substitutions (RASs) specific for each genotype/subtype (Marascio, 2019). However, Real-world data are needed to demonstrate the potential role of RASs and polymorphisms (Marascio, 2019). Still, a more specific HCV test (anti-HCV IgM) (Gerard, 1996) can be used in the second or third instance instead of the PCR. Similarly, HCVcAg testing in serum is an excellent alternative to HCV polymerase chain reaction in Africa (Mohamed, 2017). Despite the excellent performance of cAg quantification, its implementation on a large scale in resource-limited countries presents some limitations, it requires a fully equipped laboratory and a trained staff; yet these conditions are often limited to big cities in resource limited countries (Duchesne, 2017). However, EIA-3 test is reported sensitive compared to AxSYM HCV Acon® and PCR (Benouda, 2009). The Mixed Ag/Ab EIA test with good sensitivity and specificity could be a useful alternative tool in settings where direct diagnostic assay like PCR are not feasible (Yang, 2011). It could be proposed for use, especially in patients with uremia or those with HIV infection in whom an early diagnosis allows better management. Viremia sensitivity and specificity remain the same regardless of the proportion of anti-HCV seropositive persons in the cohort being tested (Chapko, 2015). Strategies involving HCV antibody

point of care testing (in venipuncture blood) followed by reflexive quantitative or drawn separated blood nucleic acid testing (NAT) are economically comparable and viremia sensitive (Chapko, 2015). However, these sensitive tests require nucleic acid testing to confirm HCV infectious status. As far as HCV serology is concerned, false negative results may occur in some contexts such as severe immunodeficiency (HIV infection, hypo- or agammaglobulinemia), solid organ transplant recipients, and patients undergoing hemodialysis (Kalantar-Zadeh, 2005; Ciotti, 2013). However, cross-reactive circulating antigens and antibodies may cause false positive results in some conditions such as pregnant women and nephritic syndrome autoimmune diseases (Kalem, 2016). False positive results of antibody screening tests for HCV may lead to unnecessary use of expensive confirmatory tests like HCV RNA or RIBA (Kalem, 2016). Our results confirm and complement the data previously reported (Ren, 2005; Pirillo, 2007). We propose an algorithm that confirms the active infection that can be used for HCV therapeutic management (discriminates patients more likely to be treated). In addition, since the combined detection (antigens and antibodies) of HCV may improve the sensitivity in contrast to antibody testing alone (Laperche, 2005; Nastouli, 2009; El-Emshaty, 2011), our method may be proposed for screening blood donors, and hemodialysis patients in countries with scarce resources provided one uses the test mix as first-line. Whether PCR and genotyping present a particular interest for both treatment and monitoring (Ciotti, 2013), our algorithm is of great interest for HCV diagnosis and screening of patients more likely to be treated in Mali. This argument is supported by other researchers who stated in light of the advances in HCV antiviral therapy, global control of HCV infection becomes feasible but depends on countries ability to identify infected people and treat them (Fourati, 2018). Today, despite the progress in HCV screening and diagnosis, it is still expensive, and efforts must be made to allow the generalized implementation of reliable tests in low and middle-income countries (Fourati, 2018). For the widespread application of these reliable tests, our algorithm may be useful in HCV management in the West African area and other low-income countries, before ulterior or complementary studies confirm our assumption.

CONCLUSION

The highest PPV was obtained by performing two EIA (4th generation, ELISA principle) tests and exploitation of the threshold (half of the maximal ratio value measured for the test, 50% TRmax). The cost of additional tests for reliable detection of the virus varied between 3548 USD and 1155 USD and the algorithm with the highest PPV (87.5%) was not the most expensive (1551 USD).

Competing interests

Authors declare no conflict of interest.

Authors' contributions

Bouare N contributed to study conception, data collection, manuscript writing, including drafting the article, analysis, and interpretation of data. Delwaide J contributed to study design and revised the manuscript; Bontems S contributed to the analysis and interpretation of data and revised the manuscript; Seidel L performed statistical analysis of data; Gerard C designed the study, supervised the analysis and interpretation of data and revised the manuscript.

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