Evaluation of Fluorescein Diacetate Vital Staining Performance in Bacteriogical Follow-Up of Tuberculosis Patients in Kinshasa

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ABSTRACT

Background: Resistants alcalo acido bacilli met during the follow-up of patients are declared as fail in the second or third month of treatment. However, the Ziehl used doesn't distinguish the dead bacilli from those which are alive.

Objective: To show the FDA pertinence in the patients monitoring under antituberculosis sufferer treatment.

Methods: Study of prospective troop, comparing the sensitivity and specificity, as so the negatives and positives predictive values of three usual analyzes technical (Ziehl, Auramine, FDA) to the tuberculosis suffer subjects. The samples tested positives by the Ziehl, in the screening (testing) center and the tuberculosis Elonga treatment, have in addition been confronted for confirmation to the cultured of the Löwenstein-Jensens medium considered as reference test, to the Kinshasa Clinics University.

Results: Sensitivity for the Ziehl was 83.3 % (IC95%:63.8-94.5) at the 2nd month, 90.5 % (IC95%: 68.2-98.3) at 3rd month and 100 % (IC95%: 80-100) at the 5th month of treatment. It's was 100 % (IC95%: 80.8-100) at the 2nd,3rd, 5th, 6th and 8th month for the Auramine. For FDA, it's was 100 % (IC95%: 82.8-100) for the 2nd month, and 100 % (IC95%: 80.8-100) at the 3th month. The results of the 3th month were identical at the 5th and 6th month of treatment. Sensitivity for the Ziehl was 83.3 % (IC95%:63.8-94.5) at the 2nd month, 90.5% (IC95%: 68.2-98.3) at 3rd month and 100% (IC95%: 80-100) at the 5th month

of treatment. It's was 100 % (IC95%: 80.8-100) at the 2nd, 3rd, 5th, 6th and 8th month for the Auramine. For FDA, it's was 100 % (IC95%: 82.8-100) for the 2nd month, and 100 % (IC95%: 80.8-100) at the 3th month. The results of 3th month were identical at 5th and in the 6th month of treatment.

Conclusion: Even though almost similar for the 3 technical after the 5th month, the indicators suggest a superiority of the FDA and the culture used as reference to the precocious steps of the follow-up.

Key words: FDA, Bacteriological Follow-Up, Tubercular, Kinshasa.

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Access this article online	
Website: **www.ijmrp.com**	**Quick Response code**
DOI: **10.21276/ijmrp.2017.3.1.013**	

INTRODUCTION

Tuberculosis is a major public health problem worldwide and especially in resource limited countries where AIDS is spreading unchecked.[1-3] These same countries suffer from worse social disruptions with very high tuberculosis incidence rate of about 150-350 cases for 100000 inhabitants.[1-3]

The Democratic Republic of Congo (DRC) belongs to the 22 countries with a heavy burden of tuberculosis.[1-4] According to the World Health Organization (WHO) 2015 tuberculosis report on control, it ranks 9th in the World and 3rd in Africa.[3] The same report places DRC in a zone where tuberculosis incidence is higher than

300 cases for 100000 inhabitants with a multidrug resistance rate (MDR-TB) estimated between 3,000-19,999 cases.[3]

According to WHO guidelines taken over by the National programs against tuberculosis, microscopic analysis of sputum remains a common mean of detection and of bacteriological monitoring of tuberculosis cases.[1-7] It uses various techniques (Ziehl, Auramine) whose sensibility and specificity are different.[1-8] However, economic conditions limit in many countries systematic use of coloration with Auramine, culture and molecular technique whose sensibility is recognized superior to that of Ziehl.[1-8]

In DRC hot Ziehl staining is the most widely used technique, in spite of its limitation in detection of tuberculosis because it allows fast detection in less than one hour the most bacilliferous and contagious patients in the environment.[1-8] This same technique of coloration (Ziehl) is used for the therapeutic follow-up, despite the fact that it doesn't allow distinguishing dead from alive bacilli. That is why therapeutic failure is sometime exaggerated or overestimated.[7-11] These failures push health staff to extend the intensive treatment phase to 3 months.[7-12]

The question is whether failures at 2nd and 3rd month are actually real failures and what these failures become at the 5th, 6th and 8th month. There is therefore a need for a more discriminating test to resolve this issue and allow fast determination of true therapeutic failures in order to avoid wasting drugs by prolonging the intensive treatment phase.[7-13]

In order to solve this question, we have carried out experiments of vital coloration technique with the Fluorescein (FDA) in the bacteriological follow-up of tuberculosis patients under treatment. This technical was used first to determine the viability of resistant acid alcohol bacilli (AFB) in the lepers[11] and then to the tuberculosis patients.[9,10,12] It allows putting in evidence only viable AFB.[9-12] The same test was used in predicting failure and resistance with a good performance of 88% to 98%.[9,10]

Hence the general objective of this study is to evaluate FDA performance in therapeutic follow-up of tuberculosis patients with a positive microscopy in relation to Ziehl used in the screening center.

OBJECTIVES

- To compare result of each technique with the culture in Löwenstein-Jensens (LJ) medium;
- To determine the sensibility, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the technique used;
- To determine the therapeutic outcome of patients being monitored.

METHODS

Place, Type and Period of study

We conducted a prospective cohort study. The aim is to validate staining test (FDA) in bacteriological monitoring of patients under treatment. The study was conducted at the Center of Detection and Treatment of Tuberculosis (CDST Elonga) and at the University of Kinshasa Clinics (CUK) from January 2011 to May 2012. Analysis was carried out at CDST and the other at Mycobacteria laboratory of CUK.

Patients and Samples

One hundred sixty-four positive Ziehl patients (new cases and relapses) were recruited among patients from the active screening population. Included in the study were patients suspected of pulmonary tuberculosis and all relapse cases coming for testing and having given clear consent. Excluded from this study were all patients with a negative bacilluscopy, and patient who refused to participate in the study.

Sputum were collected in the spittoons for various analysis (Ziehl, Auramine, FDA and culture) by a team of 3 trained medical biologists and 2 physician biologists. Löwenstein-Jensens culture used as gold standard was realized in parallel on the sputum to compare positive or negatives results and also to isolate, identify and carry out the antibiogram.

Coloration Proceedings

The different procedures used for the Ziehl, Auramine and culture are those in the PATI 4, the technical guide of National Program for Tuberculosis Control (PNT).[4] On the other hand the FDA procedure is that used by the Mycobacteria laboratory at the Antwerp Institute of Tropical Medicine according to the standard rules.[9,10] The smears for the FDA were unfixed, dried and covered with FDA reagent during 30 minutes in the dark. This reagent was prepared weekly by diluting 100µl of FDA stock solution in 10 ml of phosphate buffer at pH 6.8 containing 0.05% of Tween 80. The smears were rinsed with water and discolored with acid alcohol for 1 to 2 minutes. Aqueous phenol 5% was used to kill the bacilli for 10 minutes, followed by rinsing with water and drying in the dark. Reading was carried out under fluorescence microscope with a 25X objective for identification living AFB that appears fluorescent green. Reading was done independently without knowledge of result of the known culture. The discordant slides were read by the physician. For all these coloration methods used, numbers of AFB were expressed quantitatively.[9,10]

Quantification was realized as follows: for the Ziehl: no AFB per 300 fields corresponded to0; 1 to 9 AFB in 100 microscopic fields, the exact number of AFB was specified; from 10 to 99in 100 fields were considered 1+; 1 to 10 AFB per field for 50 microscopic fields were considered 2+ and more than 10AFB per field for 20 microscopic fields corresponded to 3+.[4] For the Auramine: no AFB observed in 300 fields microscopic corresponded to 0; 1 to 5 AFB per 300 microscopic fields, the number of AFB was specified; 9 to 10 AFB in one microscopic field matched 1+; 10 to 99 AFB per microscopic field corresponded to 2+ and above 100 AFB corresponded to 3+.[4] Reading FDA was done in the following manner: no fluorescents AFB per 100 fields, corresponded to 0; 1 to 9 fluorescents AFB per field for 100 microscopic fields, exacts number AFB is specified, 10 to 99 fluorescents AFB per 100 microscopic fields, corresponded to 1+; 1 to 10 fluorescents AFB for one microscopic field corresponded to 2+ and above 10 AFB fluorescents per microscopic field corresponded to 3+.[9,10]

Löwenstein-Jensens Culture Procedure, Strained Identification and Antibiogram

Concerning the culture considered as reference, samples were treated by the Petroff's methods which use 4% caustic Soda.[4,6] After centrifugation, 0.2 ml of inoculum were stocked in two tubes containing pure LJ media for isolation and a LJ containing tube incorporated with acid thiophen-2 carboxylic for biochemistry identification of the strains. The tubes were incubated at 37°C until the 12th week. These tubes were observed during the first three days to detect signs of contamination and thereafter once a week for looking growth of germs.

Positive culture was determined by the presence of cauliflower colonies in the middle slope.[7,8] The AFB verification was done by realizing Ziehl's technique. Number colonies were determined according to the notation, recommended by PATI 4.[4] Less than 50 colonies, specify exact number; 50 to 100 colonies were considered 1+; 100 to 200 colonies considered 2+; almost confluence culture 3+; confluence culture 4+.[4] Beside acid thiophen- 2 carboxylic, Niacin test was also used after isolation for the identification of the strains.[6] The direct technique of proportion

method of Canetti et al. was used for the antibiogram on LJ.[6] The different drugs were tested as follows: Isoniazid (0.2 µg/ml), Rifampicin (40 µg/ml), Dihydrostreptomycin (4 µg/ml) and Ethambutol (2 µg/ml).

After dilution, anti-tuberculosis drugs were incorporated into the media (LJ). The seeding of 0.2 ml inoculums was carried out respectively on the tubes containing the different molecules and on that containing exclusively pure LJ medium. On the other hand, the inoculum at 10^{-2} dilution was inoculated only in the tube containing pure LJ medium.[6] The reading was done on the 28 th, and 42 nd day of incubation while taking into account the growth on the control tube with the dilution of 10^{-2}.[6]

Therapeutic Regime

Detected patients were submitted to a therapeutic regime according to the category. For category I (new cases), patients received Rifampicin; Isoniazid; Ethambutol and Pyrazinamide (2RHEZ) for two months in intensive phase and four months of Rifampicin and Isoniazid (4RH) in continuation phase.[4] On the other hand, those of the category II (relapses) received first Streptomycin, Rifampicin, Isoniazid, Ethambutol and pyrazinamide (2SRHEZ) for two months, then Rifampicin; Isoniazid; Ethambutol and Pyrazinamide (1RHEZ) for one month in intensive phase and

finally 5 months of Rifampicin; Isoniazid and Ethambutol (5RHE) in continuation phase. According PATI 4, new cases (NC) were followed for 6 months and relapses cases for 8 months.[3,4]

HIV Serology

In addition, HIV serology was performed using the Determine and Unigold test, in all tuberculosis patients after informed consent in accordance with WHO and PNT guidelines.[1-4,13]

Ethical Considerations

The study was approved by Ethic committee of the School of the Public of the University of Kinshasa (N° ESP/CE/081/2010).

Statistical Analysis

Data were recorded in the laboratory register and then transferred to the Excel software. They were analyzed using EPI info version 5 software. This software allowed statistical processing of the information. The mean and standard deviation were calculated for quantitative variables. The results of the FDA, Ziehl and Auramine were compared to those obtained by cultivating LJ considered as the gold standard. Sensitivity, specificity, positive and negative predictive values were calculated. The exact Fisher test as well as the Pearson chi-square was used as needed. The significance level was set at 5% and the confidence interval (CI) at 95 %.

Table 1: Positivity degree of different tests during diagnostic

Tests	AFB indication					
	+		++		+++	
	n	%	n	%	n	%
Ziehl	45	28.1	57	35.6	58	36.3
Auramine	20	12.5	60	37.5	80	50
FDA	40	25	51	31.9	69	43.1
LJ	24	15	30	18.8	106	66.2

Table 2: The negativation to the different controls

Tests	Month-control									
	2		3		5		6		8	
	n	%	n	%	n	%	n	%	n	%
Ziehl										
• Negative	109	68.1	136	85	139	86.9	139	86.9	2	18.2
• Positive	51	31.9	24	15	21	13.1	21	13.1	9	81.8
Auramine										
• Negative	84	52.5	132	82.5	139	86.9	139	86.3	2	18.2
• Positive	76	47.5	28	17.5	21	13.1	21	13.7	9	81.8
FDA										
• Negative	134	83.8	139	86.9	139	86.9	139	86.9	2	18.2
• Positive	26	16.2	21	13.1	21	13.1	21	13.1	9	81.8
Culture										
• Negative	134	83.8	139	86.9	139	86.9	139	86.9	2	18.2
• Positive	26	16.2	21	13.1	21	13.1	21	13.1	9	81.8

RESULTS

From the nine hundred sixty patients, 164 patients with a positive Ziehl agreed to participate in the study. Of these, 11 were relapsed cases. However, four patients, 2 died in the first month of treatment and 2 lost in the follow-up were excluded from the study. Seventy-one patients (44.4%) came from CDST Elonga and 89 (55.6%) from CUK. There was no significant difference (P= 0.18). There were 98 men (61.3%) and 62 Women (38.7%) and the difference was significant (p=0.05). The average age was 32.7

for man with a standard deviation of 12.50 and 32.6 for women with a standard deviation of 10.65. The difference between the mean age was not significant (p=0.98). On the other hand, the age group most affected was 18 to 50. The HIV serology realized was negative for 130 (81.3%) patients and positive for 30 (18.7%). Of the 30 (18.7%) HIV positive patients 19 (63.3%) were from category I; whereas 11 (36.7%) were from category II (Relapses). The one hundred sixty patients were followed bacteriologically,

realizing the various coloration technique; Ziehl, Auramine and the vital coloration (FDA) and the culture on LJ. All the smears realized and colored by Ziehl technique, Auramine and FDA were initially positive at different degrees as shown in Table 1.

The majority of smears (3/4) (Table 1) were positive with more than one AFB for the different technique of coloration. On the other hand 28.1% was positive (1+) to Ziehl, 25% to FDA, 15% to the culture and 12.5% to the Auramine. However, 66.2% were positives (3+) to the culture during diagnostic.

The LJ culture allowed isolation of one hundred fifty-seven strains of tuberculosis Mycobacterium and 3 atypical from the 160 patients. The negativation of AFB to different tests during different controls is reported in Table 2.

Table 2 reveals that 109 Ziehl (68. 1%) were negative in the 2nd month and 136(85%) in the 3rd month. On the other hand for Auramine, the negativation rate was 84 (52.5%) in the 2nd month and 132 (82.5%) in the 3rd month of treatment. Finally for FDA and the culture 134 (83.8%) were negative in the 2nd month and 139 (86.9%) in the 3rd month of treatment.

At the 5th and de 6th month of treatment, the total conversion rate was 139 (86.9%) for all technique used in bacteriological follow-up with similar results. On the other hand at the 8th month of treatment, the failures number was 9 (81.8%) for all the tests used. Sensitivity, specificity, PPV and NPV for different test used and considering the culture on LJ as reference are reported in Table 3.

Table 3 shows the performance of the FDA compared to the others tests realized with sensitivity at 100%, specificity at 100%, a PPV at 100% and NPV at 100% from the second month of treatment. The outcome of these patients at the 6th and 8th month of treatment is shown in Table 4.

In Table 4, 137 patients out of 149 case from category I had favorable evolution (cured) whereas for category II, relapsed cases followed up to 8th month of treatment, only 2 cases were cured and 9 had unfavorable outcome (failed).

The antibiogram at the end of the 6th and 8th month of treatment shows 13.1% (21) MDR-TB, 8.1% for the NC (12 cases) and 81.8% for the retreatment (9).

Table 3: Sensitivity, specificity of the different tests

Months	Ziehl				Auramine				FDA			
	SS %	SP %	VPP	VPN	SS %	SP %	VPP	VPN	SS %	SP %	VPP	VPN
2	83.3 (61.8-94.5)	22.1 (15.6-30.1)	15.9 (10.2-23.7)	88.2 (71.6-96.2)	100 (82.8-100)	63.2 (54.5-71.2)	32.4 (22.3-44.4)	100 (94.7-100)	100 (82.8-100)	100 (96.6-100)	100 (82.8-100)	100 (96.6-100)
3	90.5 (68.2-98.3)	97.8 (93.3-99.4)	86.4 (64-96.4)	98.6 (94.3-99.7)	100 (80.8-100)	60.4 (51.8-68.5)	27.6 (18.3-39.3)	100 (94.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)
5	100 (80-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)
6	100 (80-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)
8	100 (80-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)	100 (80.8-100)	100 (96.6-100)

SS= Sensitivity; SP= Specificity; PPV= positive predictive value; NPV= negative predictive value; FDA= fluorescein Diacetate.

Table 4: The outcome of patients becoming relates to the result of FDA and culture in the 6th and 8th month of treatment

Categories/ Month	Success	Echec	Total
I (NC/6)	137 (91,9%)	12(8,1)	149(100%)
II (relapses/ 8	2(18,2%)	9(81,8)	11(100%)

NC: new cases

DISCUSSION

The aim of this study was to evaluate the validity of FDA in bacteriological follow-up in order to improve tuberculosis patient's treatment. Bacteriological follow-up is necessary for appreciating treatment quality and to determine rapidly and precociously failure cases. It allows also having an idea on suspected case of MDR-TB. This study showed that failures rate is actually overestimated especially at the 2nd and less often in the 3rd month of treatment.

On the other hand, from the 5th month of treatment, failure rates were identical for all the techniques used as shown in Table 2.

In the beginning, there was correlation of different techniques of coloration used. All the smears (category I and II) were positive to the Ziehl, Auramine and FDA, but with different degree of positivity as reported in Table 1. These results were confirmed by culture on LJ which allowed isolation of 160 strains of which 3 atypicals.

Sixty eight percent of Ziehl stained smears were negative in the second month, and 85% in the third month of treatment with a sensitivity ranging from 83.3% to 90,5% and specificity ranging from 22.1% to 97.8%. On the other hand 52.5% of smear colored with Auramine were negative at the 2rd months and 82.5% in the 3rd month. The sensitivity was 100% and specificity 63.2% in the 2nd month and 60.4% in the 3rd month of treatment. However, negativity rate with FDA and culture was 83.8% in the second and 86.9% in the third month of treatment with a sensitivity and specificity of 100%.

At the 5th and 6th month of treatment, the conversion rate was the same for all monitoring tests. One hundred thirty seven of 149 patients in category I had good outcome (cured) and of the 11 cases in category II followed up to the 8th month of treatment, 9 (81.8%) had unfavorable outcome (failed) as shown in Table 2. In total, 21 cases of failures had multidrug-resistant strains confirmed by Antibiogram.

Although it had shown its performance in therapeutic follow-up by fast and precocious determining the true failures in the 2nd month of treatment, this study had some limitations namely; the small number of the patients followed and only two health Centers were concerned. Another limitation to consider is the fact that only one smear instead of several was considered in order to avoid underestimation of cases, especially in case contains fewer tubercle bacilli.

Concerning the origin of patients in this study, the number was slightly high at CUK (55.6%) than at CSDT, but the difference was not significant (p = 0.18).

The predominance of the male sex observed in this study (61.3% vs. 38.7%, p = 0.005) agrees with some authors who argue that women tend to consult less frequently than men in our African settings, because of certain socio-cultural barriers.[14]

The difference in average age between men and women was not significant (p = 0.98) and it is also clear that the most affected age group was 18-50 (60%). This fact is consistent with numerous WHO statements that show the vulnerability of the economically most active layer.[1-3]

The present investigation shows that the results obtained with FDA are identical to those of the culture. This finding is consistent with the Bangladesh study in 2006, which shows the positivity of both FDA and culture on the liquid medium for all 60 cases of failure and 11 relapses of category II.[9] These observations also corroborate those describe in 2014 America which report similar results to the FDA and culture.[12] It also appears that the conversion rate varied according to each technique used in the control course especially in the second and a little bit in the third month as illustrated in Table 2.

In the second month of treatment, the rate of negativity in Ziehl was 68.1% (109) and a failure rate of 31.9% (51). The sensitivity was 83% (95% CI: 61.8-94.5), very low specificity at 22% (15.6-30.1), a very low PPV at 15.9% (IC 95%: 15.6-30.1) and good NPN at 88.2% (95% CI: 71.6-96.2).

This observation is consistent with the observation in Madagascar (2002), Cameroon (2009), Sri Lanka (2013) and Rwanda (2013), which respectively reported a non-conversion rate of 51.2%; 13.4; 16; 20% and 25% for patients followed in the second month of treatment.[7,15,16] Comparison of these rates shows that our rates were higher than those obtained in Cameroon; Sri Lanka and Rwanda. On the other hand, it is lower than that found in

Madagascar.[15-17] This diversity of rates could be explained by the number of patients in category II.

These results show that in the second month of treatment, patients continued to expectorate tuberculosis bacilli and the disparity in rates could be explained by the number of patients with MDR-TB strains and by the technique used. This is in line with an American study which found that the viability of bacilli and the outcome of culture during treatment differed significantly between patients with simple tuberculosis and MDR-TB (p = <0.001).[12]

With Auramine, however, the rate of negativity was low by 52.5% (84) with a high failure rate of 47.5% (76). The sensitivity was 100% (95% CI: 82.8-100), specificity of 63.2% (95% CI: 54.5-71.2), weak PPV at 32.4% (IC 95%: 22.3-44.4) and very good NPV of 100% (95% CI: 94.7-100).

The survey showed that the sensitivity of Auramine was good and a lower specificity compared to Ziehl. This situation is explained by the fact that smears stained with Auramine are examined with a dry objective of low magnification (× 25), which make that surface of each microscopic field observed 16 times higher than the magnification of × 100, and microscopic examination is faster and more sensitive than Ziehl.[4-7]

In view of these results of Ziehl and Auramine, it emerges that the Auramine although sensitive is not good for therapeutic follow-up because its poor specificity with the number of failures higher than Ziehl (71/56) to the 2nd month of treatment. This result is in line with those reported in Madagascar in 2002 and Bangladesh in 2012.[7,10] They found that many bacilli (50%) found in direct examination in the second month of treatment were no longer viable.[7] This is due to the presence of non-viable bacilli which have lost their ability to multiply during the healing process of pulmonary caverns.[7,18]

This study showed that at the FDA and culture, the conversion rate was identical to 83.8% (134) in the 2nd month of treatment and a low failure rate at 16.2%.[10-12,15,18] Compared with the other tests (Ziehl and Auramine) as shown in Table 2. This result is in agreement with those found in Bangladesh in 2006, which show positivity to both FDA and culture on the liquid medium, of all 60 failure cases and 11 relapses cases of category II.[9] They showed a perfect homogeneity of the results between the FDA and the culture.[9]

By comparing the conversion rate obtained using FDA to those found in Ziehl and Auramine in our series, we found an overestimation of the failure rate (non-conversion) in Ziehl (31.9 %) and especially with Auramine (47.5%) in the second month of treatment. The advantage of the FDA is that only viable bacilli are put in evidence and confirmed by growth on LJ media.[9-12]

This results is close to the one done in Bangladesh in 2012 which shows 1633 episodes of conversion and failures defined by the staining smears with Auramine.[10] They found that a negative FDA had a predictive value of 95% for a negative culture in patients undergoing first treatment, while its predictive positive value was around 95% in the case of retreatment.[10]

This phenomenon of overestimation of microscopic failures was also reported in the DRC in 2006, in a study on the management of patients with MR-TB strains under second-line of antituberculosis drugs.[18]

The FDA's performance compared to the other coloring techniques used in this survey is demonstrated by the

determination of true therapeutic failures from the second month of control with a very good sensitivity at 100% (82.8-100), a very good specificity at 100% (IC: 96.6-100), a very good PPV at 100% (95% CI: 82.8-100) and a very good NPV at 100% (95% CI: 96.6 - 100).

However, the rate of FDA and culture negativity was higher than that of 51% and 45% respectively in Madagascar in 2002 and in Bangladesh in 2006.[7,9] In their studies, the sensitivity was 99% and the specificity was 82%, the PPV was 92% and the NPV was 97% whereas ours were high. This could be explained by the fact that the last two values depend on the prevalence of tuberculosis.

By the third month of treatment, the conversion rate increased significantly with all techniques as illustrated in Table 2. In Ziehl, the conversion rate was increased to 85% (136) and the failure rate was reduced to 15% (24) with good sensitivity at 90.5% (95% CI: 68, 2-98.3), a good specificity of 97.8% (95% CI: 93.3-99.4), a good PPV at 86.4% (95% CI: 64-96.4) and a good NPV of 98.6% (95% CI: 94.3-99.7).

With Auramine, the conversion rate was not better than the one with Ziehl at 82.5% (132) with good sensitivity of 100% (95% CI: 80.8-100), a low specificity of 60.4% (51.8-68.5), a poor PPV of 27.6% (18.3-39.3) and a good NPV of 100% (95% CI: 94.6-100). There was not homogeneity of the results as shown in Table 2 and 3.

For FDA and culture, the conversion rate was very good at 86.9% (139) with homogeneous results as shown in Table 2 and 3. Sensitivity was better at 100% (95% CI 80.8-100), a better specificity of 100% (95% CI: 96.6-100), a better PPV of 100% (95% CI: 80.8-100) and better NPV of 100% (95% CI: 96.6-100). This result is in agreement with that reported in Bangladesh, which shows a perfect correlation with growth in culture at the 3rd month of treatment.[9,10]

Unlike the study conducted in the DRC in 2006, which revealed that at the 3rd month of treatment, the conversion rate by microscopy was 82.9% and increased to 91.4% to the culture.[18]

The failure rate in this series was 17.1% at microscopy and 8.6% at LJ culture.[18]

At the 5th and 6th month of treatment, the total conversion rate was 86.9% (139) for all techniques used in bacteriological monitoring with homogeneous results. The failure rate for the four tests was 13.1% with a similar sensitivity, specificity, PPV and NPV as shown in Table 3 and 4. The high failure rate encountered in this survey was due to by the presence of multi-resistant strains among NCs and retreatment cases. So viability of the germs was determined by the FDA and the culture.

These results corroborate those obtained in 2014 which shows a perfect homogeneity of FDA and culture results.[12] On the other hand, a study carried out in the DRC on the management of multiresistant tuberculosis had shown that in the 6th month, the conversion rate was 88.2% in culture and 85.3% in Ziehl.[18] The failure rate in this study is higher than ours because all the patients followed in their series had multidrug-resistant strains (MDR-TB). However, this failure rate is lower than the 90% rate reported in Bangladesh in 2006 at the 6th month of treatment, although 95% of the patients were MR-TB cases.[9]

Thus, the real failures were detected as early from the second month of treatment by the FDA and these failures were confirmed by the Löwenstein-Jensens culture by isolating the strains of Mycobacterium. On the other hand, the confirmation of failures

with the other tests (Ziehl and Auramine) was specified only at the 5th month of treatment.[7,9,10] Similar observations were reported in America and Bangladesh. It was shown that the viability of AFB predicts the results of the culture and also identifies 88-98% resistance to rifampicin.[9,10,12]

The outcome of patients in the 6th and 8th month of treatment is shown in Table 4. Concerning the therapeutic outcomes of patients in category I (NC), 137 out of 149 patients (91.9%) of which 15 with positive serology advanced well bacteriologically (recovered) , whereas 12 patients (8.1%), among two with positive HIV serology, had an unfavorable outcome (failure). These failures are higher compared to the normal rate of 3 % set up by WHO.[1-4] This high rate could be explained by lack of compliance to the treatment, infection by HIV but also by breaking up antituberculosis drug.[1-4,13]

The bacteriological follow-up of the 8th month of treatment concerned only patients in category II and all were HIV seropositive. Two patients evolved well (heal), while 9 were not cured. Three of them had atypical strains (NTM) resistant to anti-tuberculosis treatment.[10] This result was confirmed by the culture which was also positive. Studies in Bangladesh in 2006 and 2012 also noted the atypical strains.[9,10]

In total, 21 cases (13.1%) of therapeutic failures were recorded for both categories. This failure rate is higher than that of 3 % set up by WHO and higher than 4% found in Madagascar with different degrees of positivity.[1-4, 9,16,17] The high rate of failures found (13.1%) in this study could be explained by the number of relapse cases with MR-TB strains and by the presence of NTM strains resistant to anti- tuberculosis drugs.[12]

FDA staining appears to be beneficial for the follow-up of patients on TB treatment because it enabled early detection of therapeutic failure and to predict MDR-TB cases.[9,10,16,17] This early confirmation of therapeutic failures makes it possible to better adapt the chemotherapy, to reduce multidrug-resistant strains and mortality rate.

These results made available to the government through the PNT, which has the monopoly of controlling tuberculosis throughout the country, can make a difference in the follow-up of TB patients on treatment.

CONCLUSION

Bacteriological follow-up is necessary for determining cases of failures and MR-TB. It had been observed overestimation of failures in the 2nd and 3rd month of treatment by using the coloration of Ziehl and Auramine and the true failures became clear only in the 5th month with a risk of MR-TB stumps propagation. On the other hand, coloration of Fluorescein of Diacetate (FDA) is shown as an alternative for fast and precocious determination of true therapeutic failures from the 2nd month of treatment. Coloration with FDA is benefic for follow-up of patients under antituberculosis drug treatment and for detecting precociously failure case in order to take rapidly in charge MR-TB cases and stop the growth of multiresistants stumps.

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