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Laboratory diagnostic systems used in the diagnosis of tuberculosis in Ethiopia: A systematic review

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Laboratories and laboratory tests are fundamental components of tuberculosis control programme; providing testing for diagnosis, surveillance and treatment monitoring at every level of health care system. This review is therefore, aimed at comparing and summarizing data on these laboratory systems used in the diagnosis of tuberculosis in Ethiopia. A systematic review of literature addressing the diagnosis of tuberculosis in Ethiopia was carried out. Literature search was done in MEDLINE and EMBASE for articles published from 2000 to 2013. We used the following search terms: Laboratory, tuberculosis, diagnosis and Ethiopia. Only studies addressing diagnostic systems of tuberculosis and English language publications were included. A total of 267 potential studies were identified by the search. Thirty-three studies qualified for the review. The reported diagnostic systems used to diagnose tuberculosis (TB) in Ethiopia included symptom screening, physical examination, chest X-ray, histology, histopathology, cytology, smear microscopy, tuberculin skin test (TST), QuantiFERON TB gold in tube and culture. Ten (30.3%) studies utilized culture as the reference standard and only one study employed polymerase chain reaction (PCR) as a standardized test. Most studies have used more than one diagnostic system. Four (12.5%) studies reported the use of sample processing methods (liquefaction and concentration of sputum using either sodium hypochlorite or sodium hydroxide followed by centrifugation) before actual tests, twenty seven (81.8%) studies used direct smear microscopy, three (9.4%) studies used TST and QuantiFERON-TB Gold In-Tube test (QFTGIT) together and two (6.3%) studies employed cytological techniques. Chest X-ray was employed in the majority of smear negative results with symptomatic patients suggestive of tuberculosis. The results of this review suggest that there is a need for revising the diagnostic systems. Most of the laboratory tests employed was based on direct smear microscopy which is insensitive and can only detect 36% of tuberculosis cases in Ethiopia. This may result in misdiagnosis of the disease and further transmission of the disease, especially in children and populations with high human immunodeficiency virus (HIV) co-infection. Therefore, there is need for laboratories to find a rapid and efficient method for TB diagnosis as a complement to the smear microscopy.

Key words: Diagnosis, laboratory, tuberculosis, Ethiopia.

INTRODUCTION

Tuberculosis (TB) is one of the world’s most important causes of morbidity and mortality among adults. It is
caused by the bacterium *Mycobacterium tuberculosis* which most commonly affects the lungs (pulmonary TB), but may also affect any organ or tissue outside of the lungs (extra pulmonary TB). When TB is detected and effectively treated, the disease is mostly curable. One of the largest challenges in preventing morbidity and mortality from TB is the difficulty in making a timely diagnosis. Diagnostic approaches relying on symptoms, chest radiographs, tuberculin skin tests, microscopy or cultures, all have particular challenges (World Health Organization, 2011, 2008).

Early, accurate and rapid diagnosis of TB is critical for reducing TB transmission and incidence. Even though World Health Organization (WHO) has endorsed a novel, rapid and automated diagnostic system that are simple enough to be run in basic laboratories and clinics outside of a reference laboratory setting in 2010, smear microscopy is the sole method used for TB diagnosis in most laboratories in developing countries, where over 95% of TB-related deaths occur. In patients with active pulmonary TB, only an estimated 45% of infections are detected by sputum microscopy. In most cases, mycobacterial culture is generally considered to be the best available reference standard diagnostic test for TB diagnosis and is the most important first step method in detecting drug resistance (WHO, 2011).

TB remains the leading cause of morbidity and mortality in developing countries, including Ethiopia, despite the availability of short-course therapy that can be both inexpensive and effective. Early and proper diagnoses are essential for effective tuberculosis control programs; to improve treatment, to reduce transmission and to control development of drug resistance. Laboratories and laboratory networks are fundamental components of tuberculosis control, providing testing for diagnosis, surveillance and treatment monitoring at every level of the health-care system (Ridderhof et al., 2007; Steingart et al., 2007)

Care of patients with tuberculosis starts with a quality assured diagnosis. Arguably, the weakest component of health systems is laboratory services, which have been grossly neglected, understaffed and underfunded over time. Low-income and middle-income countries, which bear most of the global burden of tuberculosis, rely heavily on outdated tuberculosis diagnostic tests; including sputum smear microscopy, solid culture and chest radiography (WHO, 2011).

In high-incidence countries, TB control relies on passive case finding among individuals self-presenting to health care facilities, followed by either diagnosis based on clinical symptoms or laboratory diagnosis using sputum smear microscopy. Serial sputum specimens are required (one taken on the spot and the second brought in the following morning), which means that the people are asked to make repeated visits to the health care centers for specimen delivery and collection of results. For many patients, the costs of repeated visits to health care facilities are prohibitive, and patient dropout is a significant problem (Steingart et al., 2007).

Many smear microscopy laboratories are single rooms and understaffed with poorly maintained microscopes, and some of these laboratories lack consistent sources of electricity and clean water. There are few opportunities for the training of staff and little staff capacity to handle high-volume workloads. Quality assurance programs including quality control and external quality assessments are often lacking. Thus, there is a critical need for new, sensitive, easy and rapid point-of-care diagnostics, and also for investments in laboratory infrastructure, quality assurance programs and well-trained staff (Parsons et al., 2011).

Ethiopia is ranked 7th among the 22 countries with a high-burden of TB and third in Africa (WHO, 2009) but almost all TB laboratories in Ethiopia have only been equipped with the acid-fast staining and lack resources for culture, identification and drug susceptibility testing of mycobacteria, which present a huge hindrance for tuberculosis control in the country. Culture and drug susceptibility testing for *Mycobacterium tuberculosis* are not performed routinely in clinical microbiology laboratories. Smear microscopy contributes little to the diagnosis of pediatric TB and does not, by definition, identify smear-negative TB which may account for 24 to 61% of all pulmonary cases in people living with HIV (Getahun et al., 2007; Tessema et al., 2012).

Sputum smear microscopy remains the cornerstone of TB diagnosis in developing countries. The method depends upon the quality and bacterial load of the sputum specimen and the training and motivation of laboratory technicians. Although highly specific in most countries, smear microscopy is insensitive - it detects roughly 50% of all the active cases of TB. Sensitivity can be as low as 20% in children and HIV infected people. Furthermore, in addition, smear microscopy cannot detect bacterial resistance to anti-microbial drugs (World Health Organization, 2008).

Sputum smear examination for acid-fast bacilli (AFB) can diagnose up to 50 to 80% of cases of pulmonary tuberculosis in well-equipped laboratories (Aber et al., 1980). In low-income countries, poor access to high-quality microscopy services contributes to even lower rates of AFB detection. Furthermore, in countries with high prevalence of both pulmonary tuberculosis and HIV infection, the detection rate is even lower owing to the paucibacillary nature of pulmonary tuberculosis in patients with HIV infection. In the absence of positive sputum smears for AFB, at primary care level, most cases of pulmonary tuberculosis are diagnosed on the basis of clinical and radiological indicators (Getahun et al., 2007).

Culture is a more sensitive method for TB diagnosis than smear microscopy and it permits testing for drug resistance, but it has limitations and requires biosafety facilities that are expensive to build and maintain, and
specially trained laboratory technicians to perform the procedure (WHO, 2008).

Some national TB programmes in developing countries have no functioning TB culture facility at all. In others, TB culture is performed only at national reference laboratories or in hospital laboratories in large cities. Even where capacity exists, diagnosing TB with culture can take weeks because of the slow growth rate of TB bacilli. In most countries TB culture is reserved for retreatment cases. Specimens are often sent to distant laboratories. This can delay processing of specimens and lead to inaccurate results. Furthermore, test results must travel long distances back to the clinic and the patient (WHO, 2008).

**METHODOLOGY**

A systematic search of studies addressing the diagnosis of tuberculosis in Ethiopia was performed. All returned titles were reviewed and articles that obviously did not involve diagnosis or isolation of tuberculosis were excluded. The authors then reviewed abstracts of remaining articles to determine which studies examined diagnostic systems used in the diagnosis of tuberculosis. Bibliographies of relevant articles were also reviewed for potential articles. The two investigators independently reviewed the remaining articles, independently deciding on inclusion in the review using a predetermined eligibility criterion. Disagreements were resolved by consensus. For inclusion, the articles needed to describe a study involving the use of a diagnostic system to diagnose tuberculosis. Only English language articles were included. Each article was analyzed to determine the study setting, study design, sample characteristics, type of diagnostic system used, reference or gold standard used for comparison and findings of the diagnostic system. Duplicate publications of the same findings were excluded. Studies analyzing the diagnosis of tuberculosis without actually performing the diagnosis or isolating tuberculosis from study subjects were also excluded from the review.

**Search strategy**

We searched MEDLINE and EMBASE database for reports published in English up to the end of July, 2013. The first search was done on 20th April, 2013 and repeated on 7th July, 2013. The searches yielded 267 citations of which 38 were duplicate papers, 229 were subject to title and abstract review, 76 were subject to full text review and 33 articles were included in the final review. An over inclusive search strategy was used to ensure that no paper got missing. The key search words used were: “Tuberculosis or TB, “Mycobacterium tuberculosis or MTB”, “diagnosis or screening or isolation or tests or assays”, and “Ethiopia”. We also reviewed references of the selected papers to ensure that no paper got missing. We have also searched the website of the STOP TB Partnership’s New Diagnostic Working Group. We reviewed studies cited by articles identified by this search strategy and selected those we identified as relevant. The researcher reviewed titles and abstracts in duplicate to exclude ineligible articles. Papers meting the inclusion criteria were subject to full-text review (Figure 1).

**Inclusion and exclusion criteria**

To be eligible, articles needed were: peer-reviewed publications published in English, conducted in Ethiopia, conducted after 2000, focused on diagnosis or isolation of tuberculosis. Use of clear screening and diagnostic methods and algorithms in the diagnosis of TB was adopted and a survey methodology for data collection applied.

**Data extraction and classification**

The following data was extracted and summarized in evidence tables: Citation, year of publication, settings, study design, study population, type of diagnosis and outcomes.

**RESULTS**

The online search of MEDLINE and EMBASE yielded 287 articles. Additional potential studies were identified through searches of bibliographies. After articles that did not address the diagnosis of tuberculosis in Ethiopia were excluded, 229 articles remained. Further, 153 articles were excluded upon closer review because they did not include a diagnosis system or focused on screening of drug resistant tuberculosis. We retrieved full text articles for 76 citations, of which 43 studies were excluded based on a review of title and abstract. Articles that briefly mentioned a diagnosis system but did not give details or include how it was used in the study were also excluded. Thirty-two published peer-reviewed articles and one country wide survey from Ethiopia met the general study criteria and was included in the review (Table 1). We have also performed an updated search on 7 July, 2013 that yielded 62 titles, all of which had been identified during the previous search or were ineligible based on title or abstract. Repeated searches using the same search strategies were employed until the end of July, 2013 to ensure that no new publications were missed. Thus, we included 33 relevant studies in this review (Figure 1). Of the total 33 studies, one was population based country study (Alebachew et al, 2011) carried out in three (Urban, Rural and Pastoralists) study setting.

**Description of included studies**

Of the 267 citations identified by literature searches, a total of 33 English-language publications (32 articles, and 1 population based survey) met the eligibility criteria (Figure 1). Of the 33 studies, ten (30.3%) studies utilized culture (Aderaye et al., 2003, 2007; Alebachew et al., 2011; Beyene et al., 2008; Shah et al., 2009; Bruchfeld et al., 2002; Abebe et al., 2010, 2012; Legesse et al., 2011) and one study employed polymerase chain reaction (PCR) as the reference standard (Beyene et al., 2009). Eighteen (54.5%) studies used a cross sectional study design, three (9.1%) used comparative, two (6.1%) used a retrospective design, two (6.1%) randomized trial and one (3%) did not describe the study design employed method. Missing data were treated as “not reported” (indicated in the table as “not reported”) (Table 1). Three studies presented the data based on two separate
microscopy methods (Ziehl-Neelsen [ZN] and light emitting diode fluorescence microscopy [LED-FM]) (Alebachew et al., 2011; Yassin et al., 2013; Moges et al., 2012). Twenty five (75.8%) studies were published after 2008 and few studies included information about patients infected with HIV.

Descriptive information on population characteristics, such as sex and age category, was often reported. Most studies have used more than one diagnostic system. Twenty-four (72.73%) of the 33 studies utilized ZN stained microscopy and three (9.4%) studies used light emitting diodes fluorescent microscope (LED-FM). Four (12.15%) reported the use of some processing methods (Aderaye et al., 2007; Ali et al., 2012), three (9.14%) studies used tuberculin skin tests (TST) and QuantiFERON-TB gold In-Tube (QFTGIT) together and two (6.13%) studies employed cytological techniques. Chest X-ray was employed in the majority of studies, especially for smear negative results with symptomatic patients suggestive of tuberculosis. For the majority of studies, information about timing, manner of sputum collection, mycobacterial culture methods and quality assurance for microscopy was reported, while sputum characteristics (macroscopic appearance of sputum samples) and appropriate instructions on how to produce quality sputum was not described except in one study (Federal Ministry of Health, 2010). Eighteen (54.5 %) of the 33 studies reported about PTB alone, seven (21.29%) studies reported extra-pulmonary tuberculosis (EPTB) alone, four (12.15%) studies reported both pulmonary tuberculosis (PTB) and EPTB, three (9.14%) studies reported latent tuberculosis infection (LTBI) alone and one study reported both PTB and LTBI (Table 1).

**DISCUSSION**

Our systematic review of literature of 33 studies on diagnosis of tuberculosis showed that there is a considerable similarity between diagnosis systems used, study design and the types of TB isolated. However, comparisons of these variables were not significantly different. The diagnostic systems included the inclusion or exclusion of laboratory testing, and even their diagnostic focus (that is, pulmonary TB alone, pulmonary and extra pulmonary TB or latent TB infections) varied. Because publication dates of the articles range over the last thirteen years, some systems were developed and evaluated after the HIV epidemic and focused specifically on co-infected patients.

Smear-positive TB case detection rate is 36% in Ethiopia (Federal Ministry of Health, 2010). Lack of accu-rate and rapid diagnostics remains a major obstacle to progress in this regard. Health care facilities still heavily rely on sputum smear microscopy for the diagnosis of TB. This technique has low sensitivity and specificity (Aderaye et al., 2007). However, efforts were made to ensure quality AFB diagnosis through appropriate instruction of symptomatic individuals on how to produce quality sputum sample from their lung. In the laboratory, the
Table 1. Diagnostic systems used in the diagnosis of tuberculosis and characteristics of selected studies in Ethiopia.

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study setting</th>
<th>Study subject</th>
<th>Study design</th>
<th>Diagnosis type</th>
<th>Form of TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cambanis et al. (2006)</td>
<td>Health Center</td>
<td>PTB Suspects</td>
<td>Cross sectional</td>
<td>Smear microscopy</td>
<td>PTB</td>
</tr>
<tr>
<td>Legesse et al. (2011)</td>
<td>Community</td>
<td>Adults</td>
<td>Cross sectional</td>
<td>QFTGIT, TST</td>
<td>LTBI</td>
</tr>
<tr>
<td>Aderaye et al. (2007)</td>
<td>Hospital</td>
<td>HIV Patients</td>
<td>Comparative</td>
<td>Chest X-Ray, smear microscopy¥, culture</td>
<td>PTB</td>
</tr>
<tr>
<td>Dagnew et al. (2012)</td>
<td>University</td>
<td>Young Adults</td>
<td>Exploratory</td>
<td>QFTGIT, TST</td>
<td>LTBI</td>
</tr>
<tr>
<td>Beyene et al. (2008)</td>
<td>Rural</td>
<td>Patients</td>
<td>Cross sectional</td>
<td>Histology, Cytology, Smear Microscopy, Culture</td>
<td>EPTB</td>
</tr>
<tr>
<td>Alebachew et al. (2011)</td>
<td>Urban/rural/pastoralist</td>
<td>Persons ≥ 15 years</td>
<td>Cross sectional</td>
<td>Chest X-Ray, LED-FM, Culture</td>
<td>PTB, EPTB</td>
</tr>
<tr>
<td>Yimer et al. (2009)</td>
<td>Rural</td>
<td>Adults</td>
<td>Cross sectional</td>
<td>Smear Microscopy</td>
<td>PTB</td>
</tr>
<tr>
<td>McNerney et al. (2010)</td>
<td>Hospital</td>
<td>Adults</td>
<td>Comparative</td>
<td>Chest X-Ray, Smear Microscopy</td>
<td>PTB</td>
</tr>
<tr>
<td>Datiko et al. (2009)</td>
<td>Rural</td>
<td>PTB suspects</td>
<td>Randomized trial</td>
<td>Smear Microscopy</td>
<td>PTB</td>
</tr>
<tr>
<td>Beyene et al. (2009)</td>
<td>Hospital</td>
<td>Lymphadenopathy patients</td>
<td>Cross sectional</td>
<td>Histology, Culture, PCR</td>
<td>EPTB</td>
</tr>
<tr>
<td>Alene et al. (2013)</td>
<td>Hospital</td>
<td>Adult HIV patients</td>
<td>Retrospective</td>
<td>Chest X-Ray, smear microscopy, cytology</td>
<td>PTB, EPTB</td>
</tr>
<tr>
<td>Yassin et al. (2013)</td>
<td>Community</td>
<td>PTB suspects</td>
<td>Implementation</td>
<td>LED-FM</td>
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<td>Deribew et al. (2011)</td>
<td>Health Center</td>
<td>TB suspects</td>
<td>Cross sectional</td>
<td>Chest X-Ray, smear microscopy</td>
<td>PTB, EPTB</td>
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<td>Shah et al. (2009)</td>
<td>Hospital</td>
<td>HIV patients</td>
<td>Prospective</td>
<td>Symptom screening, physical examination, chest X-Ray, smear microscopy**, culture</td>
<td>PTB</td>
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<td>Eshete et al. (2011)</td>
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<td>Histopathology, Smear microscopy</td>
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<td>Analytical</td>
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<td>PTB</td>
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<td>Legesse et al. (2010)</td>
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<td>Comparative</td>
<td>Chest X-Ray, Smear Microscopy, QFTGIT, culture</td>
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<td>Wassie et al. (2013)</td>
<td>Urban</td>
<td>Children</td>
<td>Cohort</td>
<td>QFTGIT, TST</td>
<td>LTBI</td>
</tr>
<tr>
<td>Abebe et al. (2011)</td>
<td>Prison</td>
<td>Prisoners</td>
<td>Not reported</td>
<td>Smear Microscopy, culture</td>
<td>PTB</td>
</tr>
<tr>
<td>Wondimeneh et al. (2013)</td>
<td>Hospital</td>
<td>HIV patients</td>
<td>Cross sectional</td>
<td>Chest X-Ray, smear microscopy</td>
<td>PTB</td>
</tr>
<tr>
<td>Abebe et al. (2010)</td>
<td>Rural</td>
<td>Adult TB suspects</td>
<td>Cross sectional</td>
<td>Smear microscopy</td>
<td>PTB</td>
</tr>
<tr>
<td>Mages et al. (2012)</td>
<td>Prison</td>
<td>Prisoners</td>
<td>Case finding</td>
<td>LED-FM, cytology</td>
<td>PTB</td>
</tr>
<tr>
<td>Sharge et al. (2006)</td>
<td>Rural</td>
<td>Adults</td>
<td>Cross sectional</td>
<td>Smear microscopy</td>
<td>PTB</td>
</tr>
<tr>
<td>Muluye et al. (2013)</td>
<td>Hospital</td>
<td>Lymphadenopathy patients</td>
<td>Retrospective</td>
<td>Cytology</td>
<td>EPTB</td>
</tr>
<tr>
<td>Zenebe et al. (2013)</td>
<td>Hospital</td>
<td>EPTB suspects</td>
<td>Cross sectional</td>
<td>Smear microscopy, cytology</td>
<td>EPTB</td>
</tr>
<tr>
<td>Amare et al. (2013)</td>
<td>Hospital</td>
<td>Diabetic patients</td>
<td>Cross sectional</td>
<td>Smear microscopy, chest X-Ray</td>
<td>PTB</td>
</tr>
<tr>
<td>Shargeie et al. (2006)</td>
<td>Rural</td>
<td>Adults</td>
<td>Randomized trial</td>
<td>Smear microscopy</td>
<td>PTB</td>
</tr>
<tr>
<td>Biadglegne et al. (2013)</td>
<td>Hospital</td>
<td>Children/adults</td>
<td>Cross sectional</td>
<td>Cytology</td>
<td>EPTB</td>
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<tr>
<td>Abebe et al. (2012)</td>
<td>Rural</td>
<td>Adults</td>
<td>Cross sectional</td>
<td>Smear microscopy, cytology, culture</td>
<td>EPTB</td>
</tr>
<tr>
<td>Tadesse et al. (2011)</td>
<td>Urban/rural</td>
<td>Persons &gt; 14 Yrs</td>
<td>Cross sectional</td>
<td>Smear microscopy, chest X-Ray</td>
<td>PTB</td>
</tr>
<tr>
<td>Deribew et al. (2012)</td>
<td>Rural</td>
<td>Adults &gt; 15 Yrs</td>
<td>Cross sectional</td>
<td>Smear microscopy, Culture</td>
<td>PTB</td>
</tr>
</tbody>
</table>

QFTGIT = QuantIFERON-TB Gold In-Tube, TST = Tuberculin skin tests, PTB = Pulmonary Tuberculosis, EPTB = Extra-pulmonary Tuberculosis, LTBI = Latent tuberculosis infection, LED-FM = Light Emitting Diodes Fluorescent Microscope, ¥Primary test was done after concentration, **Re-examined after specimens are concentrated with sodium hypochlorite or sodium hydroxide.
macroscopic appearance of a sputum sample was checked and poor specimens were replaced with an immediate spot collection (Tadesse et al., 2011).

The gold standards chosen to evaluate the validity of these diagnostic systems also varied widely. Ten studies used culture as the gold standard (Aderaye et al., 2003; Aderaye et al., 2007; Alebachew et al., 2011; Beyene et al., 2008; Shah et al., 2009; Bruchfeld et al., 2002; Abebe et al., 2010, 2012; Legesse et al., 2011; Deribew et al., 2012). Others used TST and QFTGIT (Legesse et al., 2010; Dagnew et al., 2012; Wassie et al., 2013), histology, cytology and histopathology (Eshete et al., 2011; Muluye et al., 2013; Federal Ministry of Health, 2010).

Unfortunately, laboratory diagnosis is likely to depend strongly upon the experience and knowledge base of the laboratory technician; it may be less reliable in settings where technicians have less training. To allow for comparison of diagnostic tests across different studies and settings, future studies need to employ a more consistent and rapid gold standard systems. Ideally, culture would be gold standard, as it is a standard for validation that could be reliably reproduced across settings.

However, because cultures are difficult to obtain in resource limited settings and can lead to a delay in treatment, performing studies with culture as the gold standard may be difficult. In addition to using a variety of gold standards, the various studies often included very different sample populations. Some studies did not clearly describe the characteristics of the patient population or how they were selected. Some were retrospective, often utilizing record review (Alene et al., 2013; Muluye et al., 2013).

Prospective studies of diagnostic systems would evaluate a clearly defined sample of participants with a spectrum of disease representative of the study subjects to which the diagnostic systems would be applied in the study settings. It is essential that researchers should clearly describe the sampling process and inclusion criteria in such studies to allow for more accurate comparison of diagnosis systems across different populations or settings and to promote the utility of these systems.

QFTGIT and TST were also used as part of their diagnostic gold standard to differentiate latent TB from active TB disease (Legesse et al., 2010; Dagnew et al., 2012; Wassie et al., 2013). This makes it difficult to interpret the accuracy of a diagnostic system and its ability to predict a diagnosis of TB in a particular patient or patient population. This overlap also causes difficulty in determining the relative importance of particular signs or symptoms within the diagnostic system. The largest shift in the newer diagnostic systems as compared to smear microscopy is the focus on both pulmonary tuberculosis and extra pulmonary tuberculosis including latent tuberculosis. Diagnostic systems focusing simply on pulmonary TB, such as (Cambanis et al., 2006; Aderaye et al., 2007) have demonstrated higher sensitivities than those developed to diagnose both extra pulmonary and pulmonary TB. Because children have a higher incidence of extra pulmonary TB, using diagnostic systems targeted at pulmonary TB only addresses part of the diagnostic challenge.

The findings of this systematic review are limited by the study settings design and quality of the studies included. The lack of consistent and sometimes clearly defined inclusion criteria among the studies makes it difficult to compare sensitivity and specificity across the different diagnostic systems. Most of the various diagnostic systems have only been evaluated in specific geographic locations or single populations; few studies evaluate a particular diagnostic system in multiple geographic regions or patient populations. Fewer studies have compared the diagnostic tests with different specimens (Bronchoalveolar lavage, pre and post-bronchoscopy sputum for pulmonary tuberculosis) in the same population (Aderaye et al., 2007). There may be probably more TB cases in the surveyed communities or settings than reported in these studies. Furthermore, sputum samples may not be collected and examined in symptomatic individuals that may further aggravate the underestimation of the true TB prevalence.

With a better screening method and diagnostic facility, the prevalence could even be more than what was reported. However, one thing is very certain; the passive case detection approach currently implemented in Ethiopia leaves many (two-thirds of smear-positive TB cases) undetected and untreated TB cases in the community; favoring continuous spread of the disease through maintaining active TB transmission (Tadesse et al., 2011).

Conclusion

The results of this review suggest that there is a need for revising the diagnostic systems. Most of the laboratory tests employed was based on direct smear microscopy, which is insensitive and can only detect 36% of tuberculosis cases in Ethiopia. This may result in misdiagnosis of the disease and further transmission of the disease especially in children and populations with high HIV co-infection. Therefore, there is a need for laboratories to find a rapid and efficient method for TB diagnosis as a complement to the smear microscopy. The low case detection rates observed nationally can be improved by introducing enhanced case detection mechanisms and promoting favorable health seeking behaviors.

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Full Length Research Paper

Total and CD4+ T-lymphocyte count correlation in newly diagnosed HIV patients in resource-limited setting

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Few clinical settings in resource-limited countries perform CD4+ T-lymphocyte counts required as a baseline test for antiretroviral therapy. We investigated CD4 count in newly diagnosed HIV-infected patients attending our treatment centre and evaluated suitability of total lymphocyte count (TLC) as a surrogate marker for CD4+T-lymphocyte count required as a yardstick for initiating antiretroviral therapy. Usefulness of TLC as a surrogate marker for CD4+T-lymphocyte counts <200, ≤350 and <500 cells/µL for HIV-positive patients in our facility was evaluated by 180 pairs of TLC and CD4 counts from 180 newly diagnosed HIV-infected patients and results were compared by linear regression and Spearman’s correlation analytical tools. Approximately 72.8% of our patients were diagnosed late as revealed by CD4 count ≤350 cells/µL. An overall good correlation was noted between TLC and CD4+T-cell counts (r=0.65, slope=0.69), mean total lymphocyte count of 1.04 ± 0.81, 1.39 ± 1.06 and 1.57 ± 1.13 x 10⁹/L correspond to CD4 lymphocyte counts of <200, ≤350 and <500 cells/µL respectively. When considering initiating HAART for HIV-infected Nigerian clients, TLC can be considered as an inexpensive and easily accessible surrogate marker for predicting CD4+T-lymphocyte at two clinically important CD4 thresholds of CD4 count ≥350 cells/µL and <500 cells/µL.

Key words: CD4, total lymphocyte count, highly active antiretroviral therapy, enzyme immunoassay, HIV, flowcytometry.

INTRODUCTION

The CD4+ T lymphocyte count is the determination of the concentration of CD4+ T lymphocyte in the blood. The associated immune deficiency in human immunodeficiency virus (HIV) patients leading to infection
by opportunistic pathogen is ascribed to depletion of CD4 T-cells (Shearer, 1983). CD4 count can therefore be regarded as the accurate measurement of the robustness and functionality of the immune capability to protect the body against general infection. CD4+T lymphocyte cell depletion is one of the hallmarks of progression of HIV infection and a major indicator of the stage of the disease in HIV infected individuals (Hogg et al., 2001; Mellors et al., 1997; WHO, 2005). World Health Organization recommended that most treatment initiation decisions be guided by CD4 measurement and clinical staging (Balter, 2003; Hanson et al., 1995). Previous study has shown good correlation between CD4 count and development of various complications in HIV/AIDS (Stein et al., 1992). Patients with low CD4+ T lymphocyte cell count have been reported as long-time infected patients than those with higher CD4 count (Hammer, 1997). It is clear that late starters of highly active antiretroviral therapy with CD4 count <200 cells/µl have significantly poor response to therapy and a worse prognosis when compared with early starters with higher CD4+ T cell count (Cheissin et al., 2000; Ledergerber, 1999; Kilaru et al., 2004). The relative ease of CD4 cell monitoring also led to its advocacy in treatment guidelines for determining when to start, stop or change antiretroviral therapy and for deciding when to initiate prophylaxis for opportunistic infections (OIS) (Arthur and Sahan, 2006).

Retroviral disease has become a matter of relative chronicity in patients that have access to antiretroviral therapy and have benefitted greatly in marked reductions in morbidity and mortality. General treatment guidelines for the treatment of HIV-infected patients in many countries have adopted three approaches for the initiation of antiretroviral therapy. Early intervention in asymptomatic patients involves the commencement of antiretroviral therapy once the CD4 count is less than < 500 cells/µl. A less intensive approach is to recommend antiretroviral therapy when the CD4 count falls to 350 cells/µl.

In other countries where patients have limited financial resources, treatment decisions are typically delayed until the CD4 count becomes less than 200 cells/µl (Tanwater et al., 2001; BHIVA, 2000; Mellors, 1997). Recent study by the French researchers at the 7th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention in Kuala Lumpur, Malaysia (Hocqueloux et al., 2013) showed that people with HIV who start treatment with CD4 counts above 500, after the first phase of primary infection is over, are much more likely to experience substantial reductions in the reservoir of HIV-infected cells in their bodies, making them strong candidates for future research studies that seek to control HIV without medication. The French group found that people with HIV who started treatment with a CD4 cell count above 500 were 56 times more likely to experience a normalization of immune function and a reduction in HIV DNA to low levels when compared to people who started treatment at lower CD4 counts. Although it is well established that people who start HIV treatment with a CD4 count above 500 stand a better chance of achieving a CD4 cell count in the normal range (defined as 900 to 1000 cells/mm³ according to the study), studies of people treated in chronic infection have not found evidence of a substantial reduction in HIV DNA (the reservoir of HIV within cells) over time. This outcome gives strong support to earlier interventional and showed the likely future direction in HIV treatment modalities. It then becomes very vital at every health institution involved in HIV/AIDS testing and treatment to establish the usefulness of total lymphocyte count as an alternative and inexpensive immunological marker for CD4 count where flowcytometric technique is not available. This is imperative not only at CD4 count threshold of < 200 and ≤ 350 cells/µl only but also at < 500 and ≥ 500 cells/µl thresholds.

Total lymphocyte count (TLC) is a derived immunological marker calculated from white blood cell count and relative lymphocyte count. For instance, if a patient has a total white blood cell count of 6.0 × 10⁹/L and relative lymphocyte count of 40% obtained from differential leukocyte count, total lymphocyte count of such patient would be 2.4 × 10⁹/L. There are series of controversial research outcomes over the use of TLC as a surrogate marker for CD4 count estimation (Anastos, 2004; Beck et al., 1996; Gitura et al., 2007; Akinola, 2005; Desesse and Eskendir, 2008; Chigu et al., 2006; Nyawira, 2011). Decisions became difficult to take as our health care providers insist on scientific evidence on the use of total lymphocyte count as an inexpensive point-of-care alternative for absolute CD4 count. Moreover, there are limited data on the use of TLC as surrogate marker for absolute CD4+T-lymphocyte count at a CD4 threshold of < 500 cells/µl especially in a facility located in a rural and resource-limited setting in Nigeria.

Recently published PEPFAR II goals stated that an estimate of 100,000 children needed to be newly initiated on highly active antiretroviral therapy before 2013 (PEPFAR, 2011). In line with this, new sites have been established throughout Nigerian states including Ekiti.
Five new sites are receiving services from our treatment centre across Ekiti state as a comprehensive HIV treatment centre owing to lack of CD4 cyflow counter. Most of those sites across the country do not have cyflow counter to perform routine CD4 count analysis, and flowcytometry remains the reference method for the performance of CD4 count (Mellors et al., 1997). Even in centers where CD4 equipment are available, treatment decisions are sometimes delayed owing to varying factors ranging from proximity of the patients to testing and treatment sites, equipment breakdown, non-availability of reagents on consistent basis to late access to service engineers.

These therefore necessitate the need for the evaluation and validation of the correlation of TLC with CD4 count and its usefulness as a surrogate marker for CD4 count estimation. This is the first time it will be determined in this health institution in Ekiti state, South-West Nigeria. To the best of our knowledge, no research study in Ekiti state has determined the correlation between CD4 and total lymphocyte count to include CD4 count threshold of < 500 cells/µL but past studies have concentrated on correlations between absolute CD4 count and TLC at < 200 and < 350 cells/µL thresholds.

This study was carried out to determine at what stage of the disease HIV infected persons presented to our treatment facility, and to have comparison with our previous findings here and in other centres. Moreover, the total lymphocyte count as at the time of presentation will also determined and correlated with the CD4 count at different CD4 thresholds including < 500 cells/µL to establish its diagnostic utility as a surrogate marker for CD4 count.

The goal is to facilitate earlier treatment in newly diagnosed HIV patients based on total lymphocyte count results obtained from HIV positive samples at attached centres rather than travelling, in some cases, over 50 km distance to have CD4 count result done and asking the physicians to wait for results from testing sites. Delay associated with such old arrangement has been responsible for delay in initiation of highly active antiretroviral therapy, loss of follow-up, non-compliance to antiretroviral therapy, poor prognosis, immunologic failure and increased morbidity and mortality among HIV-infected patients.

METHODS

Study location

This study was carried out at the Haematology department of the Federal Medical Centre (FMC); Ido Ekiti, Nigeria over a period of three years. FMC is located in Ido Ekiti, the principal town in Ido Osi Local Government Area of Ekiti State with an estimated population of 107,000. It is geographically located in the northern part of Ekiti State which covers an estimated total area of 6353 km², 2,453 square mile and an estimated population of 2,737,186, where the routes from Kwara and Osun states converge. FMC, Ido Ekiti was upgraded in 2006 to serve as a centre for HIV/AIDS referral, diagnosis and treatment in Ekiti State and serving five contiguous states. The Centre has since that time been offering free diagnosis and antiretroviral therapy.

Study design

This study consisted of enumeration of total and CD4 T-helper lymphocyte counts of 180 HIV-infected subjects at baseline before initiation of highly active antiretroviral therapy (HAART). A model linear regression analysis was applied to the data and the sensitivity and specificity of the World Health Organization recommended TLC thresholds corresponding to CD4 count <200, ≤350 and <500 cells/µL were determined.

HIV counseling and testing

Early and accurate diagnosis of human immunodeficiency virus (HIV) in retroviral patients is sine qua non to successful management, good prognosis, effective follow-up and increased reduction of morbidity and mortality due to opportunistic infections among infected patients. All enrolled subjects were newly diagnosed for HIV at our HIV counseling and testing (HCT) site and PEPFAR-Supported laboratory at the Haematology Department according to the Centre for Disease Control and Prevention serial Algorithm II guidelines. We performed HIV testing using two rapid enzyme immunoassay (EIA) techniques. Whole blood samples obtained by capillary puncture or plasma samples separated from 4 millilitres of whole blood collected into K2EDTA spray-dried collection tubes were used for the procedures and the tests were performed according to CDC-UMD HIV rapid testing serial algorithm II guideline. We first used Determine kit, an immunochromatographic technique (Abbott Laboratories, Abbot Park, USA). Negative result by Determine ended testing but research patients were requested to repeat test 1 month to confirm negative results. Positive results observed were repeated with Unigold Kit (Trinity Biotech, Wicklow, Ireland).

Discordant results were first repeated by senior research counsellor and tester to ascertain true inconclusive results and finally tested with the tie-breaker kit-Stat-Pak (Chembio Medford, NY, USA), an immunocentrification technique. Final test results were considered positive/negative on the basis of the tie-breaker result and corresponding similar result from one of previous test procedures (WHO, 2007). Similar results were obtained from Genscreen HIV 1&2 kits (Biorad, France). The rapid HIV screening technique was an enzyme immunoassay based on the detection of antibodies to HIV in the patients’ sera or plasma. The Genscreen ULTRA HIV Ag-Ab is an enzyme immunoassay based on the principle of sandwich technique for the detection of HIV antigen and of the various antibodies associated with HIV-1 and/or HIV-2 virus in human serum or plasma. Diagnostic techniques/algorithm was quality controlled using one worldAccuracy HIV samples with already known positive and negative HIV results. Patients with two concurrent positive HIV results and who were repeatedly reactive were enrolled in the study.

Blood sample requirements, CD4 count and complete blood count analyses

Patients already confirmed HIV positive had their blood samples taken (following informed consent as part of the ethical guideline)
**Correlation is significant at p<0.004.

| **Table 1.** The Overall data showing the Mean age, CD4 and TLC, the correlation coefficient, slope and p-value of HIV infected patients |
|---|---|---|---|---|---|
| Age | CD4 count (Cells/µL) | Total lymphocyte count (x 10⁹/L) | r | Slope | p-value |
| 37.89±10.47 | 250.81±226.59 | 1.79±1.43 | 0.65** | 0.69** | 0.01 |

**Table 2.** Correlation of CD4 T-lymphocytes and total lymphocytes count of HIV-infected patients

<table>
<thead>
<tr>
<th>CD4 Count Thresholds (Cells/µL)</th>
<th>CD4 Count (Cells/µL)</th>
<th>Total lymphocyte count (x 10⁹/L)</th>
<th>r</th>
<th>Slope</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>82.96±57.03</td>
<td>1.04±0.81</td>
<td>0.30</td>
<td>0.46</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>≤350</td>
<td>140.23±104.05</td>
<td>1.39±1.06</td>
<td>0.55**</td>
<td>0.64</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>&lt;500</td>
<td>188.22±144.80</td>
<td>1.57±1.13</td>
<td>0.58**</td>
<td>0.68</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>≥500</td>
<td>687.86±224.34</td>
<td>3.29±2.27</td>
<td>0.58*</td>
<td>-0.013</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**Principle of flow cytometry for cd4 count**

The flow cytometry test operation is based on the simultaneous measurement of multiple physical characteristics of CD4 T-cells in a single file as it flows through the flow cytomer. The counter separated the CD4+ T cell from the monocytes-CD4 bearing cells and noise using a gating system. We prepared the samples and analyzed CD4 count according to the manufacturer’s instructions.

**CBC analysis and TLC calculation.**

The CBC samples were run directly on Sysmex KX-21N three-part differential haematology analyzer (Sysmex Corporation, Kobe, Japan). TLC was calculated from white blood cell count and relative lymphocyte count. Sysmex KX-21N is a three-part open tube differential haematology analyzer according to manufacturer’s instructions.

**Statistical analysis**

Data were computed using SPSS statistical software (Statistical Package for Social Sciences Inc, Chicago IL), version 17. The summary of the continuous variables were presented as means and standard deviation using one sample t-test. Correlation data obtained with the surrogate marker (total lymphocyte count) were compared to the reference flow cytometric CD4 count and the correlation coefficient was calculated. The degree of correlation between the reference CD4 count and total lymphocyte count was further established using bivariate Spearman’s analytical tool. We also determined the proportion of results classified by the total lymphocyte counts at various CD4 T-cell count thresholds relevant for clinical management of HIV-infected patients (<200, ≤350 and <500 cells/µL). A p-value =0.05 was considered significant for all statistical comparison.

**RESULTS**

A total of 180 HIV research patients comprising 54 males and 126 females aged 20-80 years, mean age 37.89±10.47 years were involved in the study. 89 of the patients who are approximately 49.4% were asymptomatic HIV-infected patients. The overall mean baseline CD4 count and total lymphocyte count were 250.81±226.59 and 1.79±1.43 x 10⁹/L, respectively as shown in Table 1. Of the 180 research patients, 51.67, 72.78 and 87.22% had CD4 count values of <200, ≤350 and <500, respectively. The remaining 12.78% had CD4 count of ≥500 cells/µL. The overall correlation coefficient between CD4 and total lymphocyte count of HIV-infected subjects was modest (r=0.65**, p< 0.01). Table 2 showed the comparison of correlation between CD4 and total lymphocyte counts based on CD4 T-lymphocyte count thresholds (<200, ≤350, <500 and >500 cells/µL) relevant to clinical management of HIV-infected Nigerians.

The correlation between total lymphocyte count and CD4 count was modest at a CD4 count threshold of ≤350 cells/µL (r=0.59**, p< 0.01) compared to a low correlation at a CD4 threshold of < 200cells/µL (0.30, p<0.05) and slightly higher correlation at a CD4 threshold of <500 and ≥500 cells/µL (r=0.58, p<0.01). The slope was further determined to establish the degree of association between total lymphocyte count and CD4...
count thresholds. The slopes for CD4 count thresholds <200, ≤350, <500 cells/µL were 0.46, 0.64, 0.68, respectively at p<0.05. CD4 count threshold of >500 cells/µL showed negative association at p> 0.05, p=0.95 specifically. Total lymphocyte count of 1.04 ± 0.81, 1.39 ± 1.06 and 1.57 ± 1.13 × 10³/L correspond to CD4 lymphocyte counts of <200, ≤350 and <500 cells/µL, respectively.

**DISCUSSION**

With the current trend in the clinical management of retroviral patients, the use of highly active antiretroviral therapy (HAART) has brought a panoramic change of positive impact in the treatment of HIV-infected patients. In fact, HIV patients live relatively normal lives as non-infected subjects when diagnosed early and aggressive interventions are adopted. However, in many resource-poor countries, most facilities do not have cyflow counter for the performance of CD4 count analysis required for the initiation of antiretroviral therapy. Amazingly, in Sub-Saharan Africa where nearly 24 million people are living with HIV, less than 8% have access to HAART partly due to lack of access to CD4 count test. (WHO, 2002). In this study, we have evaluated the relevance of TLC as a readily accessible and alternative immunological marker for CD4 count in determining the optimal time to initiate highly active antiretroviral therapy in HIV-infected Nigerians especially those presenting late to testing and treatment centres.

More than 50% (51.7%) of patients enrolled in this study had CD4 count <200 cells/µL and 57 (61.3%) of these had CD4 count less than 100cells/µL at presentation. 72.8% had CD4 count ≤350cells/µL. The World Health Organization (Nyawira et al., 2011) recommended the following parameters for initiating antiretroviral therapy:

1. Initiate ART for all patients with CD4 Count <200 cells/µL regardless of clinical stage.
2. Consider ART for all patients with stage I and II disease and CD4 count ≤350 cells/µL. Treatment should be initiated before CD4 count drops below 200 cells/µL.
3. Initiate ART for patients with stage III disease and CD4 count ≤350 cells/µL.
4. Initiate ART for patients with stage IV disease regardless of CD4 count.

We inferred that there was late diagnosis of HIV in the patients attending our treatment centre which is similar to findings by Kilaru and Co-workers (1997) in Barbados and Guarner and his group (1996) in Mexico. It is interesting to note that this study showed 13.4% cut in the overall previous findings of 86.2% among enrolled patients having a relatively advanced disease as reflected by their CD4 + cell count of <350 cells/µL in 2009. Of relevant interest is also the fact that while 65.5% of the study population in 2009 had CD4+ T-lymphocyte cell count of <200 cells/µL, the present study showed that 61.3% had CD4+ cell count of <200 cells/µL, representing a concomitant cut of 4.2% (Ajayi et al., 2009). The observed difference between the present outcomes and Ajayi and his group’s findings at this health care facility was due to improved clinical setting (multiple testing sites) approach, trained counselors and testers, more aggressive ‘Know your HIV status’ campaign and policy formulations on reduction of discrimination and stigmatization. The World Health Organization recommended that in treatment centres where there is no facility for performing absolute CD4 count test, clinicians need not wait until CD4 counters are available but total lymphocyte counts of <1200 and <1,500 µL corresponding to CD4 count of <200 and <350 cells/µL should be used as a surrogate marker. In this research study, we observed a modest overall correlation coefficient of r= 0.65 (p < 0.01) between paired CD4 and total lymphocyte counts of 180 HIV-infected Nigerians in Ekiti, South West, Nigeria. This value correlates well with that of Beck and his colleagues (1996) (r=0.64) obtained from a study involving 1535 asymptomatic HIV-infected patients in the United Kingdom. While it is slightly lower than that obtained from a South African study in a larger population (n=2774) of HIV-infected patients (r=0.70) (van Der Ryst et al., 1998) it is higher than that obtained from similar studies involving 100 and 32 HIV-infected Nigerians with correlation coefficients of r=0.51 and 0.25 respectively (Akanmu et al., 2001; Erhabor et al., 2006). Our results and those of previous studies showed that the higher the population of asymptomatic patients involved in the study, and early antiretroviral therapy intervention decision adopted, the better the correlation.

Comparison of correlations between CD4 and total lymphocyte count at different CD4 count thresholds showed a correlation coefficient of r= 0.30, slope 0.46; 0.55,**slope 0.64 and 0.58,**slope 0.68 for CD4 count <200, ≤350 and <500 cells/µL respectively (p < 0.05).

Mean total lymphocyte counts of 1.04 ± 0.81, 1.39 ± 1.06 and 1.57 ± 1.13 × 10³/L correspond to CD4 lymphocyte counts of <200, ≤350 and <500 cells/µL respectively. TLC is more positively correlated with CD4 lymphocyte counts of <200, ≤350 and ≤500 cells/µL than <200 threshold. We inferred that TLC does not accurately predict corresponding CD4 count values in clients being severely immuno-suppressed as at presentation to the HIV clinic.

There was a negative correlation between TLC and CD4 count ≥ 500 (r=0.68, slope= -0.013, p=0.95). The
cause of this negative slope was unclear but we assume that a lower population of patients (15%) with CD4 count of ≥ 500 cells involved in the study are responsible. If a study on evaluation of TLC as a suitable substitute for CD4 count at a CD4 count threshold of ≥500 cells/µL involves a higher population of CD4 count-TLC pair, a better picture may be obtained.

Conclusion

When considering initiating highly active antiretroviral therapy in HIV-infected patients, our findings of overall modest correlation of $r=0.65$ and slope=0.69 (p=0.004) between total and CD4+ T-lymphocyte count has shown that total lymphocyte count can be used as an alternative inexpensive and readily available surrogate marker at two clinically significant CD4 thresholds of ≤ 350 and < 500 cells/µL. It must be used in conjunction with the clinical status and WHO clinical staging system in determining the prognosis and the optimal time to initiate HAART in resource-poor settings without CD4 count facility. The findings of a cut of 13.4% in the overall findings of our patients presenting with relatively advanced disease as at diagnosis (CD4 count <$350 cells/µL) in 2009 and concomitant cut of 4.2% (CD4 < 200 cells/µL) showed a working system. A more aggressive campaign and public enlightenment on, ‘Know your HIV status’ in Ekiti communities and neighbouring states where patients come to our treatment facility to access care, evidently, will further lead to dramatic reduction in late diagnosis of HIV, hence, improve TLC diagnostic utility as a surrogate marker for CD4+ cell count.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Related Journals Published by Academic Journals

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- Journal of Veterinary Medicine and Animal Health
- Research in Pharmaceutical Biotechnology
- Journal of Physiology and Pathophysiology
- Journal of Infectious Diseases and Immunity
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