Original Research Article

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Comparative Study of Standard Laboratory Test with Commercially Available Assay (XCYTON) for Diagnosis of Meningitis: A Cross- Sectional Study

Shrirang Bamne[1], Rajesh Mohan Shetty[2], Sunil Karanth[3], Gopal Krishnan[4]

Abstract

Background and Aim: Meningitis is an inflammatory disease of the leptomeninges, the tissues surrounding the brain and spinal cord, and is defined by an abnormal number of white blood cells in the cerebrospinal fluid. Present study was performed with an aim to compare Standard Laboratory Test with Commercially Available Assay (Xcyton) for Diagnosis of Meningitis. *Material and Methods:* A cross sectional study of 101 patients admitted to Multidisciplinary Intensive Care Unit(MICU) at a tertiary care teaching hospital between January 2011 to December 2013 were included this study. Patients with suspected neuroinfection based on the history and clinical examination were subjected to lumbar puncture for CSF analysis after Computed tomography (CT scan) of brain. CSF was also collected in EDTA vacutainer (for XCyton) and extra sample was reserved for any further analysis. *Results:* Xcyton when compared to standard laboratory test had sensitivity and specificity of 76% and 71% while the positive predictive value (PPV) and negative predictive value(NPV) was 30% and 95%. Sensitivity and specificity was 20% and 50% when Xcyton was compared with diagnosis based on clinical and radiological criteria. Cryptococcal meningitis had 50% sensitivity, 100% specificity, 100% PPV and 98% NPV when XCyton and standard laboratory was compare. *Conclusion:* Standard laboratory tests may miss many cases of neuroinfection. Also Xcyton assessment alone may not be sufficient in evaluation of patients with suspected neuroinfection.

Keywords: Meningitis, Syndrome Evaluation System, XCyton.

Introduction

Meningitis is an inflammatory disease of the leptomeninges, the tissues surrounding the brain and spinal cord, and is defined by an abnormal number of white blood cells in the cerebrospinal fluid (CSF). Approximately 1.2 million cases of bacterial meningitis occur annually worldwide [1]. Meningitis is among the 10 most common infectious causes of death and is responsible for approximately 135,000 deaths throughout the world each year. Neurologic sequelae are common among survivors. It is a life threatening medical emergency, immediate steps must be taken to establish the cause and initiate effective and early treatment. The mortality rate of untreated disease approaches 100 percent, and, even with optimal therapy, there is a high failure rate.

The possibility of meningitis is suggested by the symptoms of fever, altered mental status, headache, and nuchal rigidity. Although one or more of these findings are absent in many patients with meningitis, all patients (99 to 100 percent) have at least one of the classic triad of fever, neck stiffness, and altered mental status [2]. The initial approach to management in a patient with suspected meningitis includes performing a lumbar puncture (LP) to determine whether the CSF findings are consistent with the diagnosis [3].

Computed tomography (CT) head should be performed before LP in adults with suspected meningitis who have one or more of the risk factors like immunocompromised state (eg., HIV infection, immunosuppressive therapy, solid organ or hematopoietic cell transplantation), history of central

Author's Affiliation: [1]Senior Resident, Department of Critical Care Medicine, Tata Memorial Hospital, Mumbai, Maharashtra 400012, India. [2]Head of ICU Manipal Hospital, Whitefield, Bengaluru, Karnataka 560066, India. [3]Head of ICU Manipal Hospital, Old Airport Road, Bengaluru, Karnataka 560017, India. [4]ICU Registrar Manipal Hospital, Bengaluru, Karnataka 560017, India,

Corresponding Author: Shrirang Bamne, Senior Resident, Department of Critical Care Medicine, Tata Memorial Hospital, Mumbai, Maharashtra 400012, India.
E-mail: researchguide86@gmail.com

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nervous system (CNS) disease (mass lesion, stroke, or focal infection), new-onset seizure (within one week of presentation), papilledema, abnormal level of consciousness or focal neurologic deficit [3-5].

However, the spectrum of CSF values in meningitis is so wide that the presence of typical CSF findings is of little value [6]. In a series of 696 episodes of community-acquired bacterial meningitis, 12 percent had none of the characteristic CSF findings [7]. Blood cultures are often positive and can be useful in the event that cerebrospinal fluid cannot be obtained before the administration of antimicrobials. Approximately 50 to 90 percent of patients with bacterial meningitis have positive blood cultures [8].

Molecular methods of diagnosis like nucleic acid amplification (NAA) methods (eg, polymerase chain reaction [PCR]) are particularly well suited for the diagnosis of CNS infections because CSF and spinal and brain tissue are normally sterile body sites, where any evidence of a microorganism is likely to represent infection, and infections, when present, are typically monomicrobial. Furthermore, CSF typically lacks common inhibitors of nucleic acid amplification methods such as heme, endonucleases, and exonucleases that can lead to false-negative results [9-12].

Targeted nucleic acid detection methods are often more sensitive than conventional culture-based or antigen detection methods and may detect organisms that are nonviable or uncultivable. However, except for herpes simplex virus (HSV) and JC (John Cunningham) virus, the true clinical sensitivity of most molecular tests for CNS infections is not known because there are few studies utilizing a reference standard (eg, brain biopsy) for comparison.

Multiplex or panel-based NAAs combine multiple individual NAAs into a single test, thereby allowing clinicians to test for an array of potential pathogens that may cause a clinical syndrome at the same time. In 2015, the first commercial multiplex NAA for infectious causes of community-acquired meningitis and encephalitis was cleared by the US Food and Drug Administration (FDA) for use as an aid in the diagnosis of these conditions [13].

Syndrome Evaluation System(SES) by XCyton (XCyton Diagnostics Ltd., Bangalore, India) is a one such multiplex NATs that provides diagnosis by rapid identification of causative organism [14-17].

In our hospital it is routine practice to undertake CSF analysis for HSV PCR, gram stain and culture (standard laboratory tests) in patients with suspected meningoencephalitis to increase the diagnostic yield. Many patients will have SES by Xcyton analysis of CSF as well. This gave us an opportunity to compare both the techniques. As there is no clear cut evidence that XCyton analysis is as good as the standard laboratory tests, we decided to undertake this study to find out if there is any benefit in doing Xcyton study either on its own or in addition to the Standard laboratory method [18-21].

Our study hypothesis was that XCyton helped to complement the Standard Laboratory tests and hence help in better diagnosis of neuroinfections. Therefore we retrospectively compared the CSF results of XCyton to standard laboratory tests in series of patients suspected to have neuroinfection at our centre. We also planned to correlate these two investigations with clinical and radiological features.

Materials and Methods

A cross sectional study of 101 patients admitted to Multidisciplinary Intensive Care Unit (MICU) at a tertiary care teaching hospital between January 2011 to December 2013 were included this study. Patients with suspected neuroinfection based on the history and clinical examination were subjected to lumbar puncture for CSF analysis after Computed tomography (CT scan) of brain. CSF was also collected in EDTA vacutainer (for XCyton) and extra sample was reserved for any further analysis. If the initial CSF report (cell count, cell type, CSF protein and CSF sugar) was suggestive of neuroinfection the sample for XCyton was sent and along with the standard laboratory tests. The results of both the CSF analysis were compared with each other. They were also compared with clinical and radiological findings of the patients.

The criteria for clinical diagnosis was by the presence of at-least two of the four symptoms (i.e, headache, fever, stiff neck and altered mental status). This is based on a study which showed two of the four of these symptoms were present in 95% of patients with meningitis [22]. The patient's past history of tuberculosis, history of retroviral infection or long term immunosuppression were also taken in to consideration for correlation with possible specific pathogen.

Neuroimaging plays an important role in the diagnosis and therapeutic management of infections involving the central nervous system. Cranial computed tomography (CT) helps to rule out acute brain edema, hydrocephalus and pathology of the skull base. Magnetic resonance imaging (MRI) is superior in depicting complications like sub/epidural empyema, vasculitic complications especially on

FLAIR (fluid attenuated inversion recovery)- weighted images. Diffusion weighted imaging (DWI) helps in early detection of parenchymal complications of meningitis and differentiation of pyogenic meningitis from other causes of ring enhancing lesions [23].

A recent expert consensus case definition (CCD) for use in research incorporates the following radiological signs in the diagnosis of TBM: hydrocephalus, infarcts, tuberculoma (s), basal meningeal enhancement, and the presence of pre-contrast basal hyperdensities [25].

Statistical Analysis

The data was coded and entered into Microsoft Excel spreadsheet. Analysis was done using SPSS version 15 (SPSS Inc. Chicago, IL, USA) Windows software program. The variables were assessed for normality using the Kolmogorov-Smirnov test. Descriptive statistics were calculated.

Results

Among 101 patients who were suspected to have neuroinfection, 95 had CSF pleocytosis and hence suggested neuroinfection, the remaining 6 patients were excluded as they had alternate diagnosis like obstructive sleep apnea, etc. The standard laboratory test was positive in 13 patients compared to 33 patients with XCyton.

Xcyton when compared to standard laboratory test had sensitivity and specificity of 76% and 71% while the positive predictive value (PPV) and negative predictive value (NPV) was 30% and 95% (Table 1). Sensitivity and specificity was 20% and 50% when Xcyton was compared with diagnosis based on clinical and radiological criteria. Here PPV and NPV was 23% and 57% respectively (Table 2). When Standard Laboratory test was compared to clinical and radiological criteria the sensitivity and specificity was 30% and 62% while the PPV and NPV was 11% and 85%. When Xcyton was compared with clinical and radiological criteria the sensitivity and specificity

Table 1: Overall comparison of standard laboratory to XCyton result

Xyton	Lab		Total
	Positive	Negative	
Positive	10	23	33
Negative	3	59	62
Total	13	82	95

Table 2: Overall sensitivity and specificity of XCyton compared to Standard Laboratory method

Statistic	Value	95% CI
Sensitivity	76.92%	46.19% to 94.96%
Specificity	71.95%	60.94% to 81.32%
Positive Predictive Value	30.30%	21.59% to 40.71%
Negative Predictive Value	95.16%	87.84% to 98.17%
Accuracy	72.63%	62.52% to 81.28%

Statistic	Value	95% CI
Sensitivity	75.00%	42.81% to 94.51%
Specificity	75.28%	65.00% to 83.81%
Positive Predictive Value	29.03%	20.07% to 39.99%
Negative Predictive Value	95.71%	89.27% to 98.36%

Table 3: Comparison of clinical, radiological and XCyton (individual organisims) with standard laboratory test

	Standard laboratory test		Sensitivity	Specificity
	Positive	Negative		
XCYTON TB Positive	4	2	66.6%	98.8%
Negative	1	88		
XCYTON HSV Positive	2	3	100%	96.7%
Negative	0	90		
XCYTON CRYPTO Positive	1	0	50%	100%
Negative	1	93		
XCYTON Bacterial Positive	0	13		
Negative	0	82		
XCYTON others Positive	3	5	75%	94.5%
Negative	1	86		
Clinically Positive	13	82		
Negative	0	0		
Radiologically Positive	4	31	30.7%	62.2%
Negative	9	51		

was 30% and 58%, while the PPV and NPV was 27% and 61% (Table 2). When Xcyton was correlated with clinical and radiological criteria authors conclude that clinicians were able to identify 12 extra cases which would have been missed if only standard laboratory tests were undertaken. At the same time, three cases were missed by XCyton and was diagnosed by our standard laboratory test. Six patients had false positive results with Xcyton when correlated clinically and radiologically. All these six patients showed multiple pathogens by Xcyton.

With respect to individual pathogens CNS tuberculosis XCyton test had diagnostic sensitivity of 80%, specificity of 97%, PPV of 66% and NPV of 98% compared to standard laboratory method. XCyton was 100% sensitive, 96% specific, PPV was 40% and NPV was 100% with respect to Herpes simplex virus (HSV) detection. Cryptococcal meningitis had 50% sensitivity, 100% specificity, 100% PPV and 98% NPV when XCyton and standard laboratory was compare (Table 3).

Discussion

Meningoencephalitis is a medical emergency requiring immediate identification and initiation of treatment to avoid mortality and morbidity. Clinical signs and symptoms are quite sensitive in identification of these infections and helps in prompt treatment initiation. A CSF analysis is helpful in confirming the diagnosis of neuroinfection and isolates the microorganism.

Neuroimaging helps in early detection of complications like hydrocephalus, infarcts, abscess, etc. It also helps to identify specific causative pathogens by the characteristic features. Examples include temporal lobe hyperintensivty in cases of HSV meningitis. Neurocysticercosis shows hypointense lesions on DWI.

Use of molecular methods for the detection of enteroviruses and Herpesviridae (eg, HSV, varicella-zoster virus [VZV], cytomegalovirus [CMV], and Epstein-Barr virus [EBV]) is standard of care when central nervous system (CNS) infection due to these viruses is suspected. Nucleic acid amplification tests (NATs) detect deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) specific to infectious organisms (eg, bacteria, viruses) as a means of diagnosis.

NAT which detects most common causes of community-acquired (including neonatal) bacterial meningitis, may be useful as an adjunct to culture, especially in patients who have already received antibiotic treatment it may also be useful in select cases when uncultivable or fastidious organisms are suspected (eg, Mycoplasma spp, Brucella spp, or Tropheryma whipplei).

In our study Xcyton has high sensitivity and low specificity with low PPV and high NPV. This is in contrast to the earlier published literature. In one study of multiplex PCR assay for detection of N. meningitidis, S. pneumoniae, and H. influenzae type b, overall specificity and positive predictive value was 100 percent; the negative predictive value was >99 percent [9]. The sensitivity and specificity of CSF PCR for the diagnosis of pneumococcal meningitis was 92 to 100 percent and 100 percent, respectively in another study [10].

In our study Xcyton had high specificity but moderate sensitivity in CSF for detection of tuberculosis. This is similar to other published data [14-16].

Our study showed that 12 additional cases were identified with Xcyton which would have been missed if only standard laboratory test were carried out. This means that standard laboratory tests on their own may not be sufficient. At the same time Xcyton analysis missed three cases. Xcyton is a very expensive test and undertaking this in every patient may not be cost-effective. We have not assessed cost effectiveness in this study, but it can be said that Xcyton assessment be considered in selected cases. One way of doing this is performing standard laboratory tests initially and if this does not correlate clinically then to perform Xcyton analysis. Xcyton has a very high incidence of false positive results (20%). It is worth noting that in our study all cases of false positive results with Xcyton showed multiple organisms which means these results are spurious caused by contamination.

The limitations of our study include its retrospective nature and small sample size. Also it included heterogenous group of patients.

Conclusion

Though commercially available test like XCyton are promoted as rapid diagnostic tests with high sensitivity and specificity. In our study sensitivity and specificity was 76% and 71% with PPV of 30% and NPV 95%. XCyton was able to diagnose twelve additional patients compared to standard laboratory test. There was a risk of missing three cases if Xcyton assessment alone was used and there was also high

incidence of false positive results with multiple pathogens being detected. Based on our study we conclude that standard laboratory tests may miss many cases of neuroinfection. Also Xcyton assessment alone may not be sufficient in evaluation of patients with suspected neuroinfection. Prospective studies may help to elucidate if XCyton analysis is beneficial in those cases where standard laboratory tests are negative but clinical evaluation otherwise suggests possible neuroinfection. Further refinement in these tests may negate the need to undertake multiple tests in the future.

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