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Biological diagnosis of meningococcal meningitis in the African meningitis belt: Current epidemic strategy and new perspectives

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Abstract

Laboratory diagnosis is an essential component in surveillance of meningococcal epidemics, as it can inform decision-makers of the *Neisseria meningitidis* serogroup(s) involved and the most appropriate vaccine to be selected for mass vaccination. However, countries most affected face real limitations in laboratory diagnostics, due to lack of resources. We describe current diagnostic tools and examine their cost-effectiveness for use in an epidemic context. The conclusion is that current WHO recommendations to use only the latex agglutination assay (Pastorex) at epidemic onset is cost-effective, but recently developed rapid diagnostic tests for the major epidemic-causing meningococcal serogroups may prove a breakthrough for the future.

Keywords: Meningococcal meningitis; Diagnosis; Africa

1. Introduction

Meningococcal epidemics occur worldwide but more than 50% of cases are reported from sub-Saharan Africa's "meningitis belt" [1,2]. Large epidemics are attributed predominantly to *Neisseria meningitidis* (Nm) serogroup A [3,4]. However serogroups C and X meningococci have been isolated during some epidemics [5–7] and, more of concern, serogroup W135 (NmW135) was identified in 2002–2003 as the main pathogen during outbreaks in Burkina Faso [8]. This serogroup is responsible for sporadic cases in many countries [9]. With the possibility of epidemics like those in Burkina Faso, and in the context of the limited availability of polysaccharide vaccines which include NmW135, decision-makers at country level in the meningitis belt need the best epidemiological and laboratory evidence in order to make the most appropriate vaccine choice. A diagnostic tool, either a single test or a test combination, which both would identify the serogroup implicated and would be rapid, cheap and simple enough to be performed in local health structures with limited resources, could result in earlier outbreak identification and better preparation for mass vaccination.

Such a tool would also prove vital at patient level, as early and correct antibacterial therapy is essential for a good outcome. The length of time needed for laboratory results to reach peripheral health structures not equipped with either laboratory or technical staff precludes the immediate benefit of such results for the individual patient. Thus, after initial declaration of a meningococcal epidemic in countries in the meningitis belt, treatment at peripheral level is often based on clinical diagnosis. Although signs and symptoms of non-meningococcal disease are indistinguishable from meningococcal disease, the treatment may not be the same, and this could have serious consequences for the patient.

Culture and PCR are both currently considered to be standard diagnostic methods. However, due to numerous obstacles that will not be solved in the near future in the countries of the meningitis belt (including cost, a need for trained staff and sophisticated equipment), these reference

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tests are rarely available, except in some capital cities or other large urban centres. Recent evaluations of the latex agglutination kit (Pastorex®, Biorad, Marne la Coquette, France), conducted in Niger and Burkina Faso under reference laboratory conditions [10], and in a Niger district laboratory in an epidemic context [11], have given promising results.

Here we provide a brief overview of current meningococcal diagnostics available at both national and district levels and provide new perspectives in light of a recently validated rapid diagnostic test.

2. Non-culture methods: latex agglutination test (LAT), Gram stain and white blood cell count (WBC)

2.1. The LAT

LATs for serogroup-specific polysaccharide (PS) have been in use for more than two decades. Although they provide more rapid results than culture or PCR, and can give positive results even after a few days of treatment, while culture or PCR cannot, much controversy has arisen over their proper use and variable performance [12–14]. More recently, the changing epidemiology of meningococcal disease compelled recommendation of kits capable of identifying meningococcal serogroup W135. The Pastorex® test (Biorad) can detect soluble meningococcal PS for NmA, B, C and W135/Y (i.e. it does not differentiate between NmW135 and NmY) in the cerebrospinal fluid (CSF) of the patient. The sensitivity and specificity of the test for the diagnosis of NmA and NmW135, evaluated both under reference laboratory conditions [10] and in a district laboratory in Niger [11] ranged from 84.9 to 88.0% and from 93.0 to 97.4%, respectively. The Pastorex® test, used at district level under laboratory conditions, has acceptable accuracy, and ideally could be used to test all CSF samples taken, regardless of the season. However, its cost is high and once the kit has been opened, its reagents have a limited shelf-life. In addition, each kit provides enough reagents for 20–25 tests; thus having this kit pre-positioned in every district-level laboratory for sporadic cases outside of the meningitis season or even for routine outbreak surveillance is too costly for most countries inside the belt. A more cost-effective option is to pre-position LAT kits at regional or central level so that they can be subsequently sent to the district laboratory in case of a suspected outbreak for preliminary identification of the responsible serogroup(s).

2.2. Gram stain

This method can be highly specific for indicating absence of epidemic-causing meningococci, if performed by a well-trained technician, when a clinical case definition is stringently applied and disease prevalence is very high, such as during an epidemic. If CSF samples can reach the laboratory quickly enough, presence of intracellular Gram-negative diplococci can only indicate epidemic-causing meningococcus. However, sensitivity is often compromised by the fragility of the bacteria and the ease of sample contamination.

We analysed laboratory data from the CSF of 412 patients obtained during a randomized non-inferiority trial conducted during an epidemic of NmA in Niger in 2003 [15]. The sensitivity and specificity of the Gram stain for indicating presence or absence of intracellular diplococci versus a gold standard of culture and/or PCR was 66.3 and 96.4%, respectively. As the Gram stain cannot indicate serogroup, any positive specimen should subsequently be tested by a LAT.

2.3. White blood cell count (WBC)

Numerous studies have reported the value of granulocyte cell counts using microscopy, to detect presumptive bacterial meningitis. We calculated the diagnostic value of the WBC (cut-off ≥ 50 cells/mm^3) using data from the same study described above, and repeated the analysis to determine the value of using WBC as an initial screening test, prior to the LAT.

Using culture and/or PCR as the gold standard for meningococcal detection, the sensitivity and specificity of the WBC ($n = 412$ CSF samples) were, respectively, 95.4 and 42.7%. Table 1 shows that, among the WBC positive CSF samples, the proportion of specimens positive by Gram stain was 59.6%, by LAT 79.1% and according to the gold standard 85.8%. Thus, in an epidemic context, a positive WBC was well correlated with the gold standard. Similar results have been reported previously [16]. Table 1 shows that the WBC threshold that we used will over-estimate the number of positive specimens by about 11% (36/323), while the LAT under-estimates the number of positive specimens by a similar proportion (34/323).

In order to find an affordable and feasible strategy to be used at peripheral level that may allow initial suspicion of meningococcal meningitis and possibly reduce the number of unnecessary LATs, we examined the performance of Gram

Table 1
Results of Gram stain, Pastorex agglutination test and the gold standard assays according to the results of white blood cell count in cerebrospinal fluids from clinical meningitis suspect patients

White blood cell count (threshold 50 cells/mm^3)	Number cerebrospinal fluid samples	Gram positive	Pastorex positive	Gold standard positive (culture and/or PCR)
Negative	53	4 (7.5%)	5 (9.4%)	15 (28.3%)
Positive	359	214 (59.6%)	284 (79.1%)	308 (85.8%)
Total	412	218 (52.9%)	289 (70.1%)	323 (78.4%)