Journal of african clinical cases and reviews / Journal africain des cas cliniques et revues

www.jaccrafrica.com ISSN 1859-5138 Open access

Original article

Contribution of the Polymerase Chain Reaction of cerebrospinal fluid in the diagnosis of bacterial meningitis

Apport de la Polymérase Chain Reaction du liquide céphalo-rachidien dans le diagnostic des méningites bactériennes

AM Gado*[1], SB Marou[2], IS Hamidou[1], A Akilou[1], MM Boulama[1], SA Kalilou[1], SS Moussa[1], D Alhousseini[3], M Boutchi[4]

Abstract

Introduction: Bacterial meningitis is a major public health concern in the Lapeysonnie Belt countries despite advances in prevention, diagnosis and treatment. Discrepancies are often recorded between the clinic and the classic paraclinical results. Can the polymerase chain reaction (PCR) of cerebrospinal fluid (CSF) reduce these discrepancies?

Methodology: We conducted a 6-month descriptive, analytical, and prospective study from January 22 to July 22, 2019 to establish concordance between clinical signs, cytobacteriological examination results, biochemistry of bacterial meningitis cases, and CSF PCR results in all patients admitted for suspected meningitis to the infectious diseases department of the Niamey National Hospital.

Results: Fifty patients were included. The age group between 4-14 years old was the most affected with 36%. Men were the most affected with 62% of cases. PCR identified germs in 35 cases, including 22 cases of Neisseria meningitidis, 12 cases of Streptococcus pneumoniae and 1 case of Haemophilus influenzae. We recorded the unusual presence of Neisseria meningitidis X (16% of cases). Meningococcal serogroup C was the leading cause of bacterial meningitis (28% of cases), followed by pneumococcus (24% of cases). The most common clinical signs were fever (70%), headache (64%), vomiting (60%) and stiff neck (58%).

Hyperproteinorachia and hypoglycorachia were found in 68% and 44%, respectively. We used ceftriaxone as an antibiotic. The clinical course was favourable in 84% of cases. We recorded 6 deaths (12%) and 2 cases of complications (4%).

Conclusion: This study highlighted the importance of PCR in refining the diagnosis of bacterial meningitis.

Keywords: bacterial meningitis, PCR, intake, diagnosis, Niger.

Résumé

Introduction : Les méningites bactériennes représentent une préoccupation majeure en matière de santé publique dans les pays de la ceinture de Lapeysonnie malgré les progrès réalisés dans

la prévention, le diagnostic et le traitement. Des discordances sont souvent enregistrées entre la clinique et les résultats paracliniques classiques. La polymerase chain reaction (PCR) du liquide céphalo-rachidien (LCR) peut-elle amoindrir ces discordances ?

Méthodologie : Nous avons effectué une étude descriptive analytique et prospective sur 6 mois du 22 Janvier au 22 Juillet 2019 afin d'établir la concordance entre les signes cliniques, les résultats de l'examen cytobactériologique, de la biochimie des cas de méningite bactérienne et les résultats de la PCR du LCR chez tous les patients admis pour suspicion de méningite au service des maladies infectieuses de l'hôpital national de Niamey.

Résultats : Cinquante patients ont été inclus. La tranche d'âge entre 4-14ans était la plus touchée avec 36%. Le sexe masculin était le plus concerné avec 62% des cas. La PCR a permis d'identifier les germes dans 35 cas dont 22 cas de Neisseria meningitidis, 12 cas de Streptococcus pneumoniae et 1 cas d'Haemophilus influenzae. Nous avons enregistré la présence inhabituelle du Neisseria meningitidis X (16% des cas). Ainsi le méningocoque du sérogroupe C'était la première cause des méningites bactériennes (28% des cas), suivi du pneumocoque (24% des cas). Les signes cliniques les plus rencontrés étaient : la fièvre (70%), les céphalées (64%), les vomissements (60%) et la raideur de la nuque (58%).

Une hyperprotéinorachie et une hypoglycorachie ont été retrouvées dans respectivement 68% et 44 %. Nous avons utilisé la ceftriaxone comme antibiotique. L'évolution clinique a été favorable dans 84% des cas. Nous avons enregistré 6 décès (12%) et 2 cas de complications (4%).

Conclusion : Cette étude a permis de souligner l'importance de la PCR pour affiner le diagnostic des méningites bactériennes.

Mots-clés : méningite bactérienne, PCR, apport, diagnostic, Niger.

Introduction

Bacterial meningitis (BM) currently remains a public health problem in the countries of the African Meningitis Belt (AMC), known as the Lapeysonnie Belt, where it is responsible for major sequelae and heavy mortality. [1 ; 2]. In this area, BM manifests itself as a hyper-endemic background to which epidemics with high morbidities and mortalities are regularly grafted in cycles of five to ten years [3]. These epidemics always start at the beginning of the dry season when it is hot, dry and dusty, reach their peak at the end of the dry season and come to an abrupt end with the onset of the first rains.

The etiology of BM consists mainly of three germs: *Neisseria meningitidis* (*Nm*), *Streptococcus pneumoniae (*Sp*) and* Haemophilus influenzae *(*Hib). Other germs may be encountered depending on age, terrain, and special circumstances. However, meningococcal meningitis remains one of the leading causes of epidemics in sub-Saharan Africa. [4]

Niger, a country located in the middle of the meningitis belt, is regularly hit by epidemics of meningococcal meningitis. One of the most severe occurred during the 1994-1995 season, resulting in more than 40,000 cases and more than 3,000 deaths [5]. Serogroup A has been responsible for most epidemics and waves of epidemics. After the introduction of the meningococcal A conjugate vaccine MenAfriVac, which has been implemented since 2010 through mass vaccination campaigns, the incidence of meningitis of this serogroup has fallen sharply and even disappeared in most vaccinated countries. Seasonal hyper-endemicity continued due to other meningococcal and pneumococcal disease in similar proportions. In addition, other meningococcal serogroups such as C, W and X can also cause epidemics. Such as the emergence of meningococcal W in 2002 in Burkina Faso and Niger [6], the emergence of *NmX in 2006 in Niger [7] and NmC in 2014-2015 in Nigeria and Niger.*

The diagnosis of BM is a medical emergency. It is therefore important to know the etiology of BM in

order to quickly implement the appropriate antibiotic therapy. However, very often there is a discrepancy between the clinical and the conventional results of cytobacteriological examination (BUC) of the CSF, hence the need for more and more use of PCR. To this end, the laboratory plays a very important role in the confirmation of meningitis cases in the event of an epidemic and during epidemiological surveillance. This confirmation requires a culture of the causative bacterium, identification of serogroups/serotypes, or even genotypes. In Niger, in addition to the classic assessment consisting of cytology, Gram staining, latex particle agglutination test and bacterial culture; The Centre for Medical and Health Research (CERMES) has PCR to improve the diagnosis of germs responsible for bacterial meningitis.

We conducted this study in order to establish the concordance between the clinical and paraclinical signs of bacterial meningitis cases, mainly with the results of PCR.

Methodology

The study was carried out by the National Hospital of Niamey (HNN) through the infectious diseases departments and the biology and biochemistry laboratories in a first phase, then CERMES for the performance of PCR in a second phase. This is a prospective analytical descriptive study that included patients hospitalized for meningitis in the infectious disease department of the HNN. It lasted 6 months from January 22 to July 22, 2019. We included all patients with clinical signs in favour of BM and CSF BCE results. Patients without CSF BCE results were not included. Data collection was based on a pre-established data collection sheet. The variables studied are socio-demographic, clinical, paraclinical and evolutionary.

The data entry and analysis were carried out with Epi Info version 7.2, Word and Excel 2007. The significance level of the results is $p \leq 0.05$.

The incompleteness of the completion of the CSF BCE forms and laboratory registers and the non-completion of examinations for some patients were the main difficulties we encountered.

Resultats

Of the 50 patients enrolled, the mean age of our sample was 15.24 years, 18 of our patients were between 4 and 14 years old (36%) and 15 were between 15 and 25 years old (30%). Men were the most represented with 31 cases or 62%. The M/F sex ratio was 1.63. Forty-three of the patients (86%) had no clinical history. Fever, headache, vomiting, and stiff neck were the most common clinical signs with frequencies of 98%, 86%, 84%, and 84%, respectively. Altered consciousness was noted in 23 patients (46%). CSF was cloudy in 44 patients (88%), hematic in 4 patients (8%) and clear in 2 patients (4%). The white blood cell count in CSF (leukorachia) ranged from 10 to 1000 elements/mm3 in 15 patients (30%), between 1000 and 5000 elements/mm3 in 7 patients (14%), between 5000 and 10000 elements/mm3 in 9 patients (18%) and more than 10,000 elements/mm3 in 19 patients (38%). The leukocyte formula showed a predominance of neutrophils in 45 patients (90%). Culture was negative in 50% of CSF samples, in the proportion of positive meningococcal was isolated in 19 cases (38%) and pneumococcal in 6 cases (12%). Of the 50 CSF samples submitted to PCR, 15 came back negative (30%), of the 35 that were positive (70%), *Nm C was found in 14 samples (28%),* Nm X in 8 samples (*16%),* Sp in *12 samples (24%) and* Hib *in* 1 sample (2%). Hypoglycorachia was observed in 30 CSF samples (60%) and hyperproteinorachia in 41 CSF samples (82%). At the complete blood count (CBC), 41 patients (82%) had hyperleukocytosis. C-reactive protein (CRP) was elevated in 20 of our patients (40%), this examination was not done in 27 patients (54%). A statistically significant relationship was established between CSF PCR positivity and the main clinical and paraclinical signs found in patients (see Table I). Of the 49 patients with fever, 35 had a positive PCR ($p = 0.03$). Headache was found in 43 patients, 32 of whom had a positive PCR ($p = 0.03$).

Neck stiffness was present in 42 patients, 29 had a positive PCR (p = 0.02). Vomiting was reported in 42 patients, 30 of whom had a positive PCR (p=0.05). Of the 47 patients with hyperproteinorachia, 34 had a positive PCR (p = 0.04). Of the 48 patients who underwent glycorachia, 22 had hypoglycorachia (p = 0.04).

About the etiological treatment, we used ceftriaxone as an antibiotic at a dose of 100 mg/kg/day in 2 intravenous (IV) injections, not exceeding 4 g/d, regardless of the patient's weight. Antibiotic therapy lasted less than 5 days in 6 patients (12%), between 5 and 7 days in 26 patients (52%) and more than 7 days in 18 patients (36%). In order to prevent hearing complications of BM, we initiated short-term corticosteroid therapy (48 hours) based on dexamethasone at a dose of 0.3 mg/kg/day as an IV injection in all patients aged 0 to 15 years (32 patients or 64%).

The majority of our patients (n=42 or 84%) were discharged from the hospital; Two of our patients, 4%, had complications such as hearing loss (1 case) and hydrocephalus (1 case) and 6 of our patients (12%) died.

Table I: Distribution of patients by classic signs and PCR results

	Number	**Positive PCR**	**Negative PCR**	**P**
Fever	49	35	14	0,03
Headache	43	32	11	0,03
Stiff neck	42	29	13	0,02
Vomiting	42	30	12	0,05
Proteinorachy	47	34	13	0,04
Hypoglycorachia	48	22	8	0,04

Table II: Main bacteria isolated by PCR during meningitis epidemics in selected countries of the Lapeysonnie belt

Isolated bacteria	Our study	Isabelle Delrieu et al. [24] Togo 2006 - 2009	Mahaman R. [10] Niger 2015	Sanou M. et al. [25] Burkina Faso 2009 - 2010	Bonko M. [4] Burkina Faso 2008
Nm A	-	-	-	59,3%	77%
Nm C	28%	-	80,6%	-	-
Nm X	16%	16%	-	-	1%
Sp	24%	-	6,9%	34,3%	18%
Hib	2%	-	0,3%	-	1%

Discussion

The mean age of our sample was 15.24 years with extremes of 3 months and 55 years. The most affected age group was 4 to 14 years old with 18 patients or 36%. This frequency is lower than those found by other authors including Béhanzin C. [8], Yacouba M. [9] and Mahaman R. [10] who reported frequencies of 45.8%, 50.73% and 55% respectively in patients under 14 years of age.

As in our case, the predominance of the male sex in the various studies is the order of the day [11 - 16]. On the other hand, Béhanzin C. [8] reported a female predominance during his study.

The most frequently reported classic clinical signs in our study were fever at a frequency of 98%, headache at a frequency of 86%, stiff neck and vomiting were reported in 84% of cases each. In general, the same tendencies are found in the majority of authors [18; 19; 20]. However, there are a few exceptions that are worth noting [11].

The macroscopic appearance of CSF samples is blurred in 88% of the cases in our study. The same observation was made by Béhanzin C. [8] in contrast to Zerarga K. [11] and Traoré A. [17] who reported lower frequencies of cloudy CSF in their series respectively 52% and 52.63%.

Leukorachia was elevated in all our patients and the leukocyte formula gave neutrophil predominance (PNN) in 90% of cases and lymphocyte predominance in only 6% of cases. This differs from the figures published by Zerarga K. [11] who had obtained leukorachia in 76% of cases and a predominance of PNN in 84% of cases and Sanon A. [12] who had found hyperleukorachia in 23.60% of cases and a predominance of PNN in 22.47% of cases.

We found hyperproteinorachia in 94% of our patients and hypoglycorachia in 60% of our patients. These results are consistent with those of several authors [11; 8; 16].

For bacteria isolated by PCR, Table II compares our results with those of some authors from the Lapeysonnie belt. Prior to 2010, many cases of BM were due to *Nm* A, which is no longer the case since the MenAfrivac vaccination campaign. In 2015, *Nm* C was the most predominant factor in BM outbreaks. For some time now, we have been seeing cases of MB due to *Nm* X. The proportion of cases of BM due to *Sp* is also increasing.

As far as antibiotic therapy is concerned, like several authors in the region, we have used ceftriaxone in all our patients (100%) in accordance with the recommendation of the World Health Organization (WHO); This is not the case for Sanon A. [12], which has only used this molecule in 10.6% of its patients.

In order to prevent hearing complications, we used dexamethasone in 32 of our patients aged 0 to 15 years (64%), which is not the case in the majority of authors who used this molecule in their series in smaller proportions [8; 12], but Zerarga K. [11] used it in 70.3% of its patients.

During our study, 42 patients (84%) had a favorable clinical course. This rate converges with those of Lhoste J. [21] and Boubacar M. [5] who reported a cure rate of 77% and 76.43% respectively.

We recorded 6 deaths (12%). Our case fatality rate is close to those of Merabet M. et al [22] and Lhoste J. [21] in its Nancy cohort which reported 11.79% and 12.1% of deaths respectively. On the other hand, the case fatality rates published by some authors are much higher than ours. Thus, Sanon A. [12], Youssouf K. [3] and Victorine M. [19] reported case fatality rates of 30.2%, 22.5% and 22% respectively at the end of their studies. This high lethality could be explained by the fact that in the studies of these authors the proportion of MB due to *Sp* was very high. Indeed, several authors such as Leimkugel J. et al. [23] have found that the lethality due to MB caused by *Sp* is very high.

Conclusion

At the end of this study, we retain that CSF PCR is the key examination for diagnosing BM by specifying the species of bacteria and their serogroups. Concordance between the classic clinical and paraclinical signs

of BMR and the PCR results is established. PCR must therefore be popularized for better therapeutic management and the development of effective and efficient meningococcal vaccines.

***Correspondence**

GADO Amadou Mahamadou

fredgado40@gmail.com

Available online : January 15, 2024

1 : Department of Infectious Diseases, Niamey National Hospital
2 : Department of Biology, Niamey National Hospital
3 : Department of Biochemistry, Niamey National Hospital
4 : Department of Biochemistry and Biology, Issaka Gazoby Maternity Hospital in Niamey

Conflict of interest : None

References

[1] Houssam, Zeggai Mohammed El Amine Toumiz « Méningites bactériennes de l'enfant ». Mémoire de fin d'étude. Université ABOU BAKR BELKAID TLEMCEN faculté de médecine département de pharmacie, p. 20

[2] Boubacar Mainassara Halima : Epidémie de méningite avant et après l'introduction du vaccin méningococcique conjugué monovalent contre le sérogroupe A dans la CAM, Thèse de Doctorat de l'université Pierre et Marie Curie 2017.

[3] Youssouf Keita : Méningite bactérienne chez les enfants âgés de 0 à 15ans hospitalisés dans le service de pédiatrie du CHU Gabriel Touré, Thèse de Docteur en médecine 2011.

[4] Bonko Massa Achille : Aspects microbiologiques des méningites bactériennes aigues dans la région sanitaire des hauts bassins à Bobo Dioulasso, Burkina Faso 2007-2008. Diplôme d'étude approfondie en biologie appliqué et modélisation des systèmes biologiques 2009.

[5] Boubacar Moctar : Méningite bactérienne chez les enfants de moins de 5ans (Aspects cliniques, évolutifs et sérotypages des principaux germes) Thèse de médecine, Niamey (Niger) 2014 N° 2233

[6] Organisation Mondiale de la Santé 2003 : Lutte contre la méningite épidémique dans les pays de la CAM.

[7] Boisier P., Djibo S., Boubacar MH. et al : Méningite à méningocoque incidence sans précédent de cas liés au sérogroupe X en 2006 au Niger. Clin Infect Dis. 2007 ; 44(5) :657-63. doi : 10.1086/511646.

[8] Béhanzin Constantin Sègla : Facteurs prédictifs de mauvais pronostic au cours de l'épidémie de méningite en 2015(Hôpital national de Niamey), Thèse de médecine Niamey 2016.

[9] Yacouba Mahamane : Surveillance microbiologique des méningites bactériennes au Niger. Etude rétrospective de 2005 à 2015. Thèse médecine 2016

[10] Mahaman R. Habibou : Evaluation de l'épidémie de méningite au Niger en 2015 ; Etude rétrospective évaluative déroulée sur 6 mois allant de janvier à juin 2015 à la Direction de la Surveillance et la Riposte aux Epidémies. Thèse médecine 2016.

[11] Zerarga Khaled : Etude épidémiologique, clinique des méningites purulentes au service des maladies infectieuses CHU de Bejaia, Thèse de Doctorat en médecine 2017.

[12] Sanon Adama : Aspects microbiologiques, cliniques, bactériologiques et évolutifs des méningites bactériennes à propos de 178 cas au CHU Souro Sanou de Bobo Dioulasso, Thèse de médecine Burkina Faso 13 Décembre 2004.

[13] Hamam Kahina : Etude épidémiologique des cas de méningite (viral et bactérienne) chez l'enfant dans 4 communes de la wilaya de Bejaia. Mémoire de fin de cycle, 27 Juin 2018.

[14] Abdias Ogobara Dougnon : Etude de l'épidémie de méningite cérébro-spinale 2005 au Mali, Thèse pour l'obtention du Doctorat en médecine.

[15] Esayas K., Markos T., Aynishet A. et al : Défis de la gestion des cas de MB dans les pays à faibles revenue. Une expérience Ethiopienne, juillet 2016 ; 21(7) : 870-8 doi : 101111/tmi. 12720.

[16] Pore Brigitte : Méningites bactériennes décapitées (Apport de la biologie et de la biochimie dans le diagnostic à l'Hôpital National de Niamey). Thèse médecine 2014.

[17] Traoré Annatou : Méningites bactériennes au Centre Hospitalier Universitaire Yalgado Ouédrago de Ougadougou. (Aspects épidémiologiques, bactériologiques, cliniques et évolutifs). Thèse Docteur en pharmacie 2007.

[18] Campagne G., Schuchat A., Djibo S. et al : Epidémiologie de la méningite bactérienne Niamey (Niger), 1981-1996. Bulletin of the world health organization 1999; 77:499-508.

[19] Marie Victorine Bambara : Méningite purulente du nourrisson dans le service des maladies infectieuses du CHN-YO de Ouagadougou (Aspects épidémiologiques, étiologiques, thérapeutiques et évolutifs), Thèse de Docteur en médecine 2001

[20] E. Pilly Trop 2016 Maladies infectieuses tropical pp 336.

[21] Lhoste J. Les méningites purulentes communautaires de l'enfant : étude rétrospective de 1998 à 2003 de deux cohortes à Dakar, Sénégal et à Nancy, France. Sciences du Vivant [q-bio] 2006. Hal-01731977

[22] Merabet M., Aouragh R., Idrissi A. : Les méningites bactériennes aigues communautaires chez les enfants de moins de 5 ans à la région Tanger-Tétouan-Al Hoceima (Maroc) 2006-2015 : Profil épidémiologique, clinique et biologique. Antropo, 40, 1-11. www.didac.ehu.es/antropo. 2018.

[23] Leimkugel J, Forgor AA, Gagneux S et al. An outbreak of serotype 1 Streptococcus pneumoniae meningitis in Northern Ghana with features that are characteristic of Neisseria meningitis epidemics JID 2005 ;192-9.

[24] Delrieu I., Seydou Y., Tall H. et al. Emergence d'une méningite à Neisseria meningitidis sérogroupe X au Togo et au Burkina Faso ; 2011 ; 6(5) :19513. doi 10.1371/journal.plosone.0019513.

[25] Sanou M., Palenfo D., Bisseye C. et al. Diagnostic moléculaire par PCR en temps réel d'agents bactériennes responsables des méningites purulentes au Burkina Faso. Med Sante Trop2013 ; 23 ; 93-99. doi : 10.1684/mst. 2013.0148. .

To cite this article :

AM Gado, SB Marou, IS Hamidou, A Akilou, MM Boulama, SA Kalilou et al. Contribution of the Polymerase Chain Reaction of cerebrospinal fluid in the diagnosis of bacterial meningitis. Jaccr Africa 2024; 8(1): 115-121