Where there is no brain imaging: safety and diagnostic value of lumbar puncture in patients with neurological disorders in a rural hospital of Central Africa.

Deby Mukendi[1,2]*, Jean-Roger Lilo Kalo[1], Tharcisse Kayembe[2], Pascal Lutumba[1,2], Barbara Barbé[3], Philippe Gillet[3], Jan Jacobs[3,4], Cedric P Yansouni[5], François Chappuis[6], Kristien Verdonck[7], Marleen Boelaert[7], Andrea S Winkler[8,9], Emmanuel Bottieau[3].

1) Institut National de Recherche Biomédicale, Kinshasa, DR Congo

2) Département de Neurologie, service de Neurologie Tropicale, Université de Kinshasa, DR Congo

3) Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

4) Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium

5) JD MacLean Centre for Tropical Diseases, McGill University Health Centre, Montreal, Canada

6) Division of Tropical and Humanitarian Medicine, Geneva University Hospitals and University of Geneva, Switzerland

7) Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium

8) Center for Global Health, Department of Neurology, Technical University of Munich, Munich, Germany

9) Centre for Global Health, University of Oslo, Oslo, Norway

*Corresponding author : debymukendi@yahoo.fr

Abstract

Analysis of cerebrospinal fluid (CSF) obtained by lumbar puncture (LP) is an essential step for the diagnostic approach of neurological disorders, in particular neuro-infections. In low-resource settings, it is even often the only available diagnostic method. Despite its key contribution, little is known on the risks and benefits of LP in the large tropical areas where hospital-based neuroimaging is not available. The objectives of this study were to assess the safety and diagnostic yield of LP in a rural hospital of central Africa and to identify predictors of CSF pleocytosis (white blood cell count > 5/µL) as surrogate marker of neuro-infections. From 2012 to 2015, 351 patients admitted for neurological disorders in the rural hospital of Mosango, Kwilu province, Democratic Republic of Congo, were evaluated using a systematic clinical and laboratory workup and a standard operating procedure for LP. An LP was successfully performed in 307 patients (87.5%). Serious post-LP adverse events (headache, backache or transient confusion) were observed in 23 (7.5%) of them but were self-limiting, and no death or long-term sequelae were attributable to LP. CSF pleocytosis was present in 54 participants (17.6%), almost always associated with neuro-infections. Presenting features strongly and independently associated with CSF pleocytosis were fever, altered consciousness, HIV infection and positive screening serology for human African trypanosomiasis. In conclusion, the established procedure for LP was safe in this hospital setting with no neuroimaging and CSF analysis brought a substantial diagnostic contribution. A set of presenting features may help accurately selecting the patients for whom LP would be most beneficial.

Introduction

Laboratory analysis of cerebrospinal fluid (CSF) obtained by lumbar puncture (LP) is a key diagnostic step to distinguish between inflammatory, infectious, metabolic, neoplastic, demyelinating, and degenerative causes of neurological disorders [1]. In low-resource settings, CSF analysis is often the only available investigation in the search for a specific and treatable neurological diagnosis, primarily infections. Basic analyses such as measurement of glucose or protein concentration, white blood cell (WBC) count and differential, and identification of pathogens by microscopy provide invaluable information for clinical management [1-2]. In particular, CSF pleocytosis, defined as a WBC count above 5/µL of CSF, is a key surrogate finding usually pointing to infections of the central nervous system (CNS). Moreover, the WBC differentiation helps to further consider subgroups of etiological pathogens which have to be urgently and specifically treated.

Safe performance of LP and accurate analysis of CSF require adequate staff training and appropriate equipment, which are far from optimal in low-resource settings. In such situations, the decision to perform an LP relies on a difficult-to-apprehend balance between the expected diagnostic benefits and the risks of the procedure. Indeed, multiple complications can occur after LP. Headache, backache, and nerve root irritation are the most frequently reported adverse events, in up to 60%, 40% and 13% of procedures, respectively [3-5]. Other complications are rare but may be life-threatening. Cerebral herniation, the most feared adverse event, occurred in about 1% of diagnostic LPs in historical studies prior to neuroimaging [3]. Other more rare but serious complications include post-LP infection (cellulitis, discitis, spinal abscess, and meningitis), transient cranial nerve palsies and bleeding (spinal subdural/epidural hematomas or subarachnoid hemorrhages) [3].

In high-income settings, patients with neurological disorders usually undergo neuroimaging to identify the etiology of their problem and to detect cerebral abnormalities that may precipitate herniation if an LP is performed. Neuroimaging has become part of the standard initial evaluation, even if there is some evidence that clinical evaluation should remain key in the decision to urgently perform, or defer, an LP [4,6,7]. In contrast, the risks and benefits of an LP have been hardly studied in the countless low-resource hospitals

where neuroimaging is not available, at the exception of some facilities specialized in HIV care [8,9]. Since evidence-based guidance is lacking, many first-line clinicians feel uncomfortable with the decision to perform an LP and too often, this leads to its inappropriate omission in situations where it is clearly indicated [10].

For a clinical study that was part of the NIDIAG project ("Better Diagnosis for Neglected Infectious Diseases", www.nidiag.org) and that investigated the causes of neurological disorders in the rural hospital of Mosango in the Democratic Republic of the Congo (DRC), we had to elaborate a standard operating procedure (SOP) on when and how to perform an LP [11,12]. This local guideline had to rely on clinical and fundoscopic examination only. To develop the SOP, we first looked at recommendations for high-income countries [6], which were then adapted to the setting of a rural hospital by a study team of neurologists with experience in tropical medicine (see the corresponding SOP in French and English as Supplemental Table). The NIDIAG study provided a unique opportunity to assess the risks and usefulness of LP in this particular setting.

The primary objectives of the present study were to report on the safety of LPs performed according to the elaborated guideline, and to determine the diagnostic yield of basic CSF analysis in NIDIAG study participants. A secondary objective was to identify the clinical and first-line laboratory predictors of CSF pleocytosis, the main observed abnormal finding, in order to improve the selection of patients with neurological disorders who would most benefit from a diagnostic LP in similar settings.

Methods

Study design

This study about LP was nested in a larger observational and prospective cohort study which was part of the NIDIAG project. The larger NIDIAG study had as objectives to (1) investigate the etiology and outcome of neurological disorders in a rural hospital of DRC and (2) assess the diagnostic value of clinical and laboratory predictors, including a set of rapid diagnostic tests (RDTs), for identifying severe and treatable CNS infections. Detailed information on the inclusion criteria, neurological workup, priority neurological infections, case definitions, and index and reference diagnostic methods is available in Mukendi D et al. [13].

Study setting

The NIDIAG study was conducted in the "Hôpital Général de Référence" (HGR) of Mosango, a health facility with very limited resources, located in Mosango health district (a rural area of 3,350 km² with about 110,000 inhabitants), Kwilu province at about 400 km from the capital, Kinshasa. General practitioners with minimal training in neurology provided the medical care. Besides the laboratory facilities set up to conduct the study (described in detail in [13]), advanced methods for neurological diagnosis, and in particular neuroimaging, were not available.

Participants

All patients older than five years presenting at the HGR of Mosango with non-traumatic and progressive neurological disorders were enrolled in the NIDIAG clinical and diagnostic study [13]. Briefly, for inclusion, at least one of the following symptoms or signs had to be present: (1) altered state of consciousness; (2) changes in sleep pattern; (3) cognitive decline; (4) changes in personality/behavior; (5) epileptic seizure (within less than 2 weeks); (6) recent, severe and progressive headache; (7) meningism; (8) new onset cranial nerve lesion; (9) new onset sensorimotor focal deficit; and (10) new onset gait/walking disorders.

Study procedures

All study participants were subjected to a thorough clinical/neurological examination and a set of laboratory reference and study assays. LP was an integral part of the diagnostic workup (except in case of contra-indication, see below). All diagnoses were established according to strict composite case definitions, with a particular focus on the following set of priority (severe and treatable) neuro-infections prevalent in the region: bacterial meningitis, unspecified meningo-encephalitis, second-stage human African trypanosomiasis (HAT), cerebral malaria, central nervous system (CNS) tuberculosis, HIV-related neurological disorders and neurosyphilis [13].

Performance of lumbar puncture, analysis of cerebrospinal fluid and patient follow-up

A set of clinical criteria that were absolute and relative contraindications for LP were established in an SOP that also described the procedure itself (Supplemental Table). Absolute contraindications included unresponsive coma (Glasgow coma scale [GSC] < 8), rapid deterioration of consciousness, recent (< 30 minutes) and/or repeated epileptic seizure, symptoms/signs of hemodynamic or respiratory failure, papilledema at fundoscopy, bleeding diathesis, skin infection at the puncture site or severe vertebral

deformities/opisthotonus. Relative contraindications included altered consciousness (GCS between 8 and 13) and focal neurological deficits; LP could be performed if these clinical manifestations were not progressing rapidly.

After the patient (or his/her representative) consented to participate in the study and contraindications were excluded, about 10 ml of CSF was collected. Investigations that were immediately performed in the study hospital included WBC count with differentiation and search for trypanosomes in all participants, as well as India ink and a reference Cryptococcal Antigen Latex Agglutination test (CrAg LAT) in HIV-positive patients. CSF was systematically inoculated into bacterial and mycobacterial growth indicator tube (MGIT) cultures and cryopreserved; bacterial species identification and additional molecular testing were done in reference laboratories in Kinshasa, DRC, and Antwerp, Belgium, as detailed in [13]. CSF pleocytosis was defined as the presence of more than five WBC/µL of CSF. For CSF WBC counts between 5 and 20/µL, a second cell count was performed and we categorized the patient as having pleocytosis when both results were above the cut-off of 5/µL. WBC differentiation with measurement of the proportion of neutrophils or lymphocytes was performed only when the WBC count was above 20/µl. Determination of glucose and protein concentration in CSF was not performed. When CSF pleocytosis was present, additional investigations included Gram and Ziehl staining in the study hospital, and Pastorex Meningitis antigen assay (Bio-Rad, Hercules, CA) and polymerase chain reaction for herpes simplex and zoster viruses in reference laboratories in Antwerp, Belgium [13].

All enrolled patients were admitted to the hospital and were offered targeted or empirical therapy according to pre-established clinical SOPs; they were re-evaluated on a daily basis (or more often if required) until discharge. All new symptoms and signs occurring during hospitalization were registered with particular attention to severe post-LP adverse events.

Study endpoints

For the safety component, the endpoints of the analysis were the frequency and pattern of serious adverse events developing within 24 hours after LP: death, or any new symptom/sign obliging the patient to be or remain bedridden, or any new infection emerging at the puncture site. Deaths occurring within 24 hours after LP were particularly scrutinized for a possible relationship with this procedure, taking into account that the

maximum reported period for post-LP herniation was approximately 12 hours [14]. For the diagnostic yield of CSF analysis, we assessed the frequency and pattern of abnormal findings in CSF as well as their association with the final diagnoses. CSF pleocytosis was chosen as the endpoint of the secondary objective, since it was by far the main CSF abnormality that required urgent intervention.

Statistical methods

For the primary endpoints of safety and diagnostic yield, frequencies of serious adverse events and abnormal findings were determined on the total population having undergone an LP. For the identification of predictors of CSF pleocytosis, we first compared baseline characteristics between patients with and without CSF pleocytosis in bivariate analysis. Because of the limited number of outcome events, only those variables that were available for all participants and found to be strongly associated with CSF pleocytosis (p-value < 0.02) were included in multivariate analysis. Also, in case two variables were clearly correlated, we kept the one that was most relevant clinically or for which the information was most complete. We then calculated the positive and negative likelihood ratios (LR) of the independent predictors of CSF pleocytosis, first for each of them individually and second, for those with good confirming power (LR+ ≥ 5), in combination. Stata 14 software was used for statistical analyses.

Ethical aspects

The study protocol and the informed consent forms were approved by the Institutional Review Board of the Institute of Tropical Medicine, Antwerp, Belgium and by the Ethical Committees of the University of Antwerp, Belgium, and the Public Health School of Kinshasa, DRC. The study was registered at clinicaltrials.gov under the identifier NCT01589289.

Results

From September 2012 to April 2015, 351 patients with neurological disorders were included prospectively in the NIDIAG cohort (median age: 40 years; range: 6-78 years; ratio women/men: 1.1). Cerebrospinal fluid could be analyzed in 307 of them (87.5%). Their baseline characteristics and presenting features are summarized in Table 1. Reasons for non-performance of CSF analysis are described in Fig 1 and consisted mainly of contraindication (n=10), refusal (n=16) or failure (n=18) of LP.

Safety of lumbar puncture

As described in Fig 2, of the 307 patients who underwent a successful LP, 23 (8.0%) died during (n=18) or after (n=5) the hospitalization, including two (0.7%, 95% CI 0.2-2.3) within 24 hours post-admission. The first case was a 30-year-old male who was admitted for fever and altered consciousness and died 16 hours post-LP with septic shock. He was found to have *Chromobacterium violaceum* bacteremia [15]. The second fatal case was a 55-year-old man who also presented with fever and altered consciousness and developed a non-reactive coma 10 hours post-LP; he was suspected of ketoacidosis from de-novo diabetes mellitus (Fig 2). We attributed both deaths to the underlying disease, and not to the LP. Among the 10 patients with contraindication to LP, two (20%) died, both within 24 hours after admission (one had opisthotonus and the other one non-reactive coma at presentation. Of note, of the remaining 34 patients in whom LP was not performed for other reasons or was unsuccessful, three died (8.8%), all of them beyond 24 hours after admission.

In the LP group (n=307), 23 patients (7.5%) developed serious adverse events, including severe post-LP headache in 12 patients (3.9%, 95% confidence interval [CI], maintaining them bedridden for a mean duration of 3 days (maximum 8 days); severe backache in another 8 patients (2.6%, 95% CI 1.3-5.0; median duration: 5 days; maximum 14 days), and a transient episode of confusion in 3 (1.0%, 95% CI 0.3-2.8), which resolved within hours. None developed symptoms and signs of infection at the puncture site or secondary meningitis requiring a new LP.

Results of CSF analysis

Of the 307 patients with CSF analyzed, WBC count was > 5/μl in 54 (17.6%) and one of the priority neuro-infections was finally diagnosed in 52 (Table 2). Additional diagnostic information was immediately provided by other CSF analyses in the study hospital for 25 of these 52 cases (48.1%): demonstration of trypanosomes in all 10 patients diagnosed with second-stage HAT, of Gram-negative (n=11) and Gram-positive cocci (n=1) in 12 of 14 confirmed cases of bacterial meningitis, of India ink positive yeasts in two of the three cases of HIV-related cryptococcal meningitis, and Ziehl–Neelsen stain-positive mycobacteria in one of the four cases

finally diagnosed with CNS tuberculosis. Of note only one case of priority neuro-infection (HIV-related cryptococcal meningitis finally diagnosed by positive CrAg LAT) had a WBC count below 5/µL of CSF.

The quantification and differentiation of CSF WBC per neuro-infection is provided in Table 2; WBC count was above 100/µL and predominantly neutrophilic in almost all cases (12/14) of bacterial meningitis, while it was below 100/µL in most cases (15/19) of unspecified meningo-encephalitis and in half (5/10) of the patients with second-stage HAT.

Predictors of CSF pleocytosis

As described in Fig 3, CSF pleocytosis was observed in the majority of patients presenting with altered consciousness (29/49, 59.2%). In contrast, its frequency was lowest in the subsets of patients who had epileptic seizures (9/76, 11.8%) or sensorimotor deficit (11/82, 13.4%) at presentation.

As shown in Table 1, the features associated with CSF pleocytosis in bivariate analysis with a p-value < 0.02 were: prior exposure to antibiotics and to antibiotics and/or antimalarials, altered consciousness and Glasgow Coma Scale < 15, moderate/severe dehydration, fever as reported by patient and fever documented at admission, neck stiffness, vomiting, anorexia, confirmed HIV infection (defined as reactivity to three different brands of HIV RDTS used sequentially in the diagnostic panel/national control program), positive RDT for HAT (HAT Sero *K-S*eT, Coris, Gembloux, Belgium [16] and positive Card Agglutination Trypanosoma Test (CATT) result. Blood WBC count above 10,000/µL and hyperglycemia (≥ 6.66 mmol/L) were also associated with CSF pleocytosis but as these test results were not available for all study patients because of technical reasons at the local hospital laboratory, we did not retain these two features in the multivariable analysis. The multiple logistic regression model included the variables listed in the footnote of Table 3, according to the selection procedure described under Methods, and identified the following independent predictors, by increasing strength of association: moderate/severe dehydration, neck stiffness, fever as reported by patient, Glasgow Coma Scale < 15, positive HAT RDT result and confirmed HIV infection.

Four predictors were found with a good confirming power (LR+ ≥ 5) for CSF pleocytosis: fever as reported by patient, Glasgow Coma Scale < 15, confirmed HIV infection, and positive HAT RDT. The presence of any of them increased the probability of CSF pleocytosis from a baseline of 17.6% in the study population to at least

50%. In contrast, the negative predictive value of any individual factor was weak (Table 3). However, when the four strongest predictors were all absent, the probability of CSF pleocytosis decreased from the baseline 17.6% to 0.6% (Table 3). Indeed, 194 of 307 patients (63.2%) who underwent an LP had none of the four predictors at presentation, and only 2/194 (0.6%) were found to have CSF pleocytosis: one patient with cerebrovascular accident (CSF WBC count of 8/µL) and a second patient with a final diagnosis of unspecified meningoencephalitis (CSF WBC count of 33/µL, with 98% of lymphocytes), who recovered completely. In other words, withholding LP in the large subset of patients in whom all four predictors were absent would have "missed" only two cases who had rather mild and non-actionable CSF abnormalities.

Discussion

In this large population of patients with neurological disorders in a rural hospital of Central Africa, the safety of a pre-established study guideline describing the contraindications and execution of LP in the absence of neuroimaging was reassuring. There were no case fatalities directly attributable to LP. Moderate-to-severe LP-related adverse events were observed in about 10% of the overall cohort but were all transient with no long-term sequelae. CSF analysis contributed important diagnostic information in about 18% of the patients admitted with neurological disorders, with CSF pleocytosis being present in almost all cases of established neuro-infection. Finally, a set of good predictors of CSF pleocytosis were identified e.g. confirmed HIV infection, Glasgow coma scale < 15/altered consciousness, fever as reported by the patient and positive screening serology for HAT (either RDT or CATT) - that could help identify patients who are most likely to benefit from an LP, and those in whom an LP can be safely forgone.

This study has several limitations [13]. It was a single-center prospective study conducted in hardship conditions, and diagnostic uncertainty remained important in the absence of advanced neurological facilities. Although useful for other low-resource settings, the findings, and in particular the identified predictors of CSF pleocytosis, are not generalizable as such to other tropical regions with different epidemiological profiles. Also, they cannot be extended to children less than 5 years, in whom the majority of LPs are performed in the tropics.

We focused on the description of the two deaths occurring within 24 hours after the LP, because if cerebral herniation due to LP occurs, it happens almost always within 12 hours of the procedure [3]. Although absolute certainty cannot be reached without neuroimaging, we consider that the clinical deterioration leading to death was delayed and was explained by the underlying pathology rather than by LP [3,5]. Our data are quite robust since the safety of LP was prospectively assessed in a large cohort of patients in whom it was clinically indicated and with low rates of refusals or unsuccessful procedures. We observed that strict adherence to a sound set of clinical criteria and to a rigorous procedure [5] did not lead to short- or long-term poor outcomes, and that severe adverse events were infrequent, transient, and manageable. This is an important finding since LP was sometimes performed in clinical situations (altered consciousness, focal deficit, immunosuppression) that would have prompted neuroimaging prior to LP in high-income settings. It should also be noted that overreliance on neuroimaging may itself be deleterious since it may unduly delay urgent LPs or appropriate treatment, and cerebral herniation might sometimes occur with normal radiological findings [4,6,19-22].

The diagnostic yield of CSF analysis strongly depends on the etiological spectrum of neurological disorders in a given region or population. In a setting where neuro-infections still represent a substantial burden [23], we observed that basic CSF analyses had an immediate impact on targeted and empirical therapies in about 1 in 5 patients suspected of having a life-threatening infection. The CSF WBC count and differentiation did bring important “generic” information, while some additional analyses (search for trypanosomes, Gram staining and India ink) provided the diagnostic clue for (almost) all targeted infections. Only the Ziehl staining on CSF did not perform well, as expected, in the few confirmed cases of CNS tuberculosis. It is however also important to highlight that etiology remained unknown in about half of the patients with CSF pleocytosis (finally diagnosed as “unspecified meningo-encephalitis”) even after performing additional reference molecular assays (data not shown). This is in keeping with previous studies in high-income countries on the high proportion of unknow etiologies in USA/Europe [24-26].

In order to improve the selection of patients with the highest probability of benefiting from an LP, we investigated the confirming powers of clinical and first-line laboratory features independently associated with CSF pleocytosis. The confirming power of a clinical feature or a laboratory test for a given condition is

reflected by the determination of its positive likelihood ratio [27]. From the baseline frequency of this condition, LRs allow in the Bayesian model to estimate post-symptom or post-test probabilities, an essential information for the clinical reasoning. Four different features (confirmed HIV infection, Glasgow coma scale < 15/altered consciousness, fever reported by the patient and positive HAT RDT/CATT result) were each found with a LR+ ≥ 5, that in turn increased the probability of finding CSF pleocytosis to at least 50%. Here, neck stiffness was a weaker predictor (LR+ < 5). This may be explained by the fact that not all patients with CSF pleocytosis had bacterial meningitis and that in adults with bacterial meningitis neck stiffness, although frequently present [28], has usually little diagnostic accuracy [29,30].

The "threshold" (here to perform an LP) represents the equipoise between the harm of a set of diseases on the one hand and the risk of this diagnostic procedure on the other hand. It is a pivotal concept in clinical decision-making [31]. All the study findings were presented to, and discussed with, Congolese neurologists and local general practitioners to draw an evidence-based field-adapted guidance tool for neurological disorders in rural hospitals of DRC. There was an agreement that a probability of CSF pleocytosis of at least 50% is largely above the threshold necessary for most clinicians to perform a diagnostic LP, even in low resource settings. Similarly, a probability of less than 1% was accepted a reasonable threshold below which an LP could be safely withhold (at least temporarily) since it would avoid a very large number of unnecessary procedures. We have therefore developed a guidance tool that combines the information on the regional diagnostic landscape that could be derived from our previous study [13] with the instructions to look in priority for a history of fever and symptoms/signs of altered consciousness, and to systematically perform rapid diagnostic tests targeting HIV and HAT. In the current guideline, we strongly recommend an LP in all patients (with no contraindication) in whom any of these features is present or positive, while we suggest to withhold it if all of them are absent or negative. A prospective study is being conducted to assess the operational feasibility of this new evidence-based guidance tool.

Conclusion

Strict adherence to a local guideline on when and how to perform LP in patients older than 5 years with neurological disorders in a rural hospital of Central Africa was safe and did not lead to any procedure-

attributable deaths or neurological sequelae, in the absence of neuroimaging. CSF pleocytosis had a very high generic diagnostic accuracy for most treatable neuro-infections and a set of clinical and laboratory features could be used to identify patients for whom an LP would be of highest, or of little, benefit.

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Figure 1. Flow chart for performance of lumbar puncture in the patients enrolled in the NIDIAG study

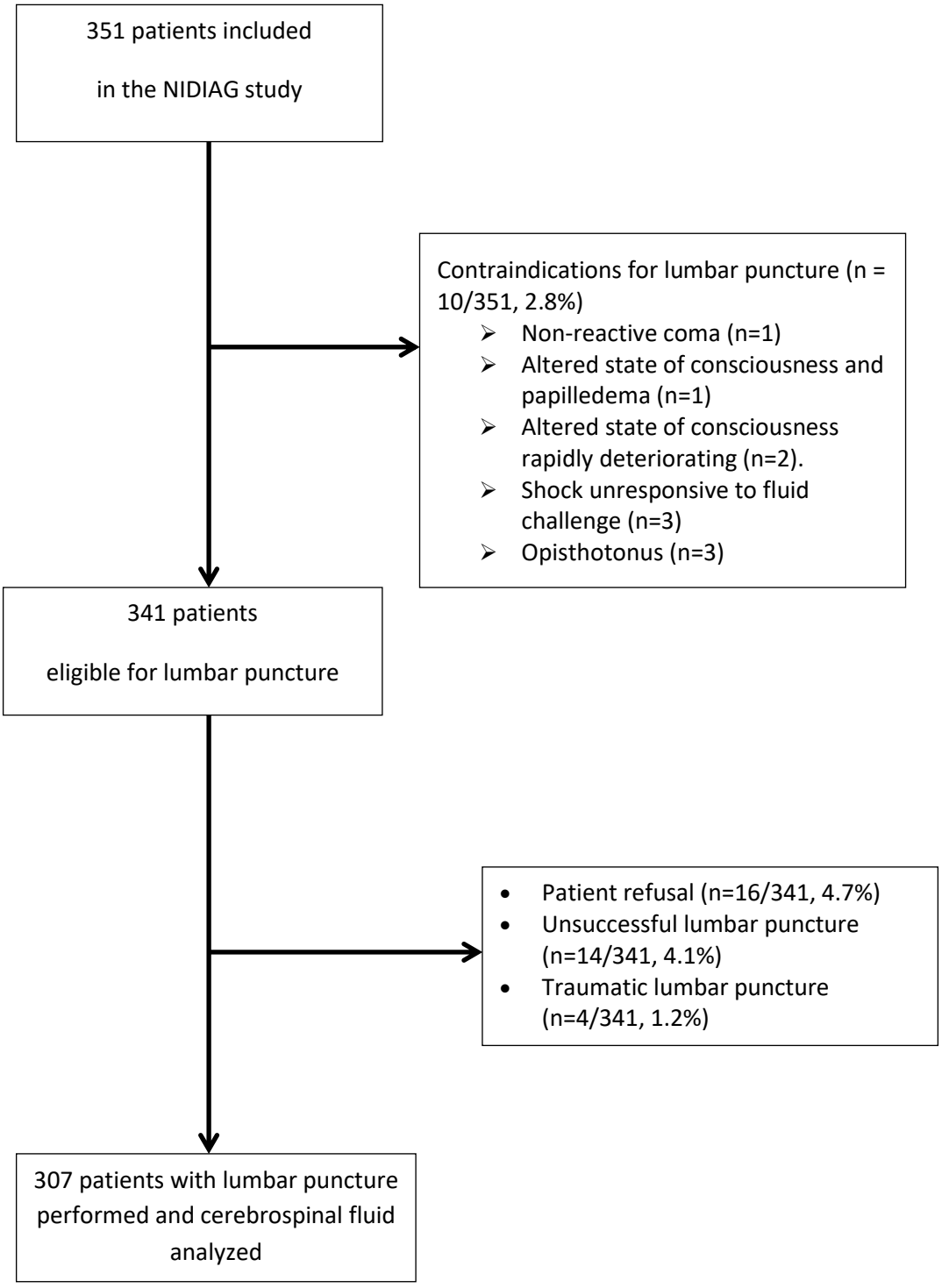

Figure 2. Distribution of deaths in patients admitted with a neurological disorder (n=351) in the "Hôpital Général de Référence" of Mosango, by lumbar puncture (LP) status

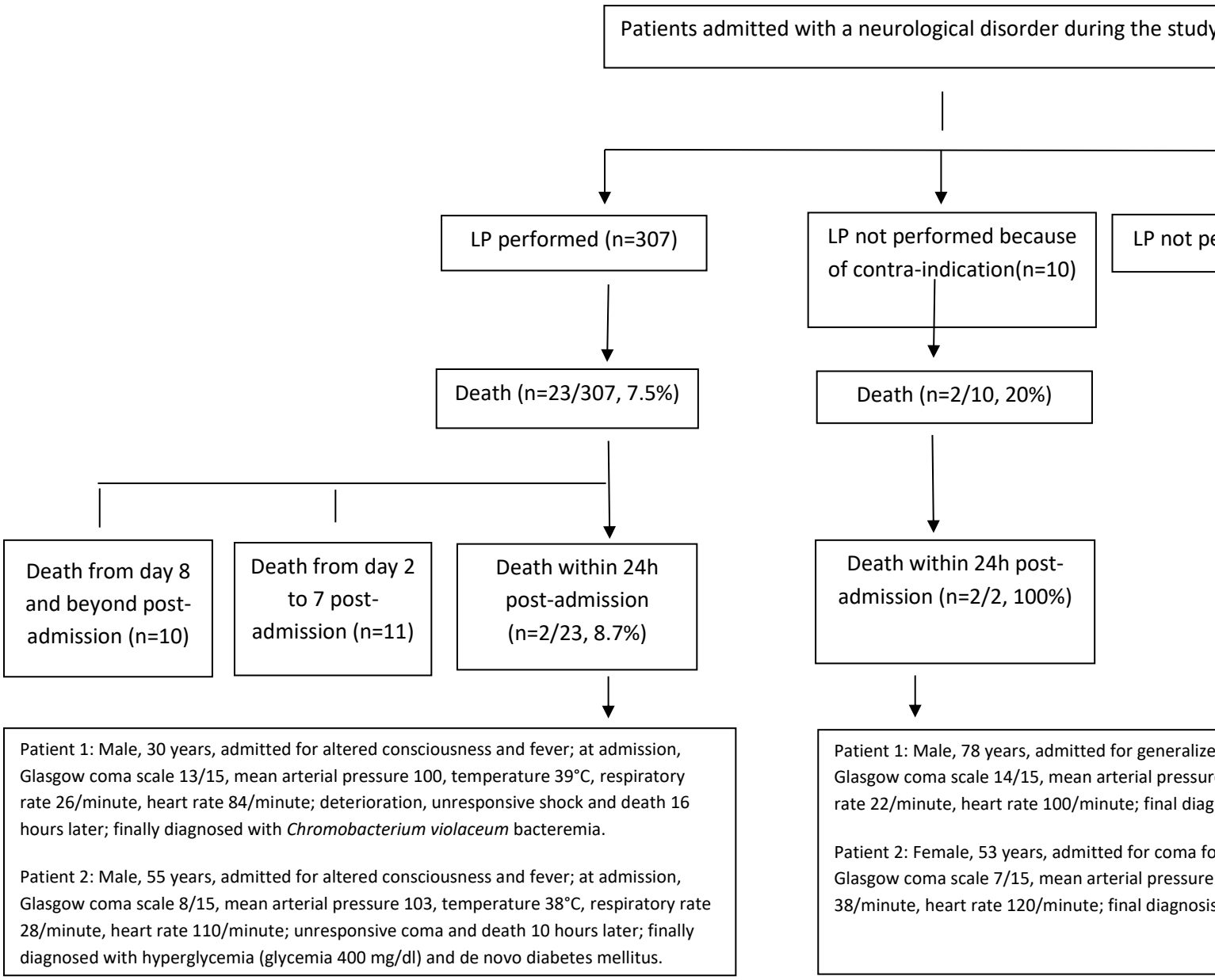

Fig 3. Frequency of cerebrospinal fluid pleocytosis (> 5 white blood cells/µl) according to neurological symptom/syndrome of presentation.

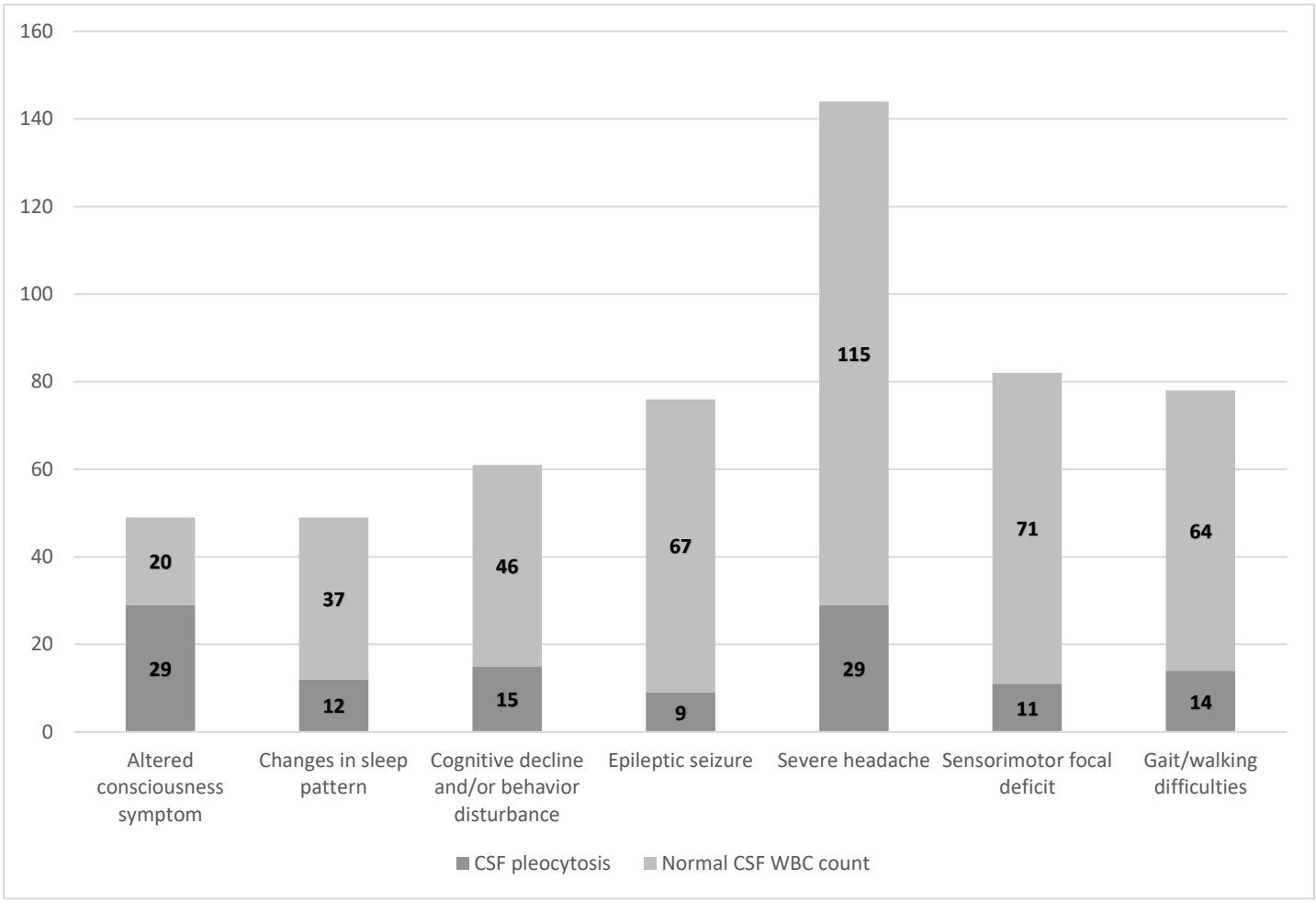

Table 1: Baseline characteristics, clinical features and laboratory findings of patients who had a successful lumbar puncture (n=307), according to CSF WBC result

Baseline characteristics	Total (n=307)	CSF WBC count		P value
		Elevated > 5/µL (n=54)	Normal (n=253)	
Epidemiological features				
Male gender	145 (47.2)	32 (59.2)	113 (44.6)	0.051
Age ≤18 years	77 (25)	20 (37)	57 (22.5)	0.026
Prior/ongoing exposure to antibiotics	85 (27.7)	26 (48.1)	59 (23.3)	0.000
Prior/ongoing exposure to antimalarials	75 (24.4)	23 (42.6)	52 (20.5)	0.001
Prior/ongoing exposure to antibiotics and antimalarials	53 (17.2)	12 (22.2)	41 (13.3)	0.288
Main neurological symptoms/signs/syndromes of presentation				
Altered consciousness, as reported by patient/family	49 (15.9)	29 (53.7)	20 (8)	0.000
Changes in sleep pattern	49 (15.9)	12 (22.2)	37 (14.6)	0.166
Cognitive decline	16 (5.2)	3 (5.5)	13(5.1)	0.900
Changes in personality/behavior	61 (19.7)	15 (27.7)	46(18.2)	0.109
Epileptic seizure	76 (24.7)	9 (16.6)	67(26.4)	0.129
Severe headache	144 (46.8)	29 (53.7)	115 (45.4)	0.270
Cranial nerve lesion	19 (6.1)	1 (2)	18 (7.1)	0.145
Sensorimotor focal deficit	63 (20.4)	10 (18.5)	53 (21)	0.688
Gait/walking difficulties	78 (25.3)	14 (26)	64 (25.3)	0.923
Neurological signs (at initial evaluation)				
Glasgow coma scale < 15	63 (20.5)	36 (66.6)	27 (10.6)	0.000
Neck stiffness at flexion, as assessed clinically	104 (33.9)	26 (48.1)	78 (30.8)	0.015
Generalized amyotrophy	24 (8.3)	7 (13)	17 (6.7)	0.121
Nystagmus (spontaneous and/or provoked)	36 (11.7)	4 (7.4)	32 (12.6)	0.277
Snout reflex	23 (7.4)	6 (11.1)	17 (6.7)	0.265
Tremor	33 (10.7)	9 (16.6)	24 (9.5)	0.121
Non neurological symptoms/signs				
Fever as reported by patient	53 (17.2)	29 (53.7)	24 (9.5)	0.000
Temperature > 37.8°C at admission	27 (8.7)	13 (24)	14 (5.5)	0.000
Moderate/severe dehydration	57 (18.5)	24 (44.4)	33 (13)	0.000
Vomiting	24 (7.8)	16 (29.6)	8 (3.1)	0.000
Anorexia	31 (10.1)	15 (27.7)	16 (6.3)	0.000
Cachexia (body mass index < 20)	41(13.3)	9 (16.6)	32 (12.6)	0.431
Diarrhea	7 (2.3)	3 (5.5)	4 (1.6)	0.076
Laboratory features				
Confirmed HIV infection*	10 (3.2)	7 (12.9)	3 (1.2)	0.000
Positive HAT-RDT (HAT Sero *K*-Set)	16/291 (5.5)	8/50 (16)	8/241 (3.3)	0.000
Positive Card Agglutination Trypanosoma Test (CATT)	19 (6.1)	11 (20.3)	8 (3.1)	0.000
Positive syphilis RDT (SD Bioline)	6 (1.9)	1(2)	5 (2)	0.949

Positive HRP2-based malaria RDT (Pf-HRP2 and pan-pLDH, SD Bioline)	28 (9.1)	7 (13)	21 (8.3)	0.280
Positive pLDH-based malaria RDT (Pf-pLDH and Pan-pLDH, Carestart)	11 (3.5)	0 (0)	11 (4.3)	0.238
WBC in blood ≥ 10,000/µL	34/242 (11)	17/43 (39.5)	17/199 (8.5)	0.000
Glycemia ≥ 6.66 mmol/L	41/288 (14.2)	12/47 (25.5)	29/241 (12)	0.015
Anemia (Hb < 9 g/dl)	16 (5.2)	5 (9.2)	11 (4.3)	0.140
Creatininemia ≥ 1.5 mg/dl	10/292 (3.4)	3/48 (6.2)	7/244 (2.8)	0.239

Note: all results are n or n/n (%)
* In DR Congo, confirmation of HIV infection relies on reactivity to three different brands of HIV RDTs, performed sequentially

CSF denotes cerebrospinal fluid; WBC white blood cells; RDT rapid diagnostic test; HAT: human African trypanosomiasis; HRP2 histidine-rich protein 2; Pf Plasmodium falciparum; pLDH parasite lactate dehydrogenase ; SD Bioline: Standard Diagnostics

Table 2: Neuro-infections diagnosed in the 54 patients found with cerebrospinal fluid pleocytosis, distributed by white blood cell quantification and differentiation

White blood cell count and differentiation	Unspecified meningo-encephalitis (n=19)	Bacterial meningitis (n=14)	Second-stage human African trypanosomiasis (n=10)	HIV-related neurological disorders (n=6)	Tuberculosis of the central nervous system (n=3)
5-20/µL	6	1	-	2	-
20-100/µL	9	-	5	1	1
Neutrophil > 50% of WBC	5		-	1	-
Lymphocyte > 50% of WBC	4		5	-	1
100-1,000/µL	4	6	4	3	1
Neutrophil > 50% of WBC	2	5	-	-	1
Lymphocyte > 50% of WBC	2	1	4	3	-
> 1,000/µL	-	7	1	-	1
Neutrophil > 50% of WBC		7	-		1
Lymphocyte > 50% of WBC		-	1		-

WBC denotes white blood cell; HIV human immunodeficiency virus

Note: Two additional patients with an alternative diagnosis had a white blood cell count of 5-20/µL, one with cerebrovascular accident and the second with *Streptococcus pyogenes* bacteremia

Table 3. Multiple logistic regression model with likelihood ratios (LRs) of independent predictors and respective post-test probabilities (PTP) of CSF pleiocytosis(n=54) in patients with neurological disorders who underwent lumbar puncture (n=307)

Features	Adjusted OR [95% CI]	Sensitivity	Specificity	LR+	LR-	PTP+ [95% CI]	PTP- [95% CI]
Moderate/severe dehydration	3.4 [1.3-9.0]	44.0	87.0	3.4	0.6	42.1 [30.2-55.0]	12.0 [16.5-8.5]
Neck stiffness	3.8 [1.3-10.8]	85.2	54.1	1.8	0.3	28.4 [22.0-35.8]	5.6 [10.5-2.8]
Fever as reported by patient	4.8 [1.9-12.0]	53.7	90.5	5.7	0.5	54.7 [41.4-67.3]	9.9 [14.1-6.8]
Glasgow Coma Scale < 15	11.0 [4.4-27.5]	66.7	89.3	6.2	0.4	57.1 [45.0-69.0]	7.4 [11.4-4.7]
Positive HAT RDT result	16.2 [4.0-66.0]	16.0	96.7	4.8	0.9	50 [28.0-72.0]	15.3 [20.0-11.5]
Confirmed HIV infection	14.9 [3.7-80.3]	13.0	98.8	10.9	0.9	70 [37.0-89.2]	15.9 [30.4-12.1]

CSF denotes cerebrospinal fluid, RDT rapid diagnostic test, HAT human African trypanosomiasis, HIV human immunodeficiency virus, LR+ positive likelihood ratio, LR- negative likelihood ratio, PTP+: post-test probability when feature present, PTP-: post-test probability when feature absent

The following selected variables were entered in the multiple logistic regression: prior/ongoing exposure to antibiotics or to antimalarials, fever as reported by patient, vomiting, anorexia, Glasgow coma scale < 15, neck stiffness, moderate/severe dehydration, positive HAT RDT result and confirmed HIV infection.