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ARTICLE

Impact of round-the-clock CSF Gram stain on empirical therapy for suspected central nervous system infections

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Abstract The impact of round-the-clock cerebrospinal fluid (CSF) Gram stain on overnight empirical therapy for suspected central nervous system (CNS) infections was investigated. All consecutive overnight CSF Gram stains between 2006 and 2011 were included. The impact of a positive or a negative test on empirical therapy was evaluated and compared to other clinical and biological indications based on institutional guidelines. Bacterial CNS infection was documented in 51/241 suspected cases. Overnight CSF Gram stain was positive in 24/51. Upon validation, there were two false-positive and one false-negative results. The sensitivity and specificity were 41 and 99 %, respectively. All patients but one had other indications for empirical therapy than Gram stain alone. Upon obtaining the Gram result, empirical therapy was modified in 7/24, including the addition of an appropriate agent (1), addition of unnecessary agents (3) and simplification of unnecessary combination therapy (3/11). Among 74 cases with a negative CSF Gram stain and without formal indication for empirical therapy, antibiotics were withheld in only 29. Round-the-clock CSF Gram stain had a low impact on overnight empirical therapy for suspected CNS infections and was associated with several misinterpretation errors. Clinicians showed little confidence in CSF direct examination for simplifying or withholding therapy before definite microbiological results.

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Introduction

Acute bacterial meningitis is a medical emergency associated with a mortality of 15 to 37 % [1–6]. Delayed empirical treatment has been reported as an independent risk factor for increased morbidity and mortality [2, 5, 7]. Cerebrospinal fluid (CSF) Gram staining accurately identifies the causative microorganism in 60 to 90 % of community-acquired and 18–60 % of nosocomial meningitis and, therefore, has the potential to help clinicians choose the most appropriate empirical regimen before definite microbiological results [8–10]. Although current Infectious Diseases Society of America (IDSA) guidelines recommend that all patients being evaluated for suspected meningitis undergo CSF Gram stain, there are no specific recommendations regarding whether this diagnostic test should be available on a 24-h basis [8]. As CSF Gram stain has always been performed round-the-clock in our centre, we conducted a 6-year retrospective investigation to assess the impact of round-the-clock CSF Gram stain on overnight empirical antibiotic prescription for suspected central nervous system (CNS) infections.

Table 1 Clinical characteristics of 241 episodes of suspected CNS infection

Characteristics	*n*=241
Demographics	
Age: median, years (range)	29 (0–96)
Sex: female	104 (43)
Risk factors	
Immunosuppression[a]	26 (11)
Otitis/sinusitis	8 (3)
Neurosurgery within 30 days	50 (21)
Presence of CSF shunt	37 (15)
Clinical presentation	
Fever	150 (62)
Headaches	95 (39)
Neck stiffness	72 (30)
Petechial rash	3 (1)
Hypotension (<90/60 mmHg)	6 (2)
Altered level of consciousness	41 (17)
Focal neurological deficit	20 (8)
Seizure	15 (6)
CSF characteristics[b]	
Pleocytosis (>10 cell/ml)	142/208 (68)
Neutrophil count>200 G/l	38/212 (18)
Protein>800 mg/l	123/213 (58)
Lactate>4 mmol/l	43/155 (28)
CSF/serum glucose<0.5	55/137 (40)
Microbiological results	
Positive Gram stain	24 (10)
Positive CSF culture	40[c] (16)
Positive CNS abscess culture	2 (<1)
Positive CSF PCR	13 (5)
Positive blood culture	14 (6)
Empirical antibiotic therapy	
Any	206 (85)
Ceftriaxone	56 (23)
Ceftriaxone+amoxicillin	38 (16)
Ceftazidime+vancomycin	24 (10)
Meropenem+vancomycin	23 (10)
Gentamicin+amoxicillin	29 (12)
Other	35 (14)
Initial suspicion of CNS infection	
Community-acquired meningitis	76 (31)
Nosocomial meningitis	64 (27)
Encephalitis	59 (25)
Neonatal sepsis	42 (17)
Definite diagnosis	
Bacterial meningitis	51 (21)
Community-acquired	23 (10)
Nosocomial	28 (12)
Aseptic meningitis	50 (21)
Viral	29 (12)
Unknown aetiology	21 (9)
Encephalitis	18 (7)
Viral	9 (3.5)
Unknown aetiology	9 (3.5)
Other non-CNS infections	60 (25)
Non-infectious diagnosis	61 (25)
In-hospital mortality	15 (6)

CNS central nervous system, *CSF* cerebrospinal fluid, *PCR* polymerase chain reaction

Data are shown in numbers (%), unless otherwise indicated

[a] Including cancer (18), inflammatory/autoimmune disease (4), solid-organ transplant recipient (1), human immunodeficiency virus (HIV) (3)

[b] The denominators of CSF biochemical tests are the total available analyses for each CSF parameter

[c] Eight positive CSF cultures were considered as contamination: *Streptococcus salivarius* (1), coagulase-negative staphylococci (6), *Kocuria kristinae* (1)

Methods

Study setting

In our 1,000-bed tertiary-care centre, a microbiology technician is available 24/7 to perform emergent CSF Gram stain, which is double-checked by a senior microbiologist the following morning.

In case of suspected community-acquired CNS infections, an institutional algorithm provides physicians criteria to initiate empirical antibiotic therapy. Clinical criteria include blood pressure<90/60 mmHg, petechiae, Glasgow Coma Scale (GCS)<9, focal neurological deficit or papillary oedema. Biological criteria comprise turbid CSF, positive CSF Gram stain, CSF neutrophils>200 G/l, CSF lactate>4 mmol/l, CSF proteins>800 mg/l or CSF/serum glucose<0.5. If one of these is present, empirical therapy with ceftriaxone is recommended, in association with amoxicillin in patients at risk for listeriosis (age>50 years, immunosuppression, alcoholism, diabetes) or signs of encephalitis. Vancomycin is not part of the empirical regimen due to the low prevalence of cephalosporin-resistant *Streptococcus pneumoniae* in Switzerland. If none of the clinical criteria are met and CSF is clear, it is recommended to wait until the result of CSF Gram stain or biochemical analysis to decide whether empirical therapy is indicated.

For nosocomial bacterial meningitis, there is no institutional algorithm, but a CSF lactate>4 mmol/l is typically used as a criterion to start empirical therapy with vancomycin and ceftazidime, cefepime or meropenem [11, 12].

Study design

In this retrospective single-centre study, all clinical suspicions of CNS infection for which CSF microscopic examination was performed outside working hours between January 2006 and September 2011 were included. Only one CSF sample per episode was considered. CSF microbiological results were retrieved from the microbiology laboratory information system. Patient medical charts were reviewed for epidemiological and clinical data. The study was approved by the institutional ethics committee; informed consent was not required.

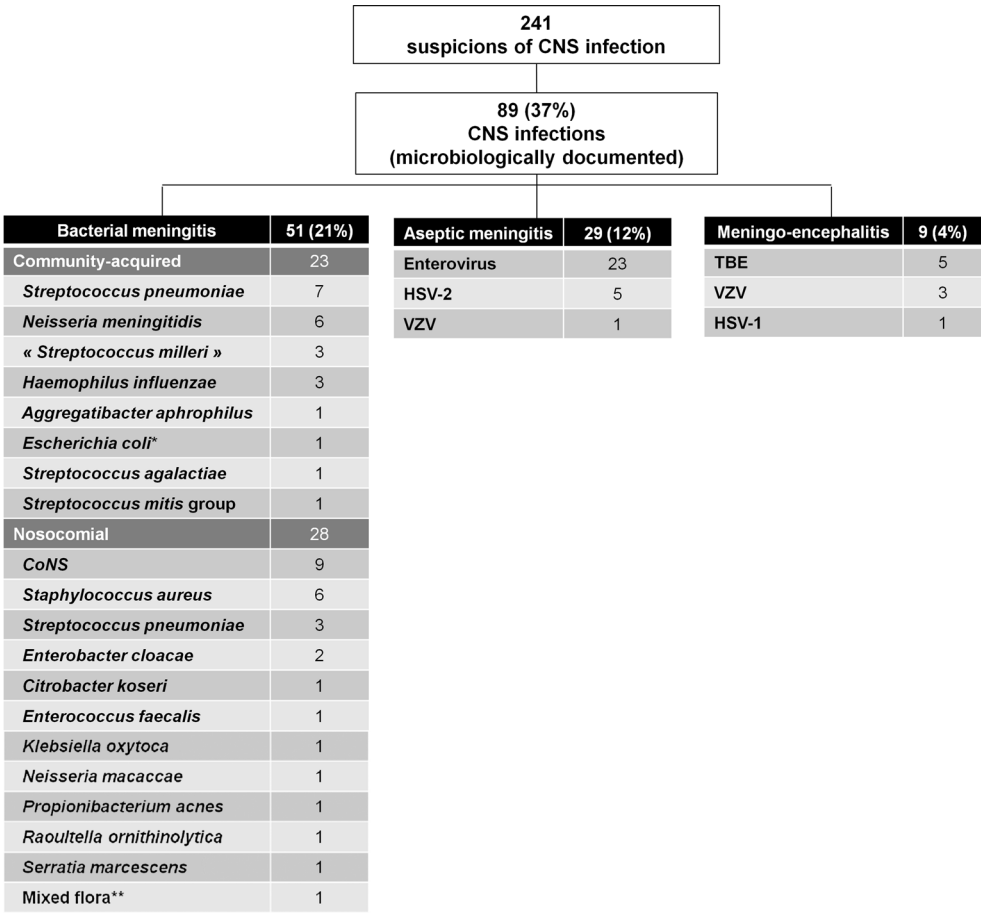
Definitions

Definite bacterial meningitis was defined as a microbiologically proven infection documented from CSF culture or polymerase chain reaction (PCR), in association with clinical signs of infection. In case of a negative CSF culture, positive CNS abscess or blood cultures were also considered if associated with CSF pleocytosis (leucocyte count≥10 cell/ml) and clinical signs of meningitis. Patients with CNS infections caused by *Mycobacterium tuberculosis*, *Treponema pallidum* or *Borrelia burgdorferi* were excluded. Meningitis was classified as nosocomial if occurring after hospital admission, within 30 days of neurosurgery or in the presence of CSF shunt.

Empirical therapy was considered appropriate when the antibiotic regimen included at least one agent with in vitro activity against the causative microorganism(s). In case of empirical combination therapy, the second agent was considered unnecessary when the causative microorganism seen on Gram stain was already covered by the first agent, based on the expected in vitro susceptibility.

Endpoints and statistical analysis

Overnight CSF Gram stain diagnostic performance for definite bacterial meningitis was assessed using the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The impact of a positive CSF Gram stain on the indication and choice of empirical therapy was assessed. Indications for antibiotic treatment according to institutional guidelines with and without Gram stain results were compared. Modifications of the empirical regimen after CSF examination results were also analysed. The impact of a negative CSF Gram stain was assessed by the proportion of patients without formal clinical and biological criteria for empirical therapy in whom antibiotics were withheld or interrupted after the results of CSF microscopic examination. Distinct and combined analyses



Bacterial meningitis	51 (21%)
Community-acquired	23
Streptococcus pneumoniae	7
Neisseria meningitidis	6
« Streptococcus milleri »	3
Haemophilus influenzae	3
Aggregatibacter aphrophilus	1
*Escherichia coli**	1
Streptococcus agalactiae	1
Streptococcus mitis group	1
Nosocomial	28
CoNS	9
Staphylococcus aureus	6
Streptococcus pneumoniae	3
Enterobacter cloacae	2
Citrobacter koseri	1
Enterococcus faecalis	1
Klebsiella oxytoca	1
Neisseria macaccae	1
Propionibacterium acnes	1
Raoultella ornithinolytica	1
Serratia marcescens	1
Mixed flora**	1

Aseptic meningitis	29 (12%)
Enterovirus	23
HSV-2	5
VZV	1

Meningo-encephalitis	9 (4%)
TBE	5
VZV	3
HSV-1	1

Fig. 1 Distribution of aetiologic pathogens in microbiologically documented central nervous system (CNS) infections. *CoNS* coagulase-negative staphylococci, *HSV* herpes simplex virus, *VZV* varicella-zoster virus, *TBE* tick-borne encephalitis. *Neonatal sepsis. **Including *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*

were performed for nosocomial and community-acquired CNS infections. Categorical variables were compared using Fisher's exact test.

Results

Study population

In total, 266 CSF samples (262 patients) were sent to the microbiology laboratory for examination outside working hours. For 22, there was no clinical suspicion of CNS infection. Three patients had meningitis caused by *M. tuberculosis* or *B. burgdorferi*. Therefore, 241 episodes (238 patients) were included. the clinical characteristics are shown in Table 1.

CNS infection was documented in 89 (36 %) cases, of which 51 (21 %) were definite bacterial meningitis (23 community-acquired, 28 nosocomial) (Fig. 1). Identification of the causative microorganism relied on CSF culture in 32, CSF PCR in 13, CNS abscess culture in two and blood cultures in four. All infections but one were monomicrobial (Table 2). Gram-positive bacteria were responsible for 32/51 (63 %) cases of definite bacterial meningitis. Two of six *Staphylococcus aureus* strains were methicillin-resistant and all ten *S. pneumoniae* were ceftriaxone-susceptible.

Diagnostic performance of overnight CSF Gram stain

Twenty-four (10 %) CSF direct examinations were interpreted as positive (Fig. 2). The pathogens were Gram-positive cocci (GPC) in 15/24. Upon validation the next morning, three cases

Table 2 Clinical and biological indications for empirical therapy in 24 patients with a positive overnight CSF Gram stain

Patient	CSF Gram	Organism	Clinical indication	CSF characteristics				
				Aspect	Neutrophils	Protein	Glucose ratio[a]	Lactate
Nosocomial meningitis (n=13)								
1	GPC	*S. aureus*	No	Haemorrhagic	171	–	–	–
2	GPC	*S. aureus*	No	Turbid	686	2,869	–	8.8
3	GPC	*S. epidermidis*	GCS<9	Turbid	22	–	–	–
4	GPC	*S. epidermidis*	No	Xanthochromia	93	1,500	0.17	–
5	GPC	*S. haemolyticus*	No	Turbid	2,170	2,544	–	–
6	GPC	*S. epidermidis*	No	Haemorrhagic	–	–	–	–
9	GPC	*S. pneumoniae*	GCS<9	Turbid	441	2,377	–	15.5
11	GPC	*E. faecalis*	Hypotension	Xanthochromia	23	6,242	0	–
15	GPC	*S. pneumoniae*	No	Turbid	506	14,806	0	14
20	GNB	*E. cloacae*	No	Turbid	55,280	5,965	0	–
21	GNB	*R.ornithinolytica*	No	Turbid	2,020	1,672	0	6.1
23	GNB	*E. cloacae*	No	Xanthochromia	1	8,110	–	–
24	GPB	–	No	Xanthochromia	0	1,631	0.2	–
Community-acquired meningitis (n=11)								
7	GPC	*S. agalactiae*	GCS<9	Turbid	4,364	–	0	13.5
8	GPC	*S. pneumoniae*	No	Clear	187	393	0.72	2.3
10	GPC	*S. pneumoniae*	GCS<9	Xanthochromia	1,188	8,025	0	18.1
12	GPC	*S. pneumoniae*	No	Clear	80	861	0.46	3
13	GPC	*S. pneumoniae*	GCS<9	Turbid	2,979	4,345	0	14
14	GPC	–	Hypotension	Clear	–	384	0.56	2.4
16	GNC	*N. meningitidis*	Petechiae	Turbid	349	–	–	–
17	GNC	*N. meningitidis*	No	Turbid	1,334	2,181	0.15	6.2
18	GNC	*N. meningitidis*	Petechiae	Turbid	15,156	66,240	0	11.7
19	GNC	*N. meningitidis*	No	Turbid	174	2,001	0	12.7
22	GNB	*H. influenzae*	Hypotension	Turbid	11,400	2,080	0.16	–

GPC Gram-positive cocci, *GNB* Gram-negative bacilli, *GPB* Gram-positive bacilli, *GNC* Gram-negative cocci

Units of CSF parameters: neutrophil count is given in G/l, protein concentration is given in mg/l and lactate concentration is given in mmol/l

[a] Glucose ratio refers to the serum/CSF glucose ratio

were found to be misclassified. Two positive CSF Gram stains showing GPC and Gram-negative bacilli (GNB) were due to artefacts, and one smear with a low quantity of microorganism was missed in a patient with *Streptococcus mitis* meningitis. In each case, the mistake was corrected in the Laboratory Interface System and attending physicians were contacted immediately.

Among 51 definite bacterial meningitis, the sensitivity, specificity, PPV and NPV of overnight CSF Gram stain were 43 % (22/51), 99 % (188/190), 92 % (22/24) and 87 % (188/217), respectively.

Impact of a positive CSF Gram stain on the indication for empirical therapy (Table 2)

Among 24 patients, three had no other clinical or biological indication for empirical therapy than CSF direct examination, including two cases of nosocomial meningitis (nos. 1 and 6) with incomplete CSF biochemical analysis. Another patient with community-acquired meningitis (no. 8) was a 19-year-old male with recurrent *S. pneumoniae* meningitis caused by undiagnosed CSF lamina cribrosa fistula, who was hospitalised within hours of symptoms onset and for whom antibiotics were immediately started after lumbar puncture. Therefore, a true impact of Gram stain was only seen in one patient (no. 6), who was not under antibiotic treatment by the time of Gram stain result.

The two patients with false-positive CSF Gram stains (nos. 14 and 24) were already receiving antibiotics before CSF Gram stain result, based on other clinical or biological criteria (Table 2). None of them had a CNS infection. The final diagnosis was *Haemophilus influenzae* endocarditis (no. 14) and intraventricular haemorrhage (no. 24).

Impact of a positive CSF Gram stain on the choice of empirical therapy (Table 3)

Empirical therapy was started before the result of CSF direct examination in 19/24 patients. In 12, the empirical regimen was not changed after Gram stain result, including five cases of nosocomial meningitis treated with a combination of ceftazidime and vancomycin while CSF Gram showed only a single type of microorganism. In seven, the empirical regimen was modified after CSF Gram stain was performed, including simplification of combination therapy of ceftriaxone and amoxicillin in three cases and broadening of the antibiotic spectrum against Gram-positive bacteria in four. Among these, three received unnecessary empirical antimicrobial agents (nos. 7, 12 and 14): vancomycin for penicillin-susceptible *S. pneumoniae* described as GPC in clusters on direct examination, addition of amoxicillin to ceftriaxone for *S. agalactiae* in order to cover listeriosis and vancomycin with gentamicin for a false-positive GPC result. For five patients with nosocomial meningitis, empirical therapy was started only after Gram stain result, including three treated with vancomycin and ceftazidime combination regardless of the Gram result.

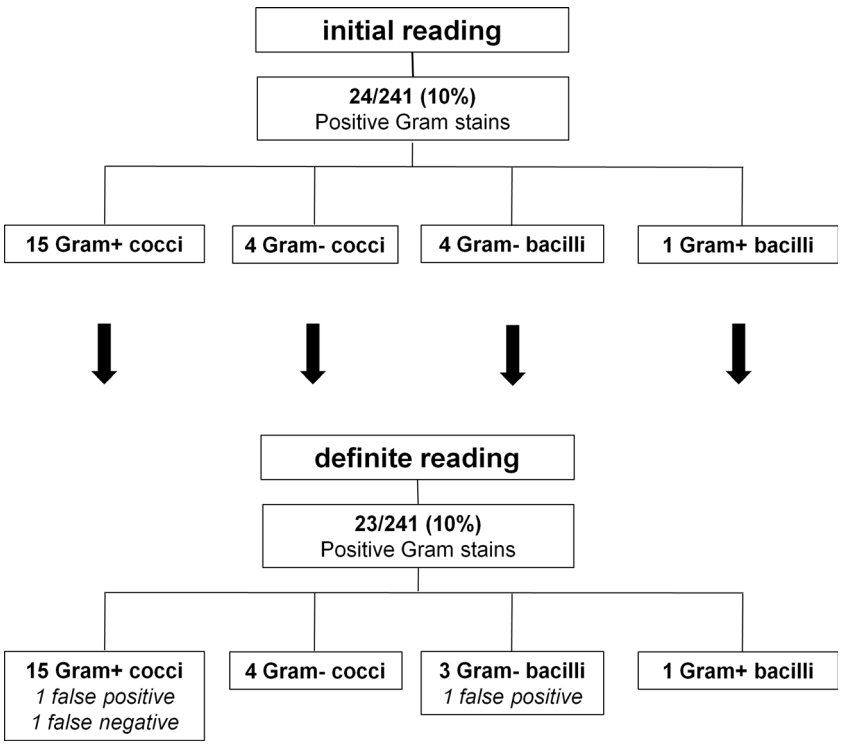


Fig. 2 Results of initial (overnight) and definite (the next morning) reading of cerebrospinal fluid (CSF) Gram stain. Discrepant results upon validation are shown in *italics*

All patients but one with a positive CSF Gram stain received an appropriate empirical therapy before definite microbiological identification. The only patient with an inappropriate regimen (no. 20) was a 12-year-old girl with *Enterobacter cloacae* post-neurosurgical meningitis treated with ceftriaxone, despite the presence of GNB on Gram stain. The treatment was modified to meropenem after identification of the responsible microorganism. In another patient with methicillin-resistant *S. aureus* (MRSA) nosocomial meningitis empirically treated with meropenem (no. 2), vancomycin was added after CSF Gram stain showed GPC in clusters (Table 3). There were no significant differences in the proportion of patients receiving appropriate empirical therapy when cases with positive and negative CSF Gram stain were compared (21/22 vs. 27/29, respectively, $p=1.0$). In the latter group, the two patients with inappropriate empirical therapy were a case of *Neisseria macacae* meningitis who received no treatment and a patient with mixed-flora post-neurosurgical meningitis (*E. coli*, *S. epidermidis*, *Pseudomonas aeruginosa*) treated with vancomycin and ceftriaxone.

Overall, an impact of a positive CSF Gram stain on the choice of empirical regimen was observed in 6/24 patients (three community-acquired and three nosocomial infections), including appropriate addition of antibiotic covering resistant GPC (no. 2), empirical regimen driven by Gram stain (nos. 9 and 23) and simplification of unnecessary combination therapy (nos. 10, 13 and 17).

Impact of a negative CSF Gram stain

Among 217 patients with a negative CSF Gram stain, 74 suspected CNS infections did not have any clinical or biological indication for empirical therapy according to institutional guidelines (Supplementary Fig. S1). Antibiotic treatment was withheld in 29/74 (39 %) and discontinued after Gram stain result in 11/74 (15 %). No CNS bacterial infection was diagnosed in any of

Table 3 Impact of a positive CSF Gram stain on the choice of empirical therapy

Patient	Meningitis	Gram	Organism	Therapy before Gram stain	Therapy after Gram stain
Empirical therapy modified after CSF Gram ($n=7$)					
2	Nosocomial	GPC	MRSA	Meropenem	Meropenem+vancomycin
7	CAM	GPC	*S. agalactiae*	Ceftriaxone	Ceftriaxone+amoxicillin
12	CAM	GPC	*S. pneumoniae*	Ceftriaxone	Ceftriaxone+vancomycin
10	CAM	GPC	*S. pneumoniae*	Ceftriaxone+amoxicillin	Ceftriaxone
13	CAM	GPC	*S. pneumoniae*	Ceftriaxone+amoxicillin	Ceftriaxone
14	CAM	GPC	–	Ceftriaxone	Ceftriaxone, vancomycin, gentamicin
17	CAM	GNC	*N. meningitidis*	Ceftriaxone+amoxicillin	Ceftriaxone
Empirical therapy unchanged after CSF Gram ($n=12$)					
1	Nosocomial	GPC	MSSA	Vancomycin, ceftazidime	Vancomycin, ceftazidime
3	Nosocomial	GPC	*S. epidermidis*	Vancomycin, ceftazidime	Vancomycin, ceftazidime
4	Nosocomial	GPC	*S. epidermidis*	Vancomycin, ceftazidime	Vancomycin, ceftazidime
11	Nosocomial	GPC	*E. faecalis*	Vancomycin, ceftazidime	Vancomycin, ceftazidime
15	Nosocomial	GPC	*S. pneumoniae*	Vancomycin, ceftazidime	Vancomycin, ceftazidime
24	Nosocomial	GPB	–	Vancomycin, ceftazidime	Vancomycin, ceftazidime
20	Nosocomial	GNB	*E. cloacae*	Ceftriaxone	Ceftriaxone
8	CAM	GPC	*S. pneumoniae*	Ceftriaxone	Ceftriaxone
16	CAM	GNC	*N. meningitidis*	Ceftriaxone	Ceftriaxone
18	CAM	GNC	*N. meningitidis*	Ceftriaxone	Ceftriaxone
19	CAM	GNC	*N. meningitidis*	Ceftriaxone	Ceftriaxone
22	CAM	GNB	*H. influenzae*	Ceftriaxone	Ceftriaxone
Empirical therapy started after CSF Gram ($n=5$)					
6	Nosocomial	GPC	*S. epidermidis*	–	Vancomycin, ceftazidime
5	Nosocomial	GPC	*S. haemolyticus*	–	Vancomycin, ceftazidime
9	Nosocomial	GPC	*S. pneumoniae*	–	Ceftriaxone
21	Nosocomial	GNB	*R.ornithinolytica*	–	Vancomycin, ceftazidime
23	Nosocomial	GNB	*E. cloacae*	–	Meropenem+amikacin

CAM community-acquired meningitis, *GPC* Gram-positive cocci, *GNC* Gram-negative cocci, *GPB* Gram-positive bacilli, *GNB* Gram-negative bacilli, *MRSA* methicillin-resistant *Staphylococcus aureus*, *MSSA* methicillin-susceptible *Staphylococcus aureus*

these 74 patients. In the subgroup of patients with community-acquired meningitis, among 59 without indication for empirical therapy, antibiotics were withheld or stopped in 34 cases (58 %) (Fig. 3). This proportion was 47 % (7/15) in the subgroup of patients with nosocomial meningitis and neonatal sepsis.

The false-negative CSF Gram stain belonged to a patient with community-acquired *S. mitis* meningitis already treated with ceftriaxone and amoxicillin for clinical suspicion of encephalitis.

Discussion

To our knowledge, this is the first study addressing the impact of CSF Gram stain on overnight therapeutic decisions for suspected CNS infections. Round-the-clock CSF Gram stain was found to have a low impact on empirical therapy for patients with suspicion of CNS infection. Indeed, most patients with a positive Gram stain had other clinical or biological criteria for empirical therapy. Overnight CSF direct examination was associated with misinterpretations by the clinician and/or false-positive results, leading to the administration of unnecessary antibiotics. On the other hand, while most clinicians did not rely on a positive result to simplify combination therapy, empirical treatment could be withheld or stopped before definite microbiological results in the majority of patients without formal indications for antibiotic therapy.

CSF Gram stain is a simple, inexpensive and rapid test with 60–90 % sensitivity and 97 % specificity for the diagnosis of bacterial meningitis [3, 13, 14]. However, the performance varies greatly depending on CSF bacterial load, the type of microorganism and previous antibiotic exposure [1, 3, 15, 16]. Indeed, reported sensitivities range from 30 to 50 % for *Listeria monocytogenes* and GNB, to 75 %, 86–97 % and 90–97 % for *N. meningitidis*, *H. influenzae* and *S. pneumoniae*, respectively [8, 17–19]. Moreover, the accuracy of Gram stain examination may be further reduced overnight when performed by less-experienced laboratory technicians. The low sensitivity (43 %) of overnight CSF Gram stain in this study was likely related to the important proportion of nosocomial meningitis, mainly caused by staphylococci and Enterobacteriaceae, for which Gram stain sensitivity has been reported to be as low as 18 to 38 % [9, 10].

Surprisingly, a positive CSF Gram stain had little impact on the overnight antibiotic management of suspected CNS infections. Although the result of this test improved empirical therapy in some patients, only two cases of nosocomial meningitis (one without other clinical or biological criteria for empirical therapy and one with inadequate antibiotic spectrum) could have been treated inappropriately if overnight CSF Gram stain would not have been part of the decisional algorithm. Indeed, the majority of patients with bacterial meningitis, including four patients with nosocomial meningitis not receiving antibiotics before Gram stain result, had other criteria for empirical therapy than CSF microscopic examination alone. Accordingly, the choice of antibiotic regimen rarely relied on CSF Gram stain. Combination therapy was left unchanged in 8/11 patients despite the fact that only a single type of microorganism was seen in CSF, while mixed-flora meningitis is a rare event, reflecting the reluctance of clinicians to simplify empirical therapy before definite microbiological results. On the other hand, misinterpretation of Gram stain result by laboratory technicians or physicians led to the addition of unnecessary

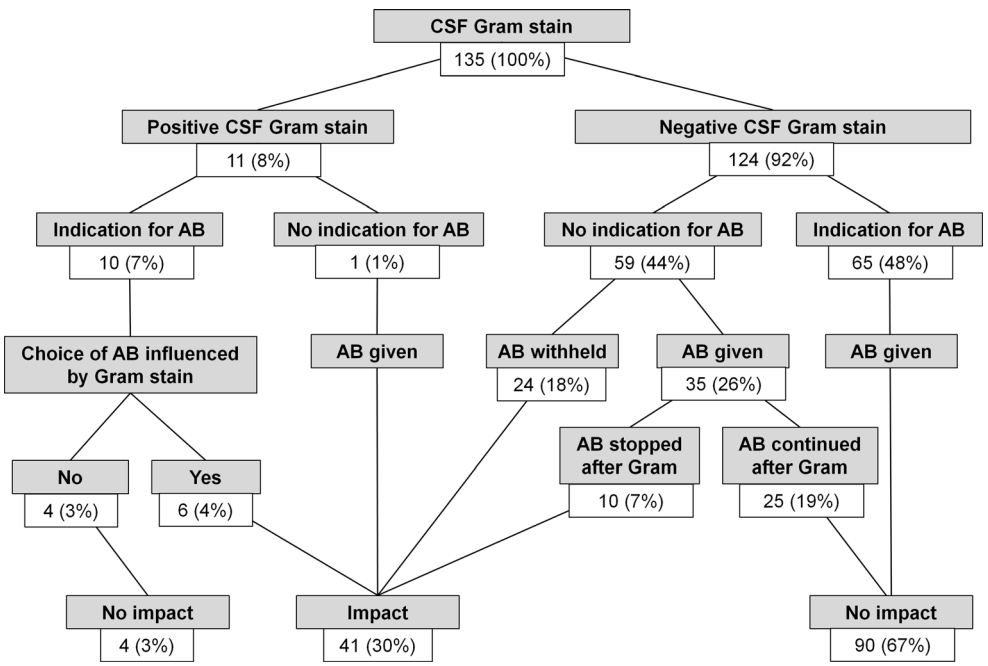


Fig. 3 Overall impact of overnight positive and negative CSF Gram stain on empirical antibiotic prescription in patients with community-acquired meningitis. *AB* antibiotics. Indication for AB: clinical or biological indication for empirical therapy other than CSF Gram stain

antibiotics in three patients and could not avoid an inappropriate empirical therapy in one patient with nosocomial meningitis.

The impact of a negative CSF Gram stain seemed somewhat more interesting. Although more than half of patients without formal indications for empirical therapy still received antibiotics despite the institutional algorithm, the knowledge of a negative CSF Gram stain allowed physicians to withhold or stop antibiotics before definite microbiological results in 54 % of them. As CSF Gram stain was part of the algorithm, the absence of overnight test would have likely resulted in all patients without CNS infection receiving unnecessary antibiotics, while waiting for the result of CSF Gram stain the next morning. In that regard, round-the-clock Gram stain probably reduced the overprescription of empiric therapy for suspicion of non-severe CNS infections in our institution.

CSF Gram stain is prone to false-positive as well as false-negative results, with important clinical implications. Factitious meningitis due to contaminated lots of commercial lumbar puncture trays, usually by non viable GNB, has been reported as a cause of false-positive Gram stains in up to 40 % of cases [20]. Conversely, false-negative CSF smears can result from low bacterial concentration. In 2,031 consecutive cases of community-acquired meningitis, visible microorganisms were found on direct examination in 25 % of samples with a bacterial load$\leq 10^3$ colony-forming units (CFU)/ml, 60 % in the range 10^3–10^5 CFU/ml and 97 % at concentrations $>10^5$ CFU/ml [16]. In the present study, all three false results were due to erroneous Gram stain interpretation by laboratory technicians. None had deleterious consequences on patient outcome. In all cases, CSF examination was performed by less-experienced technicians usually working outside the bacteriology unit, underlining the importance of inter-observer variability and the need to have this test performed by trained personnel. Indeed, a high rate of erroneous results could rightly undermine the confidence of attending physicians on this test and might further increase the proportion of inadequate antibiotic therapy.

This study had several limitations. The retrospective nature of the analysis limited the accurate assessment of empirical therapy for suspected CNS infection outside the actual clinical context, which might explain some of the observed deviations from the institutional algorithm. Although clinical and microbiological datasets were complete for all patients, some information was not available for several patients, such as the precise timing of antibiotic initiation in relation to lumbar puncture. Another limitation was the heterogenous population, comprising both community-acquired as well as nosocomial meningitis, two entities for which the performance of CSF Gram stain could differ. However, we chose to include all CSF samples for suspected CNS infections to obtain representative data of real-life situations outside working hours, although we acknowledge that our findings can only be extrapolated to institutions with a similar mix of CNS infections and similar management algorithms. Finally, we were not able to perform a cost-effectiveness analysis of overnight Gram stain, which is a mandatory step before deciding whether this test should still be performed round-the-clock.

In conclusion, overnight CSF Gram stain had a relatively low impact on therapeutic decisions. Most patients received appropriate empirical therapy for other clinical or biological indications than CSF Gram stain alone. Clinicians rarely relied on CSF Gram stain to simplify combination therapy in patients with established bacterial meningitis. However, Gram stain allowed withholding or stopping unnecessary antibiotics in more than half of patients without formal indication for empirical therapy. This might support proposing round-the-clock CSF Gram stain for guiding overnight empirical therapy in case of suspected CNS infections, despite its limitations.

Conflict of interest The authors declare that they have no conflict of interest.

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