Usefulness of Gram stain examination of peritoneal fluid in postoperative peritonitis to guide empirical antibiotherapy

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Received: 22 March 2019 / Accepted: 22 May 2019 / Published online: 29 May 2019
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Abstract

Purpose In postoperative peritonitis, Gram stain examination (GSE) of peritoneal fluid has been proposed as a guide for the prescription of glycopeptides and antifungal therapy in empirical antibiotherapy. No data support this approach for Gram-positive cocci. We aimed to evaluate the performance of GSE in predicting the results of the culture of peritoneal fluid.

Methods In this retrospective single-center study, concordance between GSE and culture of peritoneal fluid was assessed for different types of microorganisms. Factors associated with concordance of the two tests were evaluated in the subpopulation of Gram-positive cocci peritonitis.

Results Among the 152 episodes, the GSE was negative in 57 cases. The negative predictive value and the positive predictive value were 41% and 87% for Gram-positive cocci (GPC), 31% and 86% for Gram-negative bacilli, and 78% and 94% for fungi. GSE is not a reliable guide for the choice of empirical antibiotherapy and cannot reliably rule out the presence of GPC at culture. If we aim to achieve a high rate of adequacy, the systematic use of glycopeptide in the empirical antibiotherapy may be considered.

Conclusion GSE shows poor performance to predict the results of culture of peritoneal fluid in postoperative peritonitis. Avoiding covering resistant GPC cannot be based on the result of GSE.

Keywords Gram stain examination · Postoperative peritonitis

Introduction

Postoperative peritonitis (PP) is a life-threatening complication of abdominal surgery. Compared to community-acquired peritonitis, PP is associated with a high rate of multidrug-resistant (MDR) bacteria [1, 2]. All bacteria found at culture should be treated since reoperation [3–6]. It has been shown that inadequate empirical antibiotherapy (EA) was associated with unfavorable outcome [1, 7]. However, there is no reliable criterion to guide the choice of EA. In the setting of peritonitis, it is recommended to cover Gram-negative bacilli (GNB) and anaerobes. All proposed EA for PP also target beta-lactam-sensitive GPC [5, 6]. To achieve a high adequacy rate, it is proposed to target resistant GPC when risk factors for MDR bacteria are present [8]. Eligible antibiotics in this case are glycopeptides and tigecycline [5, 6].

Some authors and guidelines, including those published by IDSA, suggest guiding the prescription of antibiotics against resistant GPC by the presence of risk factors for

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colonization, a known previous colonization, or the result of Gram stain examination (GSE) of peritoneal fluid [6]. On the contrary, the last French guidelines indicate that GSE is not reliable enough to be used as a guide for the choice of EA. In fact, there is no evidence that GSE can reliably predict the presence of GPC, especially in the setting of PP, in which patients have been previously exposed to antibiotics. Previous antibiotic therapy is known to potentially alter the performance of GSE in other settings, such as in pneumonia [9]. Conversely, no study confirmed the lack of consistency of this approach. We focused on GPC because the result of GSE has no influence on the treatment of GNB, which are systematically targeted. The role of GSE for fungi in peritonitis has already been evaluated in many studies.

No data exists on the performance of the GSE of peritoneal fluid as a guide for EA for coverage of GPC. The goal of the study was to test the ability of GSE to predict the presence of GPC at culture. For the purpose of comparison, we also evaluated the performance of GSE to predict the presence of GNB and fungi. The secondary goal was to identify factors potentially influencing the performance of GSE in predicting the presence of GPC.

Materials and methods

Study population

From January 2008 through December 2011, all adult patients with PP requiring admission to the intensive care unit (ICU) were included prospectively in a database and their medical charts were reviewed retrospectively. Postoperative peritonitis was defined as peritoneal infection after abdominal surgery, confirmed by macroscopic findings and peritoneal fluid culture on reoperation yielding at least one colony. In accordance with French law, because this observational study did not modify the physicians' laboratory or clinical practices, the patients or relatives were simply informed about this study; informed consent was not required. The Paris North Hospitals, Paris 7 University, AP-HP Institutional Review Board (IRB), reviewed and approved the study.

Protocol management

Patients underwent the initial abdominal surgery in our hospital or another institution. They received prophylactic or curative antibiotic therapy in the presence of abdominal infection. All patients had been admitted to the ICU because of organ dysfunction, and all had received standard intensive care management.

Susceptibility testing and empirical antimicrobial therapy

Peritoneal fluid samples were collected systematically during reoperation and sent immediately to the bacteriology laboratory. The laboratory provides a round-the-clock GSE for peritoneal fluid. The results of GSE and cultures of peritoneal fluid were collected for bacteria and fungi in all PP. Cultures were performed with identification and susceptibility testing for Gram-positive and Gram-negative bacteria, and fungi. Antibiotic susceptibility was determined by the disk diffusion method, according to the criteria of the Antibiotic Susceptibility Testing Committee of the Société Française de Microbiologie [10]. Parenteral EA was started at the time of reoperation according to the recommendations of our institutional protocol. The adjunction of vancomycin was considered in cases of prolonged hospital stay or methicillin-resistant *Staphylococcus* or amoxicillin-resistant *Enterococcus* carriage.

Data collection

Only the first episode of PP was considered for analysis. For comparison, the following information on each patient was collected after admission to the ICU: age; sex; medical past history; previous reoperation; characteristics of initial surgery: type, route, and Altemeier wound classification of [11]; duration of antibiotic prescribed at initial surgery; interval antibiotic therapy defined as the administration of antibiotics between initial surgery and reoperation; interval antibiotic therapy and broad-spectrum interval antibiotic therapy; the simplified acute physiology score (SAPS II), and the Sequential Organ Failure Assessment (SOFA) score at the time of reoperation [12, 13]; medical condition at reoperation; time to reoperation; results of GSE, identification of pathogens in peritoneal fluid; and results of antimicrobial susceptibility tests.

Definitions

Immunosuppression was defined as current immunosuppressive therapy, corticoid therapy, or uncured cancer. Interval antibiotics (IA) related to any antibiotherapy prescribed between initial surgery and reoperation. Broad-spectrum IA comprised piperacillin/tazobactam, imipenem/cilastatin, cefotaxime, or ceftazidime. Antibiotherapy at initial surgery was the prophylactic or curative antibiotherapy prescribed at the time of initial surgery. Interval reoperation was any reoperation performed before the admission in ICU. Gram stain examination was classified according to its findings: negative GSE; GSE positive for GNB; GSE positive for GPC.

Resistant GPC were defined as GPC resistant to beta-lactam: amoxicillin-resistant enterococci and methicillin-resistant staphylococci [methicillin-resistant *Staphylococcus aureus*, coagulase-negative staphylococcus (CNS)]. For each type of bacteria, culture and GSE were defined as concordant, if both were positive, or both were negative. The concordance rate between GSE and culture for one type of bacteria was calculated as follows: numbers of concordant case/total number of PP involving this type of bacteria.

Statistical analysis

Categorical variables were expressed as numbers and percentages. Continuous variables were expressed as means and percentages. The performance of GSE in predicting the presence of GNB, GPC, and fungi was evaluated by calculating sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and positive and negative likelihood ratio. Categorical variables were compared using a Chi square or exact Fisher test as appropriate, and continuous variables were compared using *t* test or Mann–Whitney *U* test as appropriate. Factors associated with concordance between GSE and culture were determined by univariate analysis for PP with GPC. The significance level was 0.05. Statistical analyses were performed with R software 3.4.2 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

During the study periods, 152 patients were included in the study. Figure 1 shows the distribution of the population according to the results of the GSE results. Among the 152 episodes, 71 were positive for GPC, 95 for GNB, and 57 were negative at GSE. There were 16 GSE positive for fungi. Tables 1 and 2 show the types of GPC and GNB found at culture according to the results of GSE. The performance of

Fig. 1 Distribution of the population according to the results of the GSE results

Table 1 Distribution of different types of GPC according to the results of GSE

	Gram-positive cocci at GSE (71 PP)	No Gram-positive cocci at GSE (81 PP)
Negative culture for GPC	9 (13)	33 (41)
Positive culture for GPC	62 (87)	48 (59)
<i>Streptococcus</i> sp.	24 (34)	7 (9)
<i>Staphylococcus</i> sp.	22 (31)	20 (25)
<i>Enterococcus</i> sp.	33 (46)	27 (33)
Resistant GPC	18 (25)	25 (31)
<i>Staphylococcus</i> sp.	12 (17)	20 (25)
<i>Enterococcus</i> sp.	6 (8)	6 (7)

GSE Gram stain examination, PP postoperative peritonitis, GPC Gram-positive cocci

GSE to predict the results of cultures of peritoneal fluid is provided in Table 3. The GSE demonstrated a poor negative predictive value for GNB, GPC, and fungi. Not surprisingly, the performance of GSE for resistant GPC was also weak. Table 4 provides the ratio of negative and positive GSE for each type of bacteria. The concordance rate clearly varied according to the type of bacteria ($p=0.015$).

Analysis performed in the subpopulation of GPC PP showed that factors associated with concordance between GSE and culture for GPC were the duration of the antibiotic therapy prescribed for the initial surgery, an ongoing antibiotic therapy at the time of reoperation, the delay of reoperation, and the cause of PP (Table 5).

Discussion

The GSE of peritoneal fluid does not reliably predict the results of culture in the setting of PP. Therefore, if we aim to optimize the rate of adequate EA for PP, GSE cannot be used for guiding antibiotherapy.

Table 2 Distribution of different types of GNB according to the results of GSE

	Gram-negative bacilli at GSE (84 PP)	No Gram-negative bacilli at GSE (68 PP)
Negative culture for GNB	12 (14)	21 (31)
Positive culture for GNB	62 (74)	57 (84)
Enterobacteriaceae	71 (85)	51 (75)
Non-fermenting bacilli	12 (14)	11 (16)
EB and NFB	11 (13)	5 (7)

GNB Gram-negative bacilli, GSE Gram stain examination, EB enterobacteriaceae, NFB non-fermenting

Table 3 Performance of GSE in predicting the results of peritoneal fluid culture for GPC, RGPC, and GNB bacilli

	Sensitivity	Specificity	PPV	NPV	LR +	LR –
All GPC	56 (61/109)	77 (33/43)	86 (61/71)	41 (33/81)	2.4	0.6
RGPC	40 (17/42)	51 (56/110)	24 (17/71)	69 (56/81)	0.8	1.2
GNB	61 (72/119)	64 (21/33)	86 (72/84)	31 (21/68)	1.7	0.6
Fungi	35 (16/46)	99 (105/106)	94 (16/17)	78 (105/135)	35	0.7

PPV positive predictive value, NPV negative predictive value, LR likelihood ratio, CI confidence interval, GPC Gram-positive cocci, GNB Gram-negative bacilli, RGPC resistant Gram-positive cocci

Table 4 Ratio of negative and positive GSE for each type of bacteria found at culture of peritoneal fluid

	Strepto sp. (n = 31)	Entero sp. (n = 60)	Staphylo sp. (n = 43)	R entero sp. (n = 13)	R Staphylo sp. (n = 32)	EB (n = 111)	NFB (n = 23)	p
Neg GSE	7 (23)	27 (45)	21 (49)	4 (31)	21 (66)	40 (36)	11 (48)	0.015
Pos GSE	24 (77)	33 (55)	22 (51)	9 (69)	11 (34)	71 (64)	12 (52)	

Strepto Streptococcus, *Entero Enterococcus*, *Staphylo Staphylococcus*, *R Strepto* resistant *Streptococcus*, *R Entero* resistant *Enterococcus*, *R Staphylo* resistant *Staphylococcus*, *EB* Enterobacteriaceae, *NFB* non-fermenting bacilli, *NegGSE* negative Gram stain examination, *Pos GSE* positive Gram stain examination

We have observed that the performance of GSE in predicting the result of cultures varies according to the type of microorganism. The poor performance of GSE to guide the coverage of resistant GPC is not surprising. Nevertheless, some current guidelines still advocate the use of Gram stain examination to guide the prescription of glycopeptide. We do need studies to validate or refute this practice. This is the only study providing data regarding this issue.

It is proposed to base the prescription of glycopeptides on the GSE, and on the presence of risk factors for multidrug resistant bacteria [5, 6]. Some studies have explored the risk factors for MDR bacteria [14, 15], but no studies have specifically addressed the issue of predicting the presence of resistant GPC.

There are two reasons that may encourage restraining the use of glycopeptide. Besides the impact on renal function [16], it has been shown that glycopeptide administration was associated with an increased MIC of bacteria responsible for subsequent infections [17]. The administration of glycopeptide is not consensual, but GSE is still used to guide EA, including in PP, in everyday practice [18]. There is no published data indicating when the coverage of resistant GPC may be safely avoided [19].

All bacteria including resistant GPC in PP have to be treated [20, 21], but some specific issues are still debated [22]. The timing of initiation of glycopeptides has never been specifically addressed. Because of their lower pathogenicity, we may consider treating CNS and enterococci after receiving the result of peritoneal fluid culture. This

specific approach has not been investigated and would contradict the need for prompt adequate EA.

In our study, we found some potential factors decreasing the performance of GSE in predicting GPC at culture. Some non-evaluated factors, like the training of the doctor performing the GSE, or night shift timings may also affect the results of GSE. In the setting of ventilator-associated pneumonia (VAP), the sensitivity of GSE varies widely with a pooled sensitivity of GSE for VAP of 79% [23].

Some limitations exist. This is a single-center retrospective study. The result of GSE is highly operator dependent. Some institutions do not perform immediate GSE or cannot perform it during night shift hours. Some factors affecting the performance of GSE may not have been recorded. The role of anaerobes which could explain the poor performance of GSE has not been explored. This study is purely microbiological and was conducted with no relation to the clinical outcome.

Conclusion

Gram stain examination of peritoneal fluid is of poor performance in predicting the results of culture. Therefore, it cannot guide the prescription of glycopeptides when treating patients with PP.

Table 5 Characteristics of patients with GPC at culture: comparison of episodes with concordant and non-concordant GSE

	No concordance (n = 48)	Concordance (n = 61)	
General characteristics			
Gender (male)	31 (65)	32 (52)	0.20
Age (years)	62 ± 18	59 ± 16	0.28
Immunosuppression	16 (33)	25 (41)	0.41
Diabetes mellitus	8 (17)	9 (15)	0.78
Antibiotherapy 3 months prior to initial surgery	9 (19)	20 (33)	0.10
Hospitalization 3 months prior to initial surgery	16 (33)	30 (49)	0.10
Initial surgery			
Upper gastrointestinal tract for initial surgery	24 (50)	25 (41)	0.35
Septic or contaminated initial surgery	24 (50)	22 (36)	0.14
Emergent initial surgery	23 (48)	21 (34)	0.15
Duration of initial antibiotherapy (days)	2 (1–4)	1 (1–2)	0.048
Interval reoperation	13 (27)	15 (25)	0.77
Interval antibiotherapy	34 (71)	34 (56)	0.11
Broad-spectrum interval antibiotherapy	21 (44)	19 (31)	0.18
Reoperation			
Current antibiotherapy at the time of reoperation	33 (69)	29 (48)	0.026
Time since initiation of current antibiotherapy (days)	2 (0–4)	0 (0–5)	0.33
Time to reoperation (days)	7 (4–9)	9 (6–17)	0.002
SAPS 2 on admission in ICU	48 ± 15	45 ± 16	0.37
SOFA on admission in ICU	7 ± 4	7 ± 4	0.67
White blood cell count (10 ⁹ /L)	17.1 ± 12	19.1 ± 12	0.33
Source of PP			
Upper gastrointestinal tract cause	20 (42)	22 (36)	0.37
Non-upper gastrointestinal tract cause	21 (44)	34 (56)	
No cause found	7 (15)	5 (8)	
Cause of PP			
Bowel perforation	8 (17)	27 (44)	0.018
Anastomosis leakage	19 (40)	18 (30)	
Other cause	14 (29)	11 (18)	
No cause found	7 (15)	5 (8)	

Categorical variables expressed as number (percentage), normally distributed variables expressed as mean ± standard deviation, and non-normally distributed variables expressed as median (IQR)

PP postoperative peritonitis

Compliance with ethical standards

Conflict of interest Pascal Augustin, Alexy Tran-Dinh, Mathieu Desmard, Sébastien Tanaka, Nathalie Grall, Mouna Ben-Rehouma, Konstantinos Arapis, Lara Ribeiro-Parenti, and Philippe Montravers declare that they have no conflict of interest.

Research involving human participants Authorization of the institutional review board was obtained.

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