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ORIGINAL ARTICLE

Risk factors and lethality of laboratory-confirmed bloodstream infection caused by non-skin contaminant pathogens in neonates☆

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KEYWORDS
Infant, newborn;
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Abstract
Objective: To evaluate risk factors and lethality of late onset laboratory-confirmed bloodstream infection (LCBI) in a Brazilian neonatal unit for progressive care (NUPC). *Methods:* This was a case-control study, performed from 2008 to 2012. Cases were defined as all newborns with late onset LCBI, excluding patients with isolated common skin contaminants. Controls were newborns who showed no evidence of late onset LCBI, matched by weight and time of permanence in the NUPC. Variables were obtained in the Hospital Infection Control Committee (HICC) database. Analysis was performed using the Statistical Package for the Social Sciences (SPSS). The chi-squared test was used, and statistical significance was defined as $p < 0.05$, followed by multivariate analysis.

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Results: 50 patients with late onset LCBI were matched with 100 patients without late onset LCBI. In the group of patients with late onset LCBI, a significant higher proportion of patients who underwent surgical procedures (p = 0.001) and who used central venous catheter (CVC) (p = 0.012) and mechanical ventilation (p = 0.001) was identified. In multivariate analysis, previous surgery and the use of CVC remained significantly associated with infection (p = 0.006 and p = 0.047; OR: 4.47 and 8.99, respectively). *Enterobacteriacea* was identified in 14 cases, with three (21.4%) deaths, and *Staphylococcus aureus* was identified in 20 cases, with three (15%) deaths.
Conclusions: Surgical procedures and CVC usage were significant risk factors for LCBI. Therefore, prevention practices for safe surgery and CVC insertion and manipulation are essential to reduce these infections, in addition to training and continuing education to surgical and assistance teams.

PALAVRAS-CHAVE
Neonato;
Recém-nascido;
Sepse;
Vigilância;
Controle de infecções

Fatores de risco e letalidade de infecção da corrente sanguínea laboratorialmente confirmada, causada por patógenos não contaminantes da pele em recém-nascidos

Resumo
Objetivo: Avaliar os fatores de risco e a letalidade da infecção da corrente sanguínea laboratorialmente confirmada (ICSLC) de início tardio em uma Unidade Neonatal de Cuidados Progressivos (UNCP) brasileira.
Métodos: Trata-se de um estudo caso-controle realizado de 2008 a 2012. Os casos foram definidos como todos os recém-nascidos com ICSLC de início tardio, excluindo pacientes isolados com contaminantes da pele comuns. Os controles foram recém-nascidos que não mostraram qualquer evidência de ICSLC de início tardio, sendo separados por peso e tempo de permanência na UNCP. As variáveis foram obtidas na base de dados da Comissão de Controle de Infecção Hospitalar (CCIH). A análise foi realizada utilizando o Pacote Estatístico para Ciências Sociais. O teste χ^2 foi utilizado e a relevância estatística foi definida como p < 0,05, seguida pela análise multivariada.
Resultados: No estudo, 50 pacientes com ICSLC de início tardio foram combinados com 100 pacientes sem ICSLC de início tardio. No grupo de pacientes com ICSLC de início tardio, identificamos uma proporção significativamente maior de pacientes que foram submetidos a procedimentos cirúrgicos (p = 0,001) e que usaram cateter venoso central (CVC) (p = 0,012) e ventilação mecânica (p = 0,001). Na análise multivariada, cirurgia prévia e uso de CVC permaneceram significativamente associados à infecção (p = 0,006 e p = 0,047; OU: 4,47 e 8,99, respectivamente). ***A Enterobacteriacea*** foi identificada em 14 casos, com três (21,4%) óbitos, e *Staphylococcus aureus* foi identificado em 20 casos, com três (15%) óbitos.
Conclusões: Procedimentos cirúrgicos e uso de CVC constituíram fatores de risco significativos para ICSLC. Portanto, práticas de prevenção para cirurgia segura, inserção e manipulação de CVC são essenciais para reduzir essas infecções, além de treinamento e educação contínua às equipes cirúrgicas e de assistência.

Introduction

The incidence of neonatal sepsis is highly variable among different hospitals. Comparison among countries also reveals wide variation, with incidence densities of 3.6 to 18.1 infections per 1,000 patient-days in the United States,[1,2] from 6.9 to 7.8 infections per 1,000 patient-days in Italy,[3,4] from 10.9 to 17.3 infections per 1,000 patient-days in Turkey,[5] and 28.6 infections per 1,000 patient-days in a German center.[6] In Brazil, a multicenter study found 25 infections per 1,000 patient-days,[7] similar to data obtained in a private hospital in São Paulo (23.8 infections/1,000 patient-days).[8]

Neonatal mortality from sepsis is high, reaching 68% in Brazil from 2000 to 2008,[9] indicating the necessity of prioritizing preventive actions for healthcare-associated infections (HAI) in this age range. HAI in newborns should be considered a serious event, since sepsis is one of the main causes of neonatal death and is one of the focus of epidemiological surveillance.[10]

Early sepsis is related to prenatal and perinatal assistance, which depends on joint action with obstetricians and the

quality of care at the level of primary assistance. During late sepsis, newborns are generally affected by microorganisms acquired after delivery by human contact or by indirect contact with a contaminated environment. Thus, horizontal transmission plays an important role in late-onset disease, and preventive interventions to minimize this exposure should be performed in neonatal units.[10,11]

Several risk factors are associated with late sepsis, including birth weight; use of invasive devices such as a central venous catheter (CVC) and mechanical ventilation (MV); delay in enteral nutrition; parenteral nutrition; and complications of prematurity, such as arterial patent ductus, bronchopulmonary dysplasia, and necrotizing enterocolitis, which often require surgical intervention.[12]

High-risk newborns are considered more susceptible to nosocomial infections such as late-onset sepsis. Underlying disease, deficient immunity, microbiota in the neonatal intensive care unit, and the invasive procedures required for assisting newborns favors nosocomial infections in these patients. Breaks in the natural barriers of the skin and intestines allow opportunistic microorganisms to disseminate into the bloodstream, which occurs mainly in premature infants due to the immaturity of the immune system.[13]

The present study aimed to evaluate the risk factors and lethality of late onset laboratory-confirmed bloodstream infection (LCBI) in the neonatal unit for progressive care (NUPC) in a referral hospital.

Methods

The NUPC of the Hospital das Clínicas of the Universidade Federal de Minas Gerais is a tertiary referral center for the municipality and state that assists newborns with various clinical conditions, especially high-risk cases. This case-control study was performed from January, 2008 to May, 2012.

Case definition

All newborns notified with late onset LCBI according to criteria of infection for neonatology defined by the National Agency of Sanitary Surveillance (Agência Nacional de Vigilância Sanitária – ANVISA)[10] were included, considering that no other site of infection was evident. All newborns had clinical symptoms defined by notification criteria and were treated for sepsis. Due to the nonspecific signs and symptoms of neonatal sepsis, and considering the possibility of other diagnoses, only cases of LCBI with recognized pathogens from blood cultures were included. Only the first episode of LCBI was included, and patients were included once.

Infections by common skin contaminant microorganisms (such as coagulase-negative *Staphylococcus* spp.) were excluded because the criteria require isolation of microorganisms in two blood samples taken from two different venous punctures and association with clinical signs or symptoms.[10] In the NUPC, blood cultures are always performed in a newborn with suspicion of bloodstream infection before antimicrobial therapy, but treatment is immediately started due to severity of infection in these patients, which may reduce the sensitivity of further samples. Besides, two blood culture samples were not always obtained, because of the technical difficulties in collecting samples for performing blood cultures in infants. However, if sepsis was suspected and coagulase-negative *Staphylococcus* was isolated in one blood culture, treatment was instituted by physicians.

Control definition

Controls were newborns in the NUPC who showed no evidence of late onset LCBI during the study period. Pairing was performed by weight in a 1:2 (case:control) proportion and time of hospitalization in the unit, considering up to seven days of difference.

Blood samples

A total of 1 mL of blood was collected when sepsis was suspected, and specimens for culture were routinely sent to the microbiology laboratory. Microorganism isolation was performed with the automated method (VITEK2), and susceptibility testing was performed by agar disk diffusion (Kirby Bauer) to confirm the resistance profile. The sensitivity profile of microorganisms considered definitions of the Hospital Infection Control Commission (HICC), based on Clinical and Laboratory Standards Institute (CLSI).

Data collection

Data of interest were obtained from the database of the HICC considering active surveillance and included: total number of patients in the NUPC, total of notified HAI, patient-day, density of infection of HAI, and all early and late onset clinical sepsis and LCBI in the period of study.

Catheter-associated LCBI included all LCBI that occurred in patients with CVC or in newborns who had the device removed within the 48-hour period before the onset of infection, other sites of infection were excluded.[10]

For the bivariate logistic analysis, the following variables were considered: gender, surgery, and use of invasive devices (CVC and MV) before the first episode of LCBI considered as case, previous notification of early onset sepsis, use of antimicrobials for less than three days for treatment of early suspected sepsis, and use of antimicrobials agents for at least seven days for treatment of early onset sepsis. For controls, events were considered up to the end of follow up, considering pairing.

Death was considered associated to infection when the event occurred during LCBI treatment.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 13.0 was used for analysis. The weight range (matching variable) and isolated microorganisms (case definition criteria) were considered only in the descriptive analysis.

In the comparative analysis between groups (case:control), the chi-squared test was used for categorical variables. Statistical significance was defined as $p < 0.05$. Logistic regression for multivariate analysis was used when the variables were significant in the univariate analysis with respect to the response variable LCBI. In the literature, p values less than 0.10 have been used for variables included in multivariate analysis; although this is a narrow cutoff value, it provides a more rigorous analysis. Several similar studies[8,14,15] also considered variables with a significance of $p < 0.10$ or $p < 0.05$ for logistic regression.

Ethical considerations

This study is part of the Surveillance and Infection Control in Neonatology activities defined by HICC, and was approved by the institutional review board (ETIC 312/08).

Results

During the study period, a total of 1,414 newborns were admitted in the NUPC, comprising 28,530 patient-days. A total of 710 episodes of HAI were notified, with a density of incidence of 24.89 infections/1,000 patient-days. Of these episodes, 152 (21.4 %) were early onset sepsis and 246 (34.7%) were late onset sepsis, with 140 and 100 episodes of clinical early and clinical late onset sepsis, respectively. The total number of patients in the NUPC, patients-days, number of HAI, and density of incidence of HAI are presented by weight range in Table 1.

Twelve and 146 episodes of early and late onset LCBI were notified, respectively. Considering late onset LCBI, coagulase-negative *Staphylococci* were isolated in 59 (40.4%). A total of 129 (88.4%) were late onset LCBI catheter-associated infections: 73 (56.59%) with recognized pathogens and 56 (43.41%) with skin contaminant pathogens.

Fifty patients with first episode of late onset LCBI with isolation of non-skin contaminant pathogens in blood cultures were considered as cases, and were matched with 100 controls without late onset LCBI. The distribution of cases and controls by weight range is shown in Table 1. The majority of studied patients ranged in weight from 751 g to 2,500 g (74 % of cases and controls).

Comparative analysis showed a greater proportion of patients who underwent surgical procedures in the group of patients with late onset LCBI, corresponding to 15 (30%) cases, compared to eight (8%) controls ($p = 0.001$). The odds ratio (OR) revealed a 4.93 higher probability of surgical procedures in patients with late onset LCBI (95% confidence interval [CI], 1.92-12.65). Additionally, a higher proportion of patients who used CVC ($p = 0.012$) and MV ($p = 0.001$) was observed in cases than controls, with an OR of 10.76 (1.39-83.10) and 3.87 (95% CI, 1.81-8.27), respectively. Statistical differences were not observed when other analyzed variables were considered (Table 2).

In the multivariate analysis (Table 2), three variables were included: prior surgery, use of CVC, and use of MV. Previous surgery and the use of CVC remained significantly associated with infection ($p = 0.006$ and $p = 0.047$, respectively), but the use of MV showed only a tendency to be associated with infection ($p = 0.058$). Surgery presented OR = 4.47 (95% CI, 1.54-12.94) and use of CVC presented OR = 2.21 (95% CI, 1.03-78.34).

A total of 26 surgeries were performed in 23 patients. The cases underwent only one procedure each, and 11 of these procedures were performed among eight controls. Among the surgical procedures performed in the 15 cases, seven (46.67%) consisted of digestive tract surgery, and four of these involved gastroschisis correction. Among the controls, the majority (n = 7, 63.63%) of procedures were surgery other than the digestive tract (Table 3).

The proportion of deaths among the cases (n = 6; 12%) did not differ statistically from the control group (n = 9; 9%) ($\chi^2 = 0.08$, $p = 0.77$). Death occurred in three (21.43%) cases with late onset LCBI with isolation of *Enterobacteriacea*, and in three (15%) cases with isolation of *Staphylococcus aureus* (Table 4). Considering the mortality of patients with late onset LCBI associated to coagulase-negative *Staphylococcus* notified during the studied period, only one (1.69%) of the 59 newborns died, but this patient also presented *Candida parapsilosis* in the following blood culture. Thus, higher proportion of death was observed in patients with late onset LCBI associated to recognized pathogens than to skin contaminant pathogens.

Discussion

In this study, a higher proportion of patients who underwent previous surgical procedures was observed among patients with late onset LCBI. Surgery is not a variable frequently cited

Table 1 Distribution of newborns according to weight range, neonatal unit for progressive care, HC/Universidade Federal de Minas Gerais, from January, 2008 to May, 2012.

Weight range	Cases n (%)	Controls n (%)	Total of patients n (%)	Total of HAI n (%)	Patient-days (%)	Incidence density of HAI
Up to 750 g	4 (8)	8 (8)	31 (2.19)	55 (7.75)	1,463 (5.13)	37.59
751 to 1,000 g	11 (22)	22 (22)	82 (5.80)	112 (15.77)	4,302 (15.08)	26.03
1,001 to 1,500 g	9 (18)	18 (18)	203 (14.36)	127 (17.89)	5,872 (20.58)	21.63
1,501 to 2,500 g	17 (34)	34 (34)	558 (39.46)	245 (34.51)	9,458 (33.15)	25.90
Higher than 2,500 g	9 (18)	18 (18)	540 (38.19)	171 (24.08)	7,435 (26.06)	23.00
Total	50 (100)	100 (100)	1414 (100)	710 (100)	28,530 (100)	24.89

HAI, healthcare associated infections.

Table 2 Univariate and multivariate logistic analysis for risk factors for laboratory-confirmed bloodstream infection, neonatal unit for progressive care, HC/Universidade Federal de Minas Gerais, from January, 2008 to May, 2012.

	Laboratory-confirmed bloodstream infection		Univariate analysis			Multivariate analysis		
	Cases n = 50	Controls n = 100	p	OR	95% CI	p	OR	95% CI
Gender								
Male n (%)	30 (60)	48 (48)	0.225	1.63	0.82-3.24	–	–	–
Previous surgery								
Yes n (%)	15 (30)	8 (8)	**0.001**	4.93	1.92-12.65	**0.006**	4.47	1.54-12.94
Previous use of CVC								
Yes n (%)	49 (98)	82 (82)	**0.012**	10.76	1.39-83.10	**0.047**	8.99	1.03-78.34
Previous use of MV								
Yes n (%)	38 (76)	45 (45)	**0.001**	3.87	1.81-8.27	**0.058**	2.21	0.97-5.01
Early onset sepsis								
Yes n (%)	11 (22)	13 (13)	0.238	1.89	0.78-4.58	–	–	–
Use of ATM for suspected early onset sepsis (≤ three days)								
Yes n (%)	14 (28)	30 (30)	0.949	0.91	0.43-1.92	–	–	–
Use of ATM for early onset sepsis (≥ seven days)								
Yes n (%)	17 (34)	32 (32)	0.951	1.10	0.53-2.25	–	–	–

95% CI, 95% confidence interval; ATM, antimicrobial agent; CVC, central venous catheter; MV, mechanical ventilation; OR, odds ratio.

Table 3 Frequency of surgical procedures performed in neonates with late onset LCBI and controls, neonatal unit of progressive care, HC/Universidade Federal de Minas Gerais, from January, 2008 to May, 2012.

Surgery type	Cases n = 50 (%)	Controls n = 100 (%)	Total n (%)
Digestive	7 (14)	4 (4)	11 (42.31)
Cardiovascular	4 (8)	2 (2)	6 (23.08)
Neurological	1 (2)	3 (3)	4 (15.38)
Genitourinary	1 (2)	1 (1)	2 (7.69)
Musculoskeletal	1 (2)	1 (1)	2 (7.69)
Thoracic	1 (2)	0	1 (3.85)
Total	15 (30)	11 (11)	26 (100)

in studies that include risk factors for sepsis in neonates. In a prospective study conducted in a pediatric intensive care unit in the United States, the incidence of nosocomial infections in postoperative patients was twice of that in patients who did not undergo surgical procedures.[14] Some authors have reported a higher risk of bloodstream infections after surgery for neonates than for older children.[16,17]

Among cases undergoing surgery, a high proportion of procedures involving the digestive tract, which is colonized by bacteria that are more aggressive in infants who remain hospitalized in intensive care units from birth, were observed.[18] Increased risk of infection should be considered in preterm infants who have immature gastrointestinal mucosal barrier, lower levels of IgA, and may who have reduced gastric acidity.[13] Mokkadas et al.[16] also described higher frequency of confirmed sepsis (87.2%) in patients with gastrointestinal anomalies that demanded surgical interventions.

A study in Turkey[5] also evaluated cases of laboratory-proven HAI, and demonstrated that lower birth weight, gestational age, and APGAR scores, in addition to longer hospitalization and antibiotic use, were associated with infection.

Regarding other risk factors, a study in Saudi Arabia[19] reported that prolonged use of devices was the only independent risk factor for the occurrence of catheter-associated LCBI. However, that analysis included infections caused by common skin contaminants. Similarly, a multicenter study in Italy[20] observed that the use of umbilical arterial and venous catheters and MV for more than five days increased the risk of sepsis in neonates. That study also showed that additional risk factors include birth weight less than 2,500 g, use of a nasogastric tube or total parenteral nutrition, and transfers from other hospitals. In a study performed by Aurita et al.,[4] also in Italy, risk factors for infection in very low birth weight newborns included

Table 4 Distribution of microorganisms isolated from patients with late onset laboratory-confirmed bloodstream infection and associated deaths, neonatal unit for progressive care, HC/Universidade Federal de Minas Gerais, from January, 2008 to May, 2012.

Microorganisms	Episodes n	Deaths n (%)
GNR	21	3 (14.29)
Non-fermenting GNR	7	0
Acinetobacter baumannii	2	0
Burkholderia cepacia	1	0
Flavobacterium meningosepticum	1	0
Pseudomonas aeruginosa	3	0
Enterobacteriaceae	14	3 (21.43)
Enterobacter spp.	6	2 (33.33)
Escherichia coli	1	0
Klebsiella spp.	6	1 (16.67)
Serratia marcescens	1	0
Fungi	7	0
Candida albicans	2	0
Candida non-*albicans*	5	0
Gram-positive cocci	22	3 (13.64)
Enterococcus faecalis	2	0
Staphylococcus aureus	20	3 (15)
Total	50	6 (12)

GNR, gram-negative rod.

gestational age below 28 weeks, a clinical risk index for infants greater than 4, and the use of continuous positive airway pressure (CPAP). Among newborns with higher birth weight, the risk factors were malformations and use of parenteral nutrition. However, the aforementioned study included patients with clinical sepsis or with infection by coagulase-negative *Staphylococcus*.

In Brazil, few other studies have evaluated risk factors specifically related to bloodstream infection in neonates. In a neonatal unit of a private hospital in São Paulo,[8] it was concluded that premature rupture of membranes, maternal illness, use of MV and CVC, and more significantly, the use of parenteral nutrition were risk factors for sepsis, regardless of laboratory confirmation. The assessment of neonatal infections that met National Healthcare Safety Network (NHSN) criteria in a university hospital in Uberlândia[15] also revealed an association between infection and use of MV, CVC, and nasogastric tube. In the present study, CVC also indicated a higher risk for LCBI, even considering only first episodes of these infections not associated to skin contaminant pathogens.

In the present study, the outcome variable was restricted to LCBI caused by pathogenic microorganisms because this provided higher specificity for defining the infection criteria. This specificity is not found in most studies in the international[3,4,5,19] or national[8,15,21] literature. Although coagulase-negative *Staphylococcus* is the most important microorganism of sepsis reported in literature,[3-5,7,8,15,21] difficulties were found in fulfilling the infection criteria associated to this microorganism because two blood culture samples are necessary to notify LCBI.[10] This fact limits comparisons of this study with others, but better enables targeted actions in the studied location.

Considering the epidemiology of LCBI, several pathogens can be responsible for neonatal sepsis. Gram-negative *Enterobacteriaceae* or non-fermenters (such as *Pseudomonas aeruginosa*) and Gram-positive bacteria, particularly *Staphylococcus* and *Streptococcus* spp., are the main groups mentioned in literature.[8,16] In addition, approximately 1% of infants in neonatal intensive care units and 2% to 4.5% of newborns with low birth weight have septicemia caused by fungi, mainly *Candida* spp.[22]

In a ten-year prospective study in a Brazilian neonatal unit,[21] Gram-negative microorganisms (*Escherichia coli* and *Klebsiella* spp.) accounted for 51.6% of cases of LCBI, Gram-positive microorganisms accounted for 37.4% of cases (mainly coagulase-negative *Staphylococcus* spp.), and *Candida* spp. was the fourth most isolated microorganism, which is consistent with the findings of the present study. Gram-positive cocci and fungi predominate in neonatal units with greater resources, while Gam-negative enteric bacilli and fungi are more commonly described in resource-limited settings.[23]

A systematic review that included 11,471 blood cultures of neonates with sepsis in developing countries revealed that Gram-negative bacteria were isolated in at least 60% of positive samples.[24] A total of 42% of positive samples in this study had isolated Gram-negative bacteria.

Factors related to Gram-positive agents as causes of nosocomial infections in newborns are prolonged hospitalization, use of venous catheterization, parenteral lipids, skin lesions, and other invasive procedures. Cross-transmission through hands is another important dissemination route. *S. aureus* agents are less frequent causes of neonatal infections, but exhibit high virulence due to the susceptibility of this population, with three-fold higher risk of complications and morbidity, and a mortality rate reaching 55%,[25,26] regardless of the antimicrobial resistance. In this study, *S. aureus* was the most frequent microorganism isolated in LCBI with recognized pathogens.

Bloodstream infection in neonates is still a major cause of neonate morbidity and mortality.[27] In this study, a high mortality was observed in neonates with LCBI associated to *Enterobacteriaceae* (21.43%) *and S. aureus* (15%). The letality rate for LCBI associated to Gram-negative bacteria in neonates is even higher in the literature (40% to 90%).[13] Fungi account for an overall mortality of 25% to 50% in neonatal infections,[13,22] although death associated to these microorganisms was not observed in the present study.

HAI prevention practices should be prioritized in neonatal units because of the high-risk population. These practices should include early enteral feeding, use of breastfeeding, and reduced time of hospitalization in the intensive care unit, as well as training and continuing education for the healthcare team.[13,23] Given the importance of surgery as a risk factor for LCBI in the studied neonatal unit, the relevance of guidelines for safe surgery should be emphasized. All professionals involved in the procedure should use antiseptic surgical hand scrub

and follow appropriate antisepsis techniques.[28] In addition, this study focused on late onset LCBI and 98% of studied cases used CVC, revealing the importance of appropriate practices for insertion and manipulation of CVC used for intravenous fluids, medications, blood products, and parenteral nutrition. Therefore, professionals should adopt comprehensive standard procedures for preventing catheter-associated infections; multidisciplinary education programs and infection surveillance are recommended.[29,30]

The adoption of a surveillance and control of infection program in the neonatal unit has been described as an efficient and low-cost program.[11] These activities are carried out continuously in the studied NUPC in association with the HICC, and studies on surgical procedures are priorities for intervention, along with participation and adherence to prevention practices by the surgical team.

In conclusion, this study found that a significative higher proportion of surgery procedures and use of CVC was observed in patients with late onset LCBI. In addition, infants who remain hospitalized in the neonatal unit since birth, including those who require surgical treatment, undergo a greater number of interventions, are exposed to invasive devices such as CVC, and possibly, are colonized by more pathogenic agents. Therefore, prevention practices are essential to reduce these infections. Furthermore, these practices should be monitored, and training and continuing education should be provided to the surgical team.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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