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Original Research

CRITICAL CARE MEDICINE

Steady-State Pharmacokinetics and BAL Concentration of Colistin in Critically Ill Patients After IV Colistin Methanesulfonate Administration

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Background: **Infections caused by multidrug-resistant gram-negative bacteria have caused a resurgence of interest in colistin. To date, information about pharmacokinetics of colistin is very limited in critically ill patients, and no attempts have been made to evaluate its concentration in BAL.**
Methods: **In this prospective, open-label study, 13 adult patients with ventilator-associated pneumonia caused by gram-negative bacteria were treated with colistin methanesulfonate (CMS) IV, 2 million International Units (174 mg) q8h, a usually recommended dose, for at least 2 days. Blood samples were collected from each patient at time intervals after the end of infusion. BAL was performed at 2 h. Colistin was measured by a selective, sensitive high-performance liquid chromatography-based method. Pharmacokinetic parameters were determined by noncompartmental analysis.**
Results: **Patients received 2.19 ± 0.38 mg/kg (range, 1.58-3.16) of CMS per dose. At steady state, mean ± SD plasma colistin maximum (Cmax) and trough (Ctrough) concentrations were 2.21 ± 1.08 and 1.03 ± 0.69 μg/mL, respectively. Mean ± SD area under the plasma concentration-time curve from 0 to 8 h (AUC_{0-8}), apparent elimination half-life, and apparent volume of distribution were 11.5 ± 6.2 μg × h/mL, 5.9 ± 2.6 h, and 1.5 ± 1.1 L/kg, respectively. Cmax/minimum inhibitory concentration (MIC) ratio and AUC_{0-24}/MIC ratio (MIC = 2 μg/mL) were 1.1 ± 0.5 and 17.3 ± 9.3, respectively. Colistin was undetectable in BAL. Nephrotoxicity was not observed.**
Conclusions: **Although the pharmacodynamic parameters that better predict the efficacy of colistin are not known in humans, in critically ill adult patients the IV administration of CMS 2 million International Units (174 mg) q8h results in apparently suboptimal plasma concentrations of colistin, which is undetectable in BAL. A better understanding of the pharmacokinetic-pharmacodynamic relationship of colistin is urgently needed to determine the optimal dosing regimen.**

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Abbreviations: AUC = area under the plasma concentration-time curve; CF = cystic fibrosis; CL/fm = apparent total body clearance of formed colistin; Cmax = maximum concentration; CMS = colistin methanesulfonate; Ctrough = minimum concentration at predose; HPLC = high-performance liquid chromatography; Kel = apparent elimination rate constant; MIC = minimum inhibitory concentration; $t_{1/2}$ = apparent elimination half-life; Vd/fm = apparent volume of distribution of formed colistin

Colistin (also known as polymyxin E) consists of a complex mixture of polymyxins, the two main ones being colistin A (polymyxin E_1) and colistin B (polymyxin E_2).[1] Introduced in the late 1950s, colistin was the first antibiotic with notable in vitro activity against *Pseudomonas aeruginosa*,[2] although it was relegated to a second-line antibiotic because of its potential systemic toxicity, including neurotoxicity and nephrotoxicity.[3-5] Recently, however, there has been a resurgence of interest in its use for the treatment of infections caused by some gram-negative bacteria, especially *Acinetobacter baumannii* and *P aeruginosa*, which are resistant to almost all currently available antibiotics, except colistin.

Colistin is administered systemically (parenterally) as colistin methanesulfonate (CMS). CMS, which is inactive, is converted to colistin (the active form with antimicrobial activity) both in vitro and in vivo by hydrolysis of methane sulphonate radicals.[6,7] Colistin is a bactericidal antibiotic that acts as a detergent on the cytoplasmic membrane of bacteria. A study conducted on *P aeruginosa* isolates from patients with

cystic fibrosis (CF) has shown that it has a concentration-dependent killing and a modest post-antibiotic effect.[8]

Most available data on colistin and CMS pharmacokinetics in humans have been obtained evaluating its concentrations in plasma and urine by microbiological assays,[9,10] which are unable to quantify colistin and CMS separately. Recently, two reliable high-performance liquid chromatography (HPLC)-based methods have been described. These methods enable colistin and CMS to be measured in biologic specimens accurately and separately.[11,12]

Colistin is generally used in patients with CF and in critically ill patients affected by multidrug-resistant gram-negative bacteria.[13-19] Information about pharmacokinetics is necessary to establish the optimal dosage in critically ill patients, in whom the pharmacokinetics of many antibiotics is altered,[20,21] but to date such information is very limited.[17-19] Moreover, the concentration of colistin in BAL after IV infusion has never been determined. The aim of this study was to evaluate the plasma pharmacokinetics and BAL concentration of colistin at steady state in critically ill patients after IV administration of CMS.

Materials and Methods

Study Population

The pharmacokinetics and concentration of colistin in BAL were evaluated in 13 adult patients (aged 20-70 years; 10 men, three women) who during their ICU stay developed ventilator-associated pneumonia caused by *A baumannii* (11 patients) and *P aeruginosa* (two patients). In nine patients, *Acinetobacter* was multidrug resistant. Patients with creatinine clearance <80 mL/min were excluded. The creatinine clearance was calculated according to the formula $ClCr = UCr \times V/PCr$, where UCr is the concentration of creatinine in the urine, V is the urine flow rate, and PCr is the plasma concentration of creatinine.

Simplified acute physiology II score at admission was 48.2 ± 17.1, and the sequential organ failure assessment score on the day of sampling was 4.6 ± 2.8 (Table 1). A bacteriological diagnosis of ventilator-associated pneumonia was available in all patients. The study protocol was approved by the local ethical committee (Comitato di Bioetica, Fondazione IRCCS Policlinico S. Matteo, Pavia; approval number 1015, 10-02-2007).

Treatment Schedule

Patients were treated with CMS (Colimicina; UCB Pharma; Pianezza, Italy) 2 million International Units (174 mg) IV every 8 h (the CMS content is not reported in milligrams in the product information, but the manufacturer provided us this information in writing). The dose of CMS (174 mg) was the actual dose of the methanesulfonate species and not the colisitin-equivalent dose. CMS was dissolved in 50 mL of saline solution and administered over 30 min by a volumetric pump. The solutions were prepared just before administration. Potential nephrotoxicity was assessed by daily measurements of serum creatinine.

Pharmacokinetic Procedures

Evaluation of colistin concentrations in plasma and BAL was performed after at least 2 days of treatment with CMS. Blood samples were obtained immediately before and at 1, 2, 3, 4, 6, and 8 h after the beginning of the infusion. Samples were immediately centrifuged (1,000 *g*, 10 min) at 4°C and plasma stored at -80°C until analysis.

BAL was collected at 2 h after the start of the CMS infusion. The lavage was performed as described elsewhere.[22] Briefly, the fiberscope was inserted into the right middle lobe. Four 50-mL aliquots of sterile saline were instilled and each immediately aspirated into a trap; aliquots were pooled and immediately stored at -80°C. The concentration of colistin in the BAL obtained from a different patient receiving CMS as aerosol was evaluated and used as internal control. Plasma and BAL samples were collected and stored in polypropylene tubes. The maximum time that plasma and BAL was stored at -80°C was 3 months.

Colistin Assay

The concentration of colistin in human plasma and BAL was determined using the HPLC method described by Li et al[11] with minor modifications: this is a sensitive method that discriminates colistin from CMS. Briefly, after protein precipitation the supernatant was transferred to solid phase extraction C18 cartridges (Sep-Pak; Waters Corp; Milford, MA), in which derivatization with 9-fluorenylmethyl chloroformate was performed. Elution of the derivatives was followed by evaporation to dryness, redissolution, and injection of 50 μL into the HPLC.

The HPLC system consisted of Merck-Hitachi equipment, including an L-6200 pump Hitachi, an autosampler model L-7200, and an L-2480 Fluorescence Detector connected to a D-2500 integrator. The column used was a Zorbax SB C18, 75×4.6 mm, 3.5 μm, heated to 35°C. The mobile phase was a mixture of acetonitrile, tetrahydrofuran, and water delivered at a flow rate of 1 mL/min. The injection volume was 50 μL, the run time was 7 min, and the detector settings were excitation wavelength 260 nm, emission wavelength 315 nm. Calibration curves were prepared in drug-free plasma containing colistin sulfate, whereas those for colistin in BAL were prepared in saline solution. The concentrations of colistin were calculated by multiplying the obtained concentrations of colistin sulfate by 1,163/1,403, where 1,163 was the average molecular weight of colistin A and B, and 1,403 was the average molecular weight of the corresponding salt. Values of colistin for each patient were the sum of colistin A plus colistin B. Calibration curves were linear in the range 0.1 to 10 μg/mL and the accuracy quality control samples varied from 96% to 102% of the nominal concentration for intra-day and inter-day analysis, with a precision (coefficient of variation) of $\leq 10\%$. The limit of quantification was 0.1 μg/mL and the limit of detection was 0.05 μg/mL.

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Table 1—*Demographic and Clinical Characteristics of the Patients*

Patients	Age, y	Sex	Body Weight, kg	Admission Diagnosis	SAPS II Score[a]	SOFA Score[b]	Creatinine Clearance,[b] mL/min	Pathogen	Susceptibility
1	42	M	55	Tetraplegia	48	2	109.9	*P aeruginosa*	Carbapenem
2	47	M	77	Drug poisoning	74	1	112	*A baumannii*	Carbapenem
3	20	M	75	Head injury	76	2	161	*A baumannii*	Carbapenem
4	23	M	80	Head injury	43	6	215	*P aeruginosa*	Carbapenem
5	21	F	70	ICH	37	8	95.5	*A baumannii*	Carbapenem-resistant
6	40	M	90	Tetraplegia	27	4	121	*A baumannii*	Carbapenem-resistant
7	40	M	87	Head injury	27	4	129	*A baumannii*	Carbapenem-resistant
8	63	M	85	Glioblastoma	41	4	118.1	*A baumannii*	Carbapenem-resistant
9	31	M	74	ARDS	64	11	115.2	*A baumannii*	Carbapenem-resistant
10	61	F	110	Ischemic stroke	61	7	120.5	*A baumannii*	Carbapenem-resistant
11	61	F	88	Ischemic stroke	61	5	110	*A baumannii*	Carbapenem-resistant
12	70	M	93	Brain tumor	34	3	118.8	*A baumannii*	Carbapenem-resistant
13	70	M	74	Head injury	34	3	104.2	*A baumannii*	Carbapenem-resistant

A baumannii = *Acenitobacter baumannii*; F = female; ICH = intracerebral hemorrhage; M = male; *P aeruginosa* = *Pseudomonas aeruginosa*; SAPS II = Simplified Acute Physiology score II; SOFA = Sequential Organ Failure Assessment.
[a]Evaluated at admission.
[b]Evaluated the day of sampling.

Chemicals and Reagents

Colistin sulfate and 9-fluorenylmethyl chloroformate were purchased from Sigma-Aldrich (St. Louis, MO) and netilmicin sulfate from Schering-Plough (Madison, NJ). Stock solutions of colistin and netilmicin sulfate were prepared in water. All the solutions were stored at 4°C and remained stable for at least 60 days. Acetonitrile, tetrahydrofuran, acetone, and water were of HPLC grade (C. Erba reagent SpA; Milano, Italy). Trifluoroacetic acid purity was > 99% (Applied Biosystems; Foster City, CA). Sodium hydrogen carbonate, sodium hydroxide, and boric acid were of analytical grade (JT Baker Chemical Co; Phillipsburg, NJ).

Pharmacokinetic and Statistical Analysis

Noncompartmental pharmacokinetic parameters were calculated by standard methods. Maximum concentration (Cmax) was taken 0.5 h after the end of the CMS infusion (that is, 1 h after the start of the infusion). Colistin trough concentrations (Ctrough) were measured just before the morning dose. The area under the plasma concentration-time curve ($AUC_{0\text{-}8}$ for the 8-h regimen) was calculated using the trapezoidal rule from 0 to 8 h. Colistin AUCs were expressed as $AUC_{(0\text{-}24)}$ ($= 24/\tau \times AUC\tau$, where τ is the dosing interval). Because the fraction of CMS that forms colistin cannot be determined, we referred to the unknown fraction of CMS metabolized to colistin as fm (colistin formed). The apparent total body clearance (CL/fm), corrected for body weight (CL, L/h/kg), was obtained by dividing the ratio of the daily dose and the $AUC_{(0\text{-}24)}$ by body weight. The apparent volume of distribution (Vd/fm) was calculated by dividing CL/fm by the colistin elimination rate constant (Kel). Demographic and pharmacokinetic data were presented as the mean ± SD. Pharmacokinetic and statistical calculations were performed with the KINETICA 4.0 software (InnaPhase Corp; Philadelphia, PA).

Results

Pharmacokinetics of Colistin

The mean ± SD dose of CMS administered, calculated from the most accurate approximation of each patient's weight, was 2.19 ± 0.38 mg/kg per dose (range 1.58-3.16). The time course of the plasma concentration of colistin is shown in Figure 1.

A wide interindividual variability was seen in all pharmacokinetic parameters. The maximum concentration of colistin occurred 1 h after beginning CMS infusion, confirming the previously reported relatively rapid conversion of CMS to colistin.[1] At steady state, mean ± SD plasma Cmax and Ctrough were 2.21 ± 1.08 and 1.03 ± 0.69 μg/mL, respectively. Mean ± SD $AUC_{(0\text{-}8)}$ and Vd/fm were 11.5 ± 6.2 μg × h/mL and 1.5 ± 1.1 L/kg, respectively. The apparent elimination half-life ($t_{1/2}$) averaged 5.9 ± 2.6 h, and the CL/fm was 0.26 ± 0.18 L/h/kg. Pharmacokinetic parameters are detailed in Table 2. Assuming a minimum inhibitory concentration (MIC) breakpoint of 2 μg/mL, Cmax/MIC ratio and $AUC_{0\text{-}24}$/MIC ratio were 1.1 ± 0.5

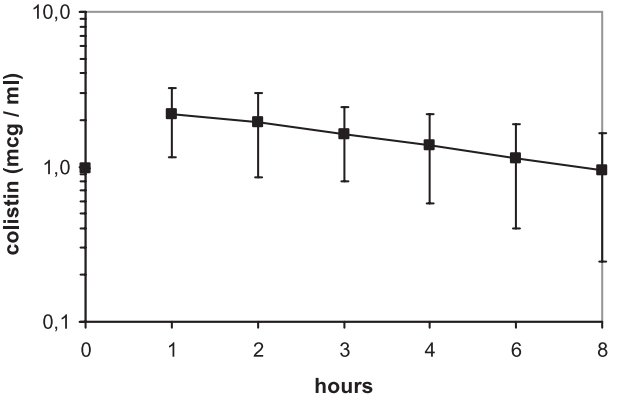

Figure 1. Plasma concentration of colistin at steady state. Two million International Units (174 mg) of colistin methanesulfonate (CMS) were administered IV in 30 min at time 0. Colistin in plasma was measured at the indicated time points. Data are presented as mean ± SD.

Table 2—*Pharmacokinetics Parameters*

Patient	Dose, mg/kg per Dose	Sampling Day	Cmax, μg/mL	Ctrough, μg/mL	$AUC_{(0-8)}$ μg × h/mL	Kel, per h	$t_{1/2}$, h	CL/fm, L/h/kg	Vd/fm, L/kg
1	3.16	6	2.52	0.66	14.76	0.09	7.9	0.21	1.77
2	2.26	3	1.93	0.66	6.94	0.30	1.4	0.33	1.08
3	2.32	4	0.68	0.23	2.96	0.25	1.7	0.78	3.10
4	2.18	6	1.93	0.25	8.77	0.18	5.6	0.25	1.35
5	2.48	5	3.85	1.93	20.88	0.49	7.4	0.12	0.61
6	1.93	3	1.33	1.20	7.51	0.13	3.9	0.26	1.94
7	2.00	5	1.77	0.58	9.15	0.26	6.9	0.22	0.83
8	2.04	4	2.54	0.33	6.77	1.10	10	0.30	0.27
9	2.34	3	2.49	1.55	15.58	NC	NC	0.15	NC
10	1.58	3	2.4	1.67	13.43	0.12	7.9	0.12	0.99
11	1.97	6	4.65	2.43	25.08	0.12	5.8	0.08	0.51
12	1.87	3	1.39	1.03	9.84	0.05	6.9	0.19	3.80
13	2.34	7	1.29	0.90	8.3	0.14	5	0.33	1.89
Mean	2.19	4.5	2.21	1.03	11.54	0.27	5.9	0.26	1.51
SD	0.38	1.4	1.08	0.69	6.20	0.29	2.6	0.18	1.06

AUC = area under the plasma concentration-time curve; CL/fm = apparent total body clearance of formed colistin; Cmax = maximum plasma colistin concentration; Ctrough = minimum plasma colistin concentration at predose; Kel = apparent elimination rate constant; NC = not calculated; $t_{1/2}$ = apparent elimination half-life; Vd/fm = apparent volume of distribution of formed colistin.

and 17.3 ± 9.3, respectively. Serum creatinine concentration was 0.76 ± 0.16 mg/dL before treatment and 0.73 ± 0.22 mg/dL at the end of the treatment with CMS.

Concentration of Colistin in BAL

To the best of our knowledge, colistin has never been measured in BAL. Two hours after the start of CMS infusion, colistin was undetectable in BAL but was present at a relevant concentration in the BAL of a patient who received CMS by aerosol, used as internal control (0.48 μg/mL).

Discussion

Pharmacokinetics of Colistin

Information about the pharmacokinetics of colistin in critically ill patients is very limited.[17-19] Moreover, the dose regimens commonly used until now are based on pharmacokinetic studies in which colistin was measured by microbiological assays that were not able to discriminate between CMS (inactive) and colistin (the active form). In this study conducted in critically ill patients with ventilator-associated pneumonia, we have investigated the steady-state plasma pharmacokinetics and the concentration of colistin in BAL after IV administration of CMS 2 million International Units (174 mg) q8h, corresponding to 2.19 ± 0.38 mg/kg per dose, a regimen frequently used in clinical practice. We measured colistin using a recently introduced, selective and accurate HPLC-based method.[11] Comparing our pharmacokinetic data with others obtained in critically ill patients, we found that our data are consistent with those of Markou et al,[17] whereas the $t_{1/2}$ we found was shorter than that reported by Plachouras et al.[19] Although the creatinine clearance values were higher in our patients (Table 1) than those reported by Plachouras et al,[19] colistin is mainly cleared by nonrenal mechanisms.[13] Therefore, the higher creatinine clearance values observed in our patients could explain this discrepancy only in part. Other comparisons of our data with those published previously are difficult, because pharmacokinetic data on colistin are limited in humans and because colistin was measured with different methods. A limit of our pharmacokinetic analysis is that, in order to avoid the distribution phase at least in part, we chose Cmax of colistin 0.5 h after the end of the infusion (that is, 1 h after the start of the infusion). This affected some pharmacokinetic parameters, such as Cmax, AUC_{0-8}, and Vd. Optimization of dosage regimens is important to cure infections and also to reduce the risk of resistance to colistin, an event recently reported.[23] Dosage regimens with intervals of 8, 12, and 24 h have all been used in clinical practice in patients with normal renal function. Bergen et al[24] simulated the pharmacokinetics of colistin formation in humans following these three intermittent dosage regimens in an in vitro pharmacokinetic/pharmacodynamic model and found no difference in overall bacterial killing (two strains of *P aeruginosa*) across the 72-h treatment period. The 8-h regimen appeared most effective at minimizing the onset of resistance. Moreover, the same study[24] and a recent study in a murine model of bacterial infection[25] have suggested that the AUC/MIC ratio of total and unbound colistin is the index best predicting the antibacterial activity against *P aeruginosa*, superior to

Cmax/MIC, suggesting that time-averaged exposure to colistin is more important than the achievement of high peak concentrations.[25] The same conclusion was reached for polymyxin B from studies conducted in an in vitro pharmacokinetic/pharmacodynamic model with once-, twice-, and thrice-daily dosing against *P aeruginosa*.[26] Thus, the available evidence indicates that the 8-h dosing regimen is preferable.

The pharmacodynamic parameters best predicting colistin efficacy and their optimal values have not yet been established in humans. This task will be particularly complex in critically ill patients. In fact, these patients are often hypoalbuminemic, whereas acute-phase proteins (such as α1-acid glycoprotein) are often increased, affecting the amount of bound and free colistin. According to the Societée Francaise de Microbiologie and the United States Clinical and Laboratory Standards Institution, the breakpoint of susceptibility based on colistin sulfate is ≤ 2 μg/mL, and > 2 μg/mL is the resistance breakpoint, whereas the criteria set by the British Society for Antimicrobial Chemotherapy are ≤ 4 μg/mL as susceptible and > 8 μg/mL as resistant.[1] Our study was designed to evaluate the plasma pharmacokinetics and BAL concentrations of colistin, not its pharmacodynamic profile or clinical efficacy. Therefore, on the basis of the magnitude of plasma concentrations and reported MIC breakpoint values, we can only speculate that, after IV administration of CMS at a commonly used dosage, the plasma concentrations of colistin might be suboptimal.

Despite the findings of apparently suboptimal plasma concentrations of colistin in humans treated with CMS, reports have shown that colistin can be effective in the treatment of infections sustained by gram-negative multidrug-resistant bacteria.[13,27-30] It cannot, therefore, be excluded that the classic pharmacodynamic parameters of other antibiotic therapy may not apply to colistin.

A few in vitro and clinical studies have suggested that IV administration of CMS in combination with other antimicrobial agents could be useful in the treatment of infections caused by multidrug-resistant *A baumannii* and *P aeruginosa*.[13] A combination therapy of nebulized CMS and oral ciprofloxacin has been used for aggressive eradication of lower respiratory tract infections with encouraging results.[31] A recent retrospective study has shown that the outcome of ventilator-associated pneumonia was better in patients who received inhaled colistin along with IV colistin than IV colistin alone,[32] and the results of a case series study led to the suggestion of using inhaled colistin as monotherapy for multidrug-resistant gram-negative nosocomial pneumonia.[33] The synergistic effects of two antimicrobial treatments might allow the use of the usually recommended doses of CMS, thereby reducing the risk of renal toxicity that would occur if higher doses of CMS were to be used to maximize the efficacy of colistin.

Because of its nephrotoxicity, CMS was abandoned during the 1970s when aminoglycosides appeared. The doses of CMS administered in our study did not cause renal impairment, a finding consistent with other reports.[17,28,29] Although a study performed in patients with CF has recently shown that CMS may be less nephrotoxic than aminoglycosides,[34] the risk of renal toxicity is still to be considered.[30,35]

Concentration of Colistin in BAL

The most relevant and original finding of our research is that colistin was undetectable in BAL. We did not perform a pharmacokinetic study of colistin in BAL, but it is unlikely that sampling at other times might have allowed detection of colistin, and it is also unlikely that the use of higher doses would have changed our results at a clinically significant level. This finding is worrying, because the success of an antibiotic therapy depends on the susceptibility of the infecting bacteria and the concentration of antibiotic achieved at the site of infection.[20,21] There is little information on the concentration of colistin at infection sites. Studies performed in animals in the 1970s showed that colistin diffuses, penetrates, and accumulates in lung (and other tissues).[36,37] Colistin is a hydrophobic antibiotic and, accordingly, low concentrations were expected to be found in BAL; however, in our patients, colistin was undetectable in BAL performed 2 h after the start of CMS infusion. Although the limit of detection of our HPLC-based method was very low (50 ng/mL), it is possible that the relatively large volumes of lavage fluid could have diluted the amount (concentrations) of colistin below this limit. Another explanation could be that, because of the presence of five free amino groups that react by electrostatic attraction to negatively charged phospholipids of membrane, colistin binds extensively to tissues, and the concentration of the free form is much less than the bound form in all tissues, lung included.[36,37] Therefore, tissue binding, rather than low tissue penetration, could be responsible for the absence of colistin in BAL.

Conclusions

In critically ill adult patients, the IV administration of CMS, 2 million International Units (174 mg) q8h, results in apparently suboptimal plasma concentration of colistin, which, moreover, was undetectable in BAL. However, because despite all these limits IV CMS has been successful in the treatment of multidrug-resistant gram-negative infections, and because colistin

binds to tissues, the pharmacokinetic-pharmacodynamic relationship of this antibiotic should be investigated further.

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Dr Carnevale: contributed to performing BAL, acquisition of clinical and microbiologic data, interpreting data, and providing final approval of the version submitted for publication.
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Dr Langer: contributed to study design, data analysis and interpretation, writing the manuscript, and providing final approval of the version submitted for publication.
Dr Regazzi: contributed to study design, data analysis and interpretation, writing the manuscript, and providing final approval of the version submitted for publication.
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