



## Pharmacokinetics of echinocandins in suspected candida peritonitis: A potential risk for resistance



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### ABSTRACT

**Introduction:** A possible increase in *Candida* resistance, especially in *Candida glabrata*, has been speculated according to poor diffusion of echinocandins to peritoneal fluid.

**Materials/methods:** Peritoneal and serum concentrations of caspofungin, micafungin and anidulafungin were analysed in surgical patients with suspected candida peritonitis. After 4 days of starting therapy, serum and peritoneal samples (through peritoneal drainage) were obtained at baseline, 1, 6, 12 and 24 h of drug administration. Micafungin and anidulafungin concentrations were determined using high-performance liquid chromatography (HPLC/F), whereas caspofungin concentrations were established by bioassay.

**Results:** Twenty-three critically ill patients with suspected abdominal fungal infection who were receiving an echinocandin were prospectively recruited. No specific criteria were applied to prescribe one specific echinocandin. No special clinical differences were observed among the three groups of patients. All were receiving antibiotic therapy, 80% required inotropic drugs, and fungal peritonitis was confirmed in 74% of them. The AUC<sub>0-24h</sub> (mg × h/L) obtained in serum and peritoneal fluid were: 126.84 and 34.38, 98.52 and 18.83, and 66.9 and 8.78 for anidulafungin, micafungin and caspofungin, respectively. The median concentration in peritoneal fluid ranged from 0.66 to 1.82 µg/mL for anidulafungin, 0.68–0.88 µg/mL for micafungin and 0.21–0.46 µg/mL for caspofungin.

**Conclusion:** The results showed moderate penetration of echinocandins into the peritoneal fluid of these patients. These levels are below the threshold of resistance mutant selection published by other authors. This could justify a potential risk of resistance in patients with prolonged treatment with echinocandins and suboptimal control of abdominal infection.

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### Introduction

The low sensitivity of echinocandins to *Candida parapsilosis* (*C. parapsilosis*) and development of resistance, especially in *Candida glabrata* (*C. glabrata*) in patients receiving prolonged treatment with echinocandins have recently been the focus of diffusion studies on these antifungals at an intra-abdominal level (Grau et al., 2015; Pérez Civantos et al., 2019; Welte et al., 2018; Dupont et al., 2017; Sganga et al., 2019). Different

pharmacokinetic/pharmacodynamic (PK/PD) studies have recently been published for echinocandins (Luque et al., 2019; Andes et al., 2011; Hall et al., 2013; Aguilar et al., 2014a; Aguilar et al., 2014b; Andes et al., 2010), but very few have focused on candidiasis peritonitis or intra-abdominal fungal infection. None of these studies have jointly analysed the behaviour of the three echinocandins in the management of abdominal fungal infection.

Intra-abdominal candidiasis (IAC) is still poorly understood compared with candidaemia. To date, data and studies on the efficacy of echinocandins in IAC are scarce, and although IAC has a high mortality rate, all current international guidelines mainly address candidaemia (Cornely et al., 2012).

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Recent studies indicate that echinocandin resistance rates among *C. glabrata* have increased worldwide (Rivero-Menendez et al., 2019; Guinea et al., 2014; Chapman et al., 2017; Hou et al., 2017). Resistance has been reported to easily develop *in vitro* (Bordallo-Cardona et al., 2017; Bordallo-Cardona et al., 2018a; Bordallo-Cardona et al., 2018b; Shields et al., 2019) and in patients after echinocandin exposure (Rivero-Menendez et al., 2019; Shields et al., 2012; Bizerra et al., 2014; Sasso et al., 2017), which occurs because of the presence of point mutations in hot-spot regions of the *FKS1* and *FKS2* genes (Rivero-Menendez et al., 2019; Shields et al., 2012; Bizerra et al., 2014). These mutations have been associated with higher minimal inhibitory concentrations (MICs) and therapeutic failure (Shields et al., 2012; Sasso et al., 2017).

This study aimed to analyse PK/PD parameters of the three echinocandins (anidulafungin, micafungin and caspofungin) in serum and peritoneal fluid (PF) in post-surgical critically ill patients with proven or suspected IAC. Other aspects related to this series, such as IAC diagnosis (including the role of multiplex quantitative real-time PCR and  $\beta$ -D-glucan in serum), aetiological agents, therapeutic response and prognosis have recently been published and complement this study (Fortún et al., 2020).

The study was prospectively performed from 2016 to 2019 at a single centre, and only patients who provided written consent were included.

## Methods

This was a prospective PK study of critically ill adult patients who were admitted to the Anaesthesiology and Surgical Critical Care Department at Ramon y Cajal Hospital, Madrid, Spain.

Inclusion criteria were age  $\geq 18$  years, a diagnosis of post-surgical nosocomial peritonitis that was refractory to  $>4$  days of antibiotics, and undergoing PF drainage.

IAC was defined following the 2013 European Consensus criteria (Bassetti et al., 2013): 'yeast detection by direct microscopy examination or growth in culture from purulent or necrotic intra-abdominal specimens obtained during surgery or by percutaneous aspiration; *Candida* growth from bile, intra-biliary ducts devices, and biopsy of intra-abdominal organs; *Candida* growth from blood cultures in the clinical setting of secondary and tertiary peritonitis in the absence of any other pathogen; *Candida* growth from drainage tubes only if placed less than 24 h. before the cultures'.

The following variables were obtained for all patients: age, gender, central catheter, parenteral nutrition, ICU, septic shock, APACHE II, intestinal perforation or leak, pancreatitis, solid tumour, chemotherapy, diabetes, previous chemotherapy, dialysis, Pittet index, *Candida* score, source of intra-abdominal candidiasis, blood cultures, candida isolates, empirical antifungal started, and 30-day mortality. Blood cultures were processed in the Microbiology Department at Ramon y Cajal Hospital using the BACTEC FX blood culture system (Becton

Dickinson Diagnostic Instrument Systems, MD, USA). Fungi were identified using mass spectrometry (matrix-assisted laser desorption/ionization time-of-flight [MALDI-TOF]; Bruker, Germany).

>Four days after starting anidulafungin (100 mg/d, first day 200 mg), caspofungin (50 mg/d, first day 70 mg), or micafungin (100 mg/d) therapy when the patients were stable, serum and peritoneal samples (through peritoneal drainage) were obtained at the following time points: baseline and at 1, 6, 12, and 24 h after antifungal administration. The samples were frozen at  $-80$  °C until analysis. Anidulafungin and micafungin concentrations were determined using a validated high-pressure liquid chromatography (HPLC/UV-F) method. Caspofungin concentrations were established using a bioassay.

## Bioassay

The bioassay involved measuring the biological activity of caspofungin in serum samples in a diffusion assay. Preparation of the medium, assay reagents and the test organism (*Candida kefyr* ATCC 28838; caspofungin MIC, 0.06  $\mu$ g/mL) have previously been described (Cendejas-Bueno et al., 2013).

## High-pressure liquid chromatography assay

A new HPLC/UV-F assay was developed using a stepwise gradient elution profile. The proposed method enables the specific quantification of echinocandin in 150  $\mu$ L of sample (CS and clinical samples) after a first step of protein precipitation and direct injection of resulting supernatant. An HPLC assay (Waters 2695 separation module) was developed using a stepwise gradient elution profile on a reverse-phase C18, 2.7- $\mu$ m CortecT3 analytical column (100  $\times$  4.6 mm) that was maintained at 25 °C in conjunction with a Cortec T3 guard column (VanGuard 3.9  $\times$  5 mm). The mobile phase consisted of acetonitrile and ammonium acetate (pH 5.5) at a flow rate of 0.6 mL/min. The stepwise gradient elution profile was programmed as follows: Solvent A (acetonitrile) was initially 35% for 1 min and then increased to 70% for 7 min, and finally decreased to 35% again for the next 3 min. Detection was performed by the specific characterisation of each compound by its UV profile. Additionally, in-series fluorescence detection was also performed (Waters 2475 multi  $\lambda$  Fluorescence Detector) because these candins have fluorescence properties. Dual detection allows a more specific and sensitive method of quantification.

The wavelengths of excitation and emission were set at 273 nm and 464 nm, respectively. The Empower Software (version 3.0, Waters Corporation, MA, USA) controlled the HPLC system control, and acquisition and processing of the data. For each candin characterisation, a comparison of retention times and a UV-F profile with authentic standards was performed.

**Table 1**

Characteristics of patients included in the study.

	Anidulafungin (n = 11)	Caspofungin (n = 8)	Micafungin (n = 4)
Male, kg, %	7/11, 63.6%	7/8, 87.5%	3/4, 75%
APACHE > 14, %	9/11, 81.8%	5/8, 62.5%	4/4, 100%
Inotropic requirements, %	9/11, 81.8%	7/8, 87.5%	4/4, 100%
Multi-organic failure, %	6/11, 54.5%	3/8, 37.5%	3/4, 75%
Confirmed bacterial peritonitis, %	11/11, 100%	8/8, 100%	4/4, 100%
Confirmed fungal peritonitis, %	8/11, 72.7%	6/8, 75%	3/4, 75%
Candidaemia, %	1/11, 9.1%	0	0
Haemodialysis, %	1/11, 9.1%	1/8, 12.5%	1/4, 25%
Weight, mean (range)	77.8 (53–98)	78.8 (67–100)	68.2 (45–80)
Serum bilirubin, mean (mg/dL, range)	1.17 (0.30–4.61)	1.28 (0.31–3.72)	1.12 (0.40–2.31)
Serum creatinine, mean (mg/dL, range)	1.07 (0.40–1.91)	0.82 (0.51–1.92)	1.20 (0.59–1.94)
Serum albumin, mean (g/dL, range)	2.24 (1.31–3.74)	2.23 (1.62–3.71)	2.23 (1.30–3.70)
Serum protein, mean (g/dL, range)	4.69 (3.62–6.31)	5.05 (3.57–6.34)	4.50 (4.20–5.21)

**Table 2**  
Echinocandin pharmacokinetic parameters (mean  $\pm$  standard deviation values).

Antifungal (n)		S	PF	Ratio PF/S (%)
AND (11)	C <sub>max</sub> (mg/L)	7.96 $\pm$ 5.40	2.57 $\pm$ 2.19	32.3
	C <sub>min</sub> (mg/L)	3.99 $\pm$ 2.73	0.64 $\pm$ 0.35	16.1
	AUC <sub>0–24h</sub> (mg $\times$ h/L)	126.84 $\pm$ 78.66	34.38 $\pm$ 20.17	27.1
MCF (4)	C <sub>max</sub> (mg/L)	8.45 $\pm$ 3.24	0.88 $\pm$ 0.69	10.4
	C <sub>min</sub> (mg/L)	2.04 $\pm$ 1.34	0.66 $\pm$ 0.47	32.5
	AUC <sub>0–24</sub> (mg $\times$ h/L)	98.52 $\pm$ 34.55	18.83 $\pm$ 14.05	19.1
CAS (8)	C <sub>max</sub> (mg/L)	5.30 $\pm$ 2.66	0.49 $\pm$ 0.39	9.2
	C <sub>min</sub> (mg/L)	1.43 $\pm$ 0.73	0.24 $\pm$ 0.27	16.9
	AUC <sub>0–24</sub> (mg $\times$ h/L)	66.90 $\pm$ 32.71	8.78 $\pm$ 7.83	13.1

AND, anidulafungin; MCF, micafungin; CAS, caspofungin; S, serum; PF, peritoneal fluid.

### PK evaluation

Data were processed using Empower Software (version 3.0, Waters Chromatography, S.A, Spain). Echinocandin PK analysis was determined using a non-compartmental model. All calculations were performed using Microsoft Excel<sup>®</sup> (Microsoft, Redmond, WA, USA) spread sheets, using the PK solver add-in program, which has demonstrated equivalence calculating PK/PD parameters compared with other specific PK programs. Plots were created with GraphPad Prism 7, (La Jolla, CA, USA). The primary PK parameters that were evaluated were the area under the concentration-time curves from 0 to 24 h (AUC<sub>0–24</sub>), maximum concentration (C<sub>max</sub> in mg/L) and minimum concentration (C<sub>min</sub> in mg/L).

The Ramon y Cajal Hospital Institutional Review Board approved the study protocol, and informed consent was obtained from the patients or their representatives.

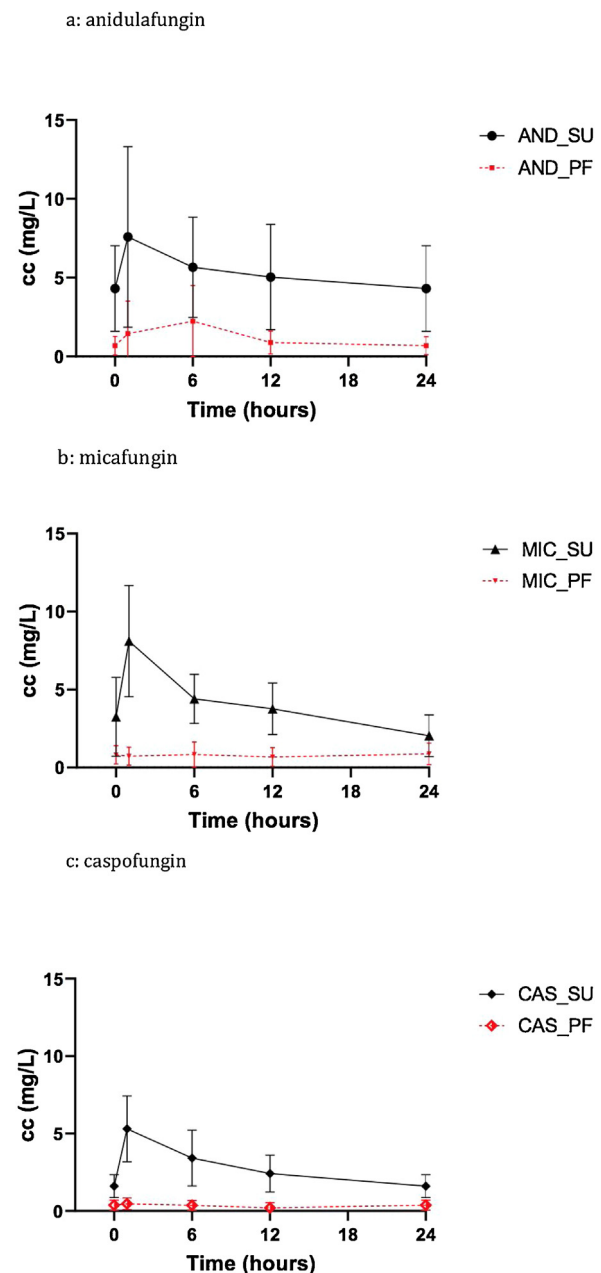
### Results

Twenty-three critically ill patients with suspected abdominal fungal infections were recruited.

At the current centre, no specific criteria are applied to prescribe a specific echinocandin. Anidulafungin, caspofungin or micafungin were prescribed in 11, eight, and four patients, respectively, in this study. Table 1 shows the principal characteristics of the patients. All patients had recently undergone surgery and had a recently implanted abdominal drain. No differences were observed among these three groups: all the patients were in critical condition, were admitted into the surgical intensive care unit, and >80% required inotropic drugs. Before antifungal therapy was started, all patients were receiving antibiotic therapy for previously confirmed bacterial peritonitis, and fungal peritonitis was confirmed in three-quarters of them. One patient in each of the drug groups required haemodialysis, and no differences in weight or serum levels of creatinine, bilirubin, protein, or albumin were observed among the three drug groups. All patients were treated with echinocandins at conventional doses.

After 4 days of therapy (steady state), serum and PF (through peritoneal drainage) were collected at baseline, and at 1, 6, 12 and 24 h after echinocandin administration. The PK/PD analysis was performed using a non-compartmental approach and the principal results are shown in Table 2 and Figure 1.

The AUC<sub>0–24h</sub> (mg  $\times$  h/L) that was obtained in serum and PF was highest for anidulafungin, followed by micafungin and caspofungin. The ratio of PF-to-serum (%) was also higher for anidulafungin and the lowest for caspofungin. In summary, the results showed a moderate penetration of echinocandins into the PF in patients with intra-abdominal infections, with a median AUC<sub>0–24h</sub> for the PF-to-plasma ratio of 0.13–0.27 at the assumed steady-state. The median concentration in PF ranged from 0.66 to 1.82  $\mu$ g/mL for anidulafungin, 0.68–0.88  $\mu$ g/mL for micafungin and 0.21–0.46  $\mu$ g/mL for caspofungin.



**Figure 1.** Observed echinocandin concentrations in serum and peritoneal fluid. Median concentrations and standard deviation at baseline, 1, 6, 12 and 24 h on day +4 of therapy (anidulafungin: 200 mg on day 1, followed by 100 mg/d thereafter; caspofungin: 70 mg on day 1, followed by 50 mg/d thereafter; micafungin: 100 mg/d (no change in doses)).

## Discussion

The peritoneal concentrations obtained for the three candins in the present study ranged from 0.21 to 0.46  $\mu\text{g/mL}$  for caspofungin to 0.66–1.82  $\mu\text{g/mL}$  for anidulafungin, and most concentrations were  $<1 \mu\text{g/mL}$ . This is consistent with results published by other authors as a safeguard of efficacy for managing patients with IAC because these levels far exceed the MIC90 that EUCAST suggests for the usual strains of *Candida albicans* (0.03  $\mu\text{g/mL}$ ), and *Candida krusei* (*C. krusei*), *C. glabrata* and *Candida tropicalis* (0.06  $\mu\text{g/mL}$ ). However, they would be insufficient for the management of *C. parapsilosis* (4  $\mu\text{g/mL}$ ) (The European Committee on Antimicrobial Susceptibility Testing, 2020).

Recently, Grau et al. conducted a similar study in which they analysed micafungin PK/PD in surgical patients. On day 3 they found an  $\text{AUC}_{0-24\text{h}}$  in plasma and PF of 56.5 (52–77.7)  $\text{mg} \times \text{h/L}$  and 23.9 (18.8–31.7)  $\text{mg} \times \text{h/L}$ , respectively, corresponding to a median PF-to-plasma ratio of 0.3 (Grau et al., 2015). The only covariates that were statistically significant and improved the fit of the model were total body weight normalised to 70 kg and serum albumin concentration normalised to 2.2 g/day, according to the high protein binding of echinocandins (Grau et al., 2015). The effect of weight on PK results for echinocandins has also previously been demonstrated for caspofungin (Hall et al., 2013) and anidulafungin (Luque et al., 2019), and some studies showed that a 25% increase in the anidulafungin dose is recommended in morbidly obese patients (Wasmann et al., 2018). However, in the present study, no morbidly obese patients were included, and all the patients in the three groups presented a homogeneous profile of weight and albuminaemia, which were close to the average values mentioned in previous studies. Thus, it is believed that the data obtained in the current study adequately represent the PK of the three candins in this type of patient.

The levels obtained in PF in the current patients were similar to those obtained by other authors, and they confirmed a moderate penetration of echinocandins into the PF in patients with IAC. Perez-Civantos et al. confirmed anidulafungin levels between 0.7–0.9  $\mu\text{g/mL}$ , with an average  $\text{AUC}_{0-24}$  of 57.9  $\text{mg} \times \text{h/L}$ , (Pérez Civantos et al., 2019) which was similar to the levels obtained by Welte et al. (0.12–0.99  $\mu\text{g} \times \text{h/mL}$ ) (Welte et al., 2018).

Andes et al. (2011) demonstrated that the AUC/MIC ratio, APACHE II score and history of corticosteroid use were significant independent predictors of a favourable response for all *Candida* species. This study analysed 493 patients who were included in two large clinical trials with micafungin. The MIC90 of *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei* was 0.008  $\mu\text{g/mL}$ , 0.016  $\mu\text{g/mL}$ , 0.016  $\mu\text{g/mL}$ , and 0.125  $\mu\text{g/mL}$ , respectively, and 1.0  $\mu\text{g/mL}$  for *C. parapsilosis* (Andes et al., 2011). In plasma, fractional target AUC/MIC ratios of 3000 and 285 were associated with positive therapeutic outcome in a population PK/PD model of patients with invasive candidiasis or candidaemia caused by other species different to *C. parapsilosis*, respectively (Andes et al., 2011).

The current results confirmed an AUC/3000 ratio in serum of 0.042, 0.032 and 0.022 for anidulafungin, micafungin and caspofungin, respectively, which would be achieved using the current EUCAST susceptibility cut-off for *C. albicans* (0.03  $\text{mg/L}$ ), but it would be sub-optimal for *C. glabrata*, *C. krusei* and *C. tropicalis* (0.06  $\text{mg/L}$ ). The AUC/285 ratio in the current study was 0.44, 0.34 and 0.23, respectively, which is below the threshold for *C. parapsilosis* (4  $\text{mg/L}$ ). Although these PK/PD parameters have not been optimised outside serum, low levels were obtained in the peritoneum in the current study, and other similar studies have also suggested this therapeutic difficulty, especially for *C. parapsilosis* and *C. glabrata*.

Despite this unfavourable PK/PD data, echinocandins have shown high success rates in the treatment of candidaemia and other forms of invasive candidiasis, including IAC, which are

caused by different *Candida* species such as *C. parapsilosis*. Recently, Sganga et al. performed a post hoc analysis to determine the efficacy and safety of anidulafungin treatment in patients with IAC from five prospective studies, and anidulafungin showed a global response rate that was similar to the anidulafungin registrational trial of candidaemia, with no differences in outcomes in patients with *C. albicans* compared with *C. glabrata*.

Recent studies have indicated that echinocandin resistance rates among *C. glabrata* clinical isolates have increased worldwide (Rivero-Menendez et al., 2019; Guinea et al., 2014; Chapman et al., 2017; Hou et al., 2017). Rivero et al. exposed *in vitro* susceptible isolates from two patients to an increasing concentration range of micafungin, and they obtained echinocandin-resistant and FKS mutant colonies after exposure to the lowest micafungin concentration that was considered to confer resistance by EUCAST (0.06  $\text{mg/L}$ ) in less than 48 h of incubation. The mutant prevention concentration (MPC), which is defined as the lowest concentration that can completely inhibit fungal growth for each isolate after 5 days of incubation, was documented in this study (Rivero-Menendez et al., 2019), and no significant differences were found between the MPC geometric mean after anidulafungin or micafungin exposure after 5 days of incubation (2.44  $\text{mg/L}$  versus 1.72  $\text{mg/L}$ ) (Rivero-Menendez et al., 2019). This finding is significant because the mean peritoneal concentrations of the three echinocandins obtained in the current study were always below these MPCs. Results obtained in the *in vitro* studies on how echinocandin-susceptible *C. glabrata* strains are able to develop resistance after exposure to low echinocandin concentrations support the fact that *C. glabrata* is able to colonise and survive in certain reservoirs of the human body, such as the abdomen (Shields et al., 2014), peritoneum (Grau et al., 2015), gastrointestinal tract (Healey et al., 2007) or mucosal surfaces (Jensen et al., 2015), because of long-term penetration of echinocandins at lower concentrations compared with those that prevent resistance acquisition.

This study had several limitations. The sample size was small, and the PK variability was high in this population, but the more significant factors that were associated with this variability such as weight and serum albumin were similar among the patients. PK/PD targets for echinocandins obtained in other studies have been developed using plasma data in patients with candidaemia, and thus, the results could not be directly applied to PF data. The PK/PD target attainment was not correlated with clinical response because of the small number of patients, with three-quarters of the patients having a microbiologically confirmed fungal infection. Finally, it did not confirm resistance to echinocandins, but the study was not designed for this purpose, and long-term *Candida* spp. isolates at the peritoneal level or in colonisation were not analysed.

In conclusion, this study confirmed, as in other similar studies, that there is poor diffusion of echinocandins into PF. Anidulafungin has a higher concentration and a higher PF-to-plasma ratio compared with micafungin and caspofungin, although this was not a differential aspect in clinical response in the few studies that focused on the use of echinocandins in IAC. The levels of echinocandins that are achieved in the peritoneum are below the concentration of resistant mutant selection that were published by other authors; this was clear for *C. parapsilosis* and for high-risk *C. glabrata*. These data can explain the development of resistance in *C. glabrata* and warn about mutant selection in patients on prolonged treatment with echinocandins and suboptimal control of abdominal infection.

## Ethical approval and consent to participate

The Ramon y Cajal Hospital Institutional Review Board approved the study protocol, and informed consent was obtained from the patients or their representatives.

## Consent for publication

Not applicable.

## Availability of supporting data and patients

PubMed, the Cochrane library and Medline databases. Patients: critically ill adult patients admitted to the Anaesthesiology and Surgical Critical Care Department at Ramon y Cajal Hospital, Madrid, Spain.

## Conflict of interest

None declared.

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## Authors' contributions

FG, AGL, MEA, EGG, PMD and JF wrote the manuscript. MCE and SM made significant alterations. All authors read and approved the final manuscript.

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