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Combined antimicrobial therapy in septic major burns based on drug serum levels and cultures to combat bacterial resistance by pharmacokinetics based on pharmacodynamics

Abstract

Introduction: Vancomycin and piperacillin-tazobactam are widely prescribed in the therapy of septic shock of critically ill ICU patients, including major burns with infections caused by susceptible Gram-positive and Gram-negative nosocomial bacteria. Therapeutic drug serum monitoring and cultures were essential to combat these infections. Therefore, combating the development of resistance is a relevant first-line tool to ensure the maintenance of these antibiotics in the therapeutic arsenal, especially in tertiary public hospitals with high demand for care for these septic patients with high-risk mortality admitted to Intensive Care Units.

Subject: Aim of the study was investigated by an open label clinical protocol in major burns during the first septic shock, after 72 hours of ICU admission to evaluate pharmacodynamics based on pharmacokinetics, that could affect the coverage of vancomycin, 1-hr. intermittent infusion, in a combined therapy with piperacillin-tazobactam by 3-hrs.-extended infusion in a dose prescribed in a creatinine clearance dependence, against susceptible pathogens.

Methods: The primary endpoint was the pharmacodynamics target based on microbiology of the isolate susceptible strains obtained from cultures, and on drug serum monitoring that was realized by two Sets of blood sampling for antibiotics serum measurements by TDM, twice a week, to reach the target recommended for both ATBs, with dose prescribed based on a renal function dependence. The recommended target for vancomycin by 1hr. intermittent infusion was considered against Gram-positive susceptible strains; while for piperacillin-tazobactam by 3hrs.-extended infusion was chosen against Gram-negative strains. The primary outcome was to evaluate the combined therapy prescribed to patients, considering vancomycin and piperacillin/tazobactam dose prescribed in Set 1 at TDM1 *vs* Set 2 at TDM 2, adjusted or maintained dose.

Results: Coverage strategy was based on the prediction index (AUC^{ss}0-24/MIC, ratio) for vancomycin effectiveness related to target attainment. In addition, coverage related to piperacillin-tazobactam was based on the prediction index of drug effectiveness (*%f* T>MIC) based on target attainment. It is important to highlight that each antibiotic coverage was monitored at the first septic shock, in the early stage in patients with high variability of renal clearance as acute kidney injury (AKI, n=10), renal function preserved or vasopressor requirements (n=22). PK-changes impacted coverage of both ATBs related to renal function dependence by each antibiotic investigated, and dose adjustment was done in real time for each patient.

Conclusion: Combined therapy of septic shock with vancomycin-piperacillin/tazobactam against nosocomial pathogens by PK/PD approach was done twice a week based on cultures, ATBs serum levels to guarantee the target attainment in a short period for all patients. Then, this strategy has been applied in the last ten years in the clinical protocols of our hospital, especially in the ICU of Burned Unit, to guarantee the individualized therapy by antimicrobial coverage, that contributes for combating the mutant´s selection of Enterobacteriaceae. and to prevent the development of bacterial resistance resulting in death on ICU by selection of MDR pathogens.

Keywords: vancomycin-piperacillin/tazobactam combined therapy, ICU major septic major burns, pharmacokinetic changes impact, PK/PD approach in a real time, target attainment based on cultures-serum levels

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Abbreviations: AKI, acute kidney injury; C-RP, C-reactive protein; CLSI, clinical laboratory standards institute, database USA; CVVHD-F, continuous venovenous haemodialysis-filtration; GSA, global sepsis alliance; ICBU, intensive care burn unit; ICU, intensive care unit; MDR, multidrug resistance; MIC, minimum inhibitory concentration; MV, mechanical ventilation; NLR, neutrophils to lymphocytes ratio; PD, pharmacodynamics; PK, pharmacokinetics; PNM, Pneumonia; PTA, probability of target attainment; RFA, renal function augmented; RFP, renal function preserved; SAPS3, simplified acute physiology score 3; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SSC, surviving sepsis campaign;

SIRS, systemic inflammatory response syndrome; TBSA, total burn surface area; TDM, therapeutic drug monitoring; UTI, urinary tract infection; WHO, world health organization

Introduction

Septic shock is a preventable event due to a potentially fatal organ dysfunction caused by a dysregulated host response to infection.[1] The clinical outcome in most high-risk cases is death in patients with nosocomial bacterial infections associated with comorbidities, including SARS-CoV-2 viral infections, demonstrated in a large international prospective clinical trial, with approximately 70 to 80% of ICU patients receiving therapy with antibiotics.[2] However, incidence of infections and mortality in the ICU have not improved over the last 35 years.[3] This fact indicates that it may be possible to improve care for septic patients and clinical outcomes in ICU. In addition, considering renal clearance in those patients, the association between increased mortality rate and antimicrobials dose adjustment in intensive care unit patients with renal impairment is reported, or even with augmented renal clearance is another common finding with worse clinical outcome in critically ill patients receiving antimicrobial therapy.[4–6]

In view of the growing challenge to the prescription of antimicrobials for adequate treatment and effective control of bacterial infectious conditions during COVID-19 pandemic, several international organizations reinforced that combating bacterial resistance, and the prevention of the development of multidrug-resistant strains is urgent. Thus, this population requires an immediate change in the behavior of the clinical team and continuous monitoring of these patients undergoing intensive care through continuous hemodynamic, respiratory, renal, and infectious surveillance. It is important to highlight that in a therapy, drug monitoring implemented with antibiotic stewardship program, it was described for the treatment of various infections based on serum levels of beta-lactam agents in patients admitted to the ICU. It was demonstrated that approximately 75% of patients did not reach the therapeutic goal against susceptible strains of Gram-negative bacteria. This fact reinforces that monitoring serum levels and cultures of antibiotics is essential to evaluate changes in pharmacokinetics that impact the coverage of the prescribed agent, measured through pharmacokinetics-pharmacodynamics based on defined targets.

Therefore, in the last 15 years another therapeutic strategies have been proposed for the most prescribed beta-lactams against nosocomial pathogens related to the dosage regimen, duration of drug infusion and frequent cultures monitoring, including also pharmacoeconomic studies related to hospital costs of ICU patients that must be carried out linked to patient care and clinical outcome to better investigate mortality. Hydrophilic antibiotics is a challenge once must be prescribed based on renal clearance dependence to treat ICU patients with renal function preserved or augmented by vasopressors requirements, acute kidney injury (AKI), or even patients undergoing continuous Veno-venous hemodialysis filtration (CVVHDF). Antimicrobial resistance is quite important in the ICU of major burns and should be considered since two – three surgical interventions, such as debridement followed by grafting, may occur weekly for each burn patient, depending on the extent and depth of the injury. Therefore, immunosuppression and systemic inflammatory response syndrome (SIRS) can be expected in a subsequent period that contributes to Gram-positive and G-negative nosocomial bacterial infections in recent surgical wounds, bones, and lungs. Pharmacokinetics based on pharmacodynamics is an essential tool in the combat of antimicrobial resistance against nosocomial pathogens based on ATB serum levels for individualization of therapy.[7,9–11,13,14]

Objective

The aim of the study was to investigate an open label clinical protocol septic major burn patient, 72 hours of ICU after admission, to evaluate pharmacodynamics based on pharmacokinetics, during the first septic shock that could affect the coverage of vancomycin in a combined therapy with piperacillin-tazobactam against susceptible pathogens isolates, both in a dose prescribed based on creatinine clearance dependence. Drug effectiveness was done in real time by pharmacokinetic/pharmacodynamics (PK/PD) for target attainment based on cultures and drug serum monitoring (TDM) for dose adjustment requirements to avoid the development of antibiotic resistance.

Methods

Study design, Ethics, patient eligibility, combined therapy in septic major burns: The study was carried on in a tertiary public clinics' hospital HC FMUSP of Medical Scholl, University of Sao Paulo, SP, Brazil. The clinical protocol was a prospective, open-label study. Ethical approval registers CAAE 07525118.3.0000.0068-v.3, Brazilian Platform was obtained by approval of the Ethical Committee of Hospital of Clinics, Medical School of University of Sao Paulo; no conflicts of interest to declare were obtained from all authors. The study was conducted from January 2019 to April 2020, with informed written consent obtained from all legally designated representatives of the patients. Adult patients from the Intensive Care Burn Unit, presenting severe thermal injury and sepsis diagnosis after 72 hrs. of ICU admission due to nosocomial pathogens were eligible as reported previously by Greenhalgh et al. were eligible for inclusion in the clinical protocol.[13]

On the other hand, therapy of patients with antibiotics including vancomycin and piperacillin/tazobactam intolerance were non eligible. Therapy of patients with antibiotics including vancomycin and piperacillin/tazobactam without intolerance were eligible. Patient selection for inclusion in the clinical protocol was major burns, adults (>18 yrs), both genders (20M/12F), TBSA < 40% or even >40%, SAPS 3< 57 or even >57, nosocomial infection by cultures 72hrs of ICU admission. Thirty-two septic adult patients' major burned (20M/12F) eligible were included in the study protocol to investigate the efficacy-safety of combined therapy (vancomycin-piperacillin/tazobactam) guided by cultures and serum drug levels. It is important to highlight that cultures were collected before the therapy with antibiotics starts. Pharmacokinetics (PK) was applied to investigate if the coverage measured by PK/PD approach is impacted in a renal function-dependence, as it frequently occurs in those ICU septic major burn patients. Demographic, clinical characteristics of patients included after 72hrs. of ICU admission, in Table 1. Therapeutic vancomycin-piperacillin at the steady state levels were monitored in real time in Set 1 by TDM1, and in Set 2 by TDM2 by dose adjustment or dose maintained. Vancomycin-piperacillin/tazobactam combined therapy was initiated at the earlier stage of the first septic shock with dose regimen recommended to ICU patients in a dependence of renal function that could be preserved, augmented by vasopressor requirements, or acute kidney injury as a function of systemic inflammatory response syndrome (SIRS).

Table 1 Burn septic patients undergoing ICU antimicrobial therapy, median (quartiles). Demographic, clinical, laboratorial data

Demographic data	**n=32**
Gender (20M/12F)	(20M/12F)
Age (yrs)	42 (37-48)
Ideal body weight (kg)	73 (64-82)
Body surface area (m2)	1.78 (1.72-1.85)
Body mass index (kg/m2)	26 (23-29)
Clinical data - ICU admission	**n=32**
SAPS3	45 (38-62)
TBSA (%)	24(18-56)
Thermal/electrical injury	26/6
Inhalation injury	24
Mechanical ventilation	22
Vasopressors	16
Accident	28
Crime	4
Laboratorial data 72hrs/ICU admission	
CLcr (mL/min)	88 (58-124)
C- reactive protein (mg/L)	193 (55 - 352)
Neutrophils/Lymphocytes ratio	7.16 (4.26-12.56)

Abbreviations: SAPS3, simplified acute physiology score III; CLcr, creatinine clearance; IQR, quartiles (25-75); ICU, Intensive care unit

Complete medical history, physical examination was obtained for each enrolled patient; laboratory data, and microbiology of isolated strains documented in blood cultures, bronchoalveolar lavage, wound/bone, and urinary tract. Susceptibility testing was done to obtain the minimum inhibitory concentration for each antimicrobial agent against each pathogen isolated according to Clinical Standard Laboratory Institute (CSLI database). Coverage of each antibiotic based on pharmacokinetic-pharmacodynamic recommended targets. Renal function based on creatinine clearance was estimated by serum creatinine levels applying Cockcroft-Gault equation measured by the COBAS Analyzer 8000 series; inflammatory biomarkers such as C-RP in serum were performed on the COBAS Analyzer 8000 series (C-RP) (Roche, trademark), Neutrophil-to-lymphocyte ratio (NLR) in blood count was measured using a Hematological Analyzer (SYSMEX brand). All results of the tests carried out in the hospital's Central Laboratory, including also cultures results that were sent to the ICU via the network. Additionally, both antibiotics serum levels were determined by liquid chromatography/ultraviolet detection (LC-UV, Shimadzu series 10, with automatized injection of purified extracts from serum samples) in the Clinical Pharmacokinetics Center, after development and validation of bioanalytical methods detailed previously.[10,11,13,14]

Vancomycin therapy in septic major burns: blood sampling for TDM & PK, PK/PD.

Vancomycin effectiveness was investigated after an empirical dose regimen 1g q12h, 1hr.-intermittent infusion by pump recommended in ICU patients of hospital during 48hrs. of started therapy, to guarantee that the steady state level was reached in septic burns with renal function preserved (RFP) or even augmented by vasopressors (RFA). In addition, patients with acute kidney injury (AKI) dose must be reduced 1g q24h. Then, blood was sampling, volume of 3-4 mL each, at the 3rd hr. (sample 1) of started infusion, and a second one, 1hr. before the next infusion (sample 2) for drug serum level's purposes. Vancomycin serum levels were measured for each patient by collecting two blood samples at Set 1 (TDM1) by applying a bioanalytical chromatographic method described previously.[10] Dose adjustment was done afterwards if required in Set 2 (TDM2) that must be done as soon as possible to confirm if the pharmacokinetic-pharmacodynamics target was achieved.

Conventional pharmacokinetics (PK) was based on the one-compartment open model after 1hr. intermittent – infusion, via calibrated pump to estimate the PK-parameters as follows: elimination rate constant [kel], biological half-life [t(1/2)], total body clearance [CL_T], apparent volume of distribution at steady state [Vd^{ss}]; it was included also the area under the curve reached at steady state.[10,11] Vancomycin effectiveness was evaluated by applying the pharmacokinetic/pharmacodynamic approach, and coverage was based on the minimum inhibitory concentration (MIC), and the area under the curve (AUC^{ss}_{0-24}), another PK-parameter. MIC data of Gram-positive strains isolated from cultures, and the area under the curve (AUC^{ss}_{0-24}), estimated by trapezoidal rule based on PK-model was applied for target attainment. Vancomycin coverage was based on AUC^{ss}_{0-24}/MIC ratio to attain the PK/PD target, equivalent to AUC^{ss}_{0-24}/MIC>400-600, recommended target at the last consensus.[9]

Piperacillin/Tazobactam therapy in burns: blood sampling for TDM & PK, PK/PD.

Thirty-two septic major burns received also therapy including piperacillin/tazobactam, a beta-lactam agent at regimen recommended in hospital according to renal function administered by 3 hrs. extended-infusion in ICU septic patients to attain the PK/PD target (100%*f*ΔT>MIC) recommended since 2015 by Abdul-Aziz et al.,[6] for beta lactam agents largely prescribed as piperacillin and carbapenem agents. It is important to highlight that piperacillin-tazobactam is the first choice recommended in our hospital against Gram-negative nosocomial pathogens, and it was prescribed to ICU septic burn patients.[6] Then, piperacillin-tazobactam was administered systemically by pump 3 hrs.-infusion, according to the institutional protocol described for major burns at dose regimen 4.5g q6h for patients with renal function preserved or augmented by vasopressors requirements for target attainment against Gram-negative susceptible strains up to MIC 16 mg/L. Also, for patients with acute kidney injury (AKI), dose regimen of 4.5g q12h is recommended, or yet 4.5g q8h for septic patients undergoing continuous Veno-venous hemodialysis filtration (CVVHDF) installed. Blood was sampling, volume of 3-4 mL each, at the 3rd hr. (sample 1) of started infusion, and a second one, 1hr. before the next infusion (sample 2) for piperacillin serum levels for PK/PD target attainment and for PK purposes done by a validated bioanalytical chromatographic method.[13]

Conventional pharmacokinetics (PK) based on the one-compartment open model after 3hrs.-extended infusion, via calibrated pump to estimate the PK-parameters as described previously. Piperacillin effectiveness was evaluated by applying the PK/PD approach recommended to estimate the coverage of beta-lactam agents that was based on the minimum inhibitory concentration (MIC: mg/L). MIC data of Gram-negative strains isolated from monitored cultures, and the kinetic parameters as follows, and respective units: trough equivalent to the free piperacillin concentration (C^{ss}_{trough}: mg/L), elimination rate constant (kel: hrs-1), time interval between consecutive doses (τ: hrs) based on one compartment PK-model. Piperacillin-tazobactam target attainment was based on equation %*f*ΔT>/MIC, that means the percentage time dose interval (%ΔT) required to maintain the minimum the free (*f*) fraction of piperacillin serum levels (C^{ss}_{trough}) before the next dose infusion. Then, piperacillin-tazobactam target recommended against Gram-negative strains coverage is equivalent 100%*f*ΔT>/MIC. It means that piperacillin

coverage after piperacillin-tazobactam, dose regimen will depend on time interval between two consecutive doses (τ) that the free minimum serum concentration (C^{ss}_{trough}) must be maintained higher than MIC data of each pathogen isolated.

Statistical analysis

Individual and population data: The actual statistics of this study conducted on 32 major burn patients included, at the first septic shock that occurred after 72hrs. of ICU admission. Software's were as follows: OFFICE 365, version 2208 (Excel); GraphPAD Instat-GraphPad Prism version 9.1.14 and version 10. Parametric and non-parametric tests (Mann Whitney and Wilcoxon) for unpaired and paired data tested) were applied to data obtained from the investigated patients. The significance of $p<0.05$ was considered.

Results

Combined therapy Vancomycin- Piperacillin/tazobactam in ICU major septic burns by PK/PD tools

It is important to highlight that targets were attained, and dose adjustments were required for both ATBs (vancomycin-piperacillin/tazobactam) based on creatinine clearance for all patients investigated as described in Table 2 and illustrated in Figure 1.

Table 2 Septic major burn patients undergoing combined antimicrobial therapy, median (IQR) Dose regimen-infusion, PK changes, PK/PD approach based on TDM-Cultures Empirical dose at TDM1 vs Dose adjusted at TDM2

ATB therapy TDM_PK PK/PD target	TDM1 dose recommended	TDM2 dose individualized	P
Vancomycin n=22/32 (RFP or RFA) TDM1 vs TDM2	1g q12h (n=22) 1hr- pump infusion	1g q8h (n=6) (vasopressor requirements)	0.0001
IBW dose normalized - Daily dose (mg/kg)	27 (24-31)	41 (37-47)	
Vancomycin n=10/32 (AKI) TDM1 vs TDM2	1g q24h (n=10) 1hr- pump infusion	1g q24h (n=4)	0.8745
IBW dose normalized - Daily dose (mg/kg)	14 (12-16)	14 (12-16)	
Vancomycin n=10/32 (AKI) TDM1 vs TDM2	1g q24h (n-10) 1hr- pump infusion	0.75g q12h (n=6)	0.022
IBW dose normalized - Daily dose (mg/kg)	14 (12-16)	21(18-23)	
Piperacillin-Tazobactam n=22/32 (RFP or RFA)	4.5g q6h (n=22) 3hrs-infusion	4.5g q6h (n=22) 3hrs-infusion	0.1377
IBW dose normalized - Daily dose (mg/kg)	247(220-281)	247(220-281)	
Piperacillin-Tazobactam n=4/32 (AKI)	4.5g q12h (n=10) 3hrs-infusion	4.5g q12h (n=4) 3hrs-infusion	0.8865
IBW dose normalized - Daily dose (mg/kg)	123(110-141)	123(110-141)	
Piperacillin-Tazobactam n=6/32 (AKI-CVVHDF)	4.5g q12h (n=10) 3hrs-infusion	4.5g q8h (n=6) 3hrs-infusion	0.0022
IBW dose normalized - Daily dose (mg/kg)	123(110-141)	185(165-211)	
Vancomycin PK changes impacting coverage	**RFP**	**RFA**	**P**
kel (hr-1)	0.079 (0.052-0.133)	0.178 (0.164-0.200)	0.0001
t(1/2) β (hrs)	8.9 (5.2-13.4)	3.9 (3.5-4.2)	0.0001
CLt (L/h)	1.8 (0.9-2.9)	4.6 (3.9-4.8)	0.0001
Vd^{ss} (L)	21.0 (17.5-27.9)	25.3 (19.6-27.6)	0.4995
	RFP	**AKI**	
kel (hr-1)	0.079(0.052-0.133)	0.049(0.034-0.053)	0.01
t(1/2) β (hrs)	8.9(5.2-13.4)	14.3(13.0-20.6)	0.01
CLt (L/h)	1.8(0.9-2.9)	0.9(0.9-1.2)	0.018
Vd^{ss} (L)	21.0(17.5-27.9)	20.8(17.4-29.9)	0.9479
Piperacillin PK changes impacting coverage	**RFP n=22**	**RFA n=10**	**P**
kel (hr-1)	0.365(0.277-0.408)	0.462(0.385-0.533)	0.0567
t(1/2) β (hrs)	1.9(1.7-2.5)	1.5(1.3-1.8)	0.0567
CLt (L/h)	11.0(10.0-14.0)	10.0 (8.0-12.0)	0.085
Vd^{ss} (L)	34.0 (23.0-44.0)	21.6 (20.8-22.5)	0.0001
	RFP n=22	**AKI n=10**	
kel (hr-1)	0.365(0.277-0.408)	0.129(0.089-0.155)	0.0001
t(1/2) β (hrs)	1.9(1.7-2.5)	5.4(4.5-7.8)	0.0001
CLt (L/h)	11.0(10.0-14.0)	1.3(1.0-1.5)	0.0001
Vd^{ss} (L)	34.0 (23.0-44.0)	9.7 (6.4-11.1)	0.0001
	AKI n=10	**CVVHDF n=6**	
kel (hr-1)	0.129(0.089-0.155)	0.163(0.127-0.185)	0.0564
t(1/2) β (hrs)	5.4(4.5-7.8)	4.3(3.7-5.5)	0.0672
CLt (L/h)	1.3(1.0-1.5)	6.5(3.4-6.8)	0.0001

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Table 2 Continued....

ATB therapy TDM_PK PK/PD target	TDM1 dose recommended	TDM2 dose individualized	P
Vd^{ss} (L)	9.7 (6.4-11.1)	34.0 (23.0-42.8)	0.0001
Microbiology of Cultures (CSLI database)	**Coverage (%)**	**Dose adjustment**	**Adjustment**
Vancomycin [PK/PD target AUC^{ss}_{24}/MIC>400-600]	**Target attainment**	**Effectiveness/Safety**	**Patients**
MIC: 0.5 mg/L/Susceptible strains	100% (32/32)	1g q24h/AKI	n=10
MIC 1 mg/L/ Susceptible strain isolated	100% (32/32)	1g q8h/RFA	n=6
Piperacillin [PK/PD target 100%*f*T>MIC]	**Target attainment**	**Effectiveness/Safety**	
MIC 0.25 up to 16 mg/L/Susceptible (RFP-RFA)	100% (22/22)	4.5 q6h	n=22
MIC 0.25 up to 16 mg/L/Susceptible (AKI)	100% (4/4)	4.5 q12h/AKI	n=4
MIC 0.25 up to 16 mg/L/Susceptible (CVVHDF)	100% (6/6)	4.5g q8h/CVVHDF	n=6

Abbreviations: CSLI, clinical standard laboratory institute; RFP, renal function preserved; RFA, renal function augmented; AKI, acute renal injury; CVVHDF, continuous veno-venous hemodialysis filtration; TDM, therapeutic drug monitoring; IQR, quartiles (25-75); IBW, ideal body weight; PK/PD, pharmacokinetic-pharmacodynamic target; ICU, Intensive care unit; MIC, minimum inhibitory concentration

Statistics: GraphPad Prism, v.9.1.4, Mann Whitney ($p<0.05$).

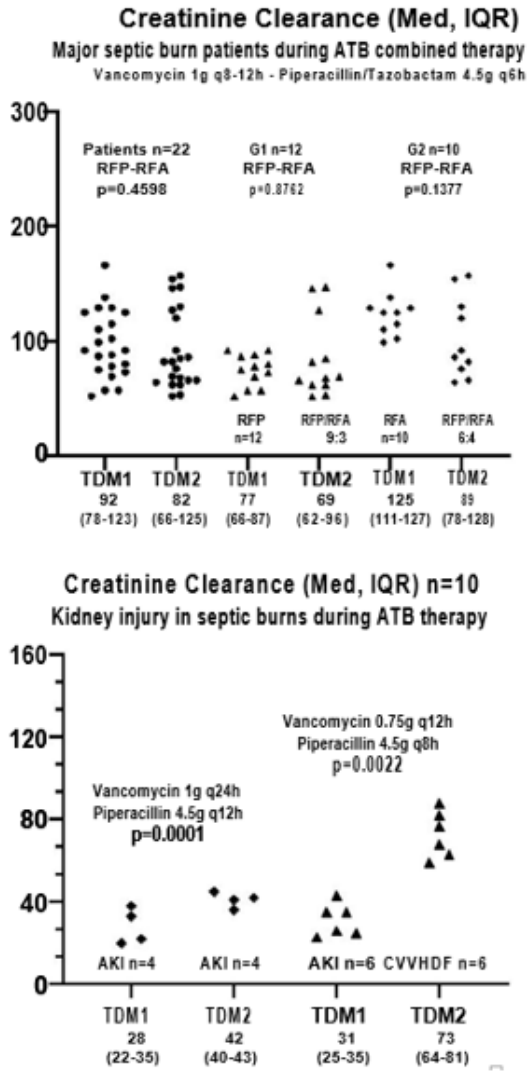

Figure 1 Creatinine clearance according to renal function in septic burns during ATB combined therapy.

1A: Renal function preserved or augmented by vasopressor requirements.

1B: Acute kidney injury or continuous Veno-venous hemodialysis filtration.

Vancomycin: The vancomycin dose regimen recommended for septic patients was based on the ideal weight normalized and the creatinine clearance. The initial drug prescription was applied to 32 patients with preserved renal function, based on creatinine clearance. On the first day of vancomycin therapy started, an increase on creatinine serum levels higher than 2.5 mg/dL occurred due to acute kidney injury (AKI) in 10/32 patients. Then, the vancomycin dose regimen was reduced to 1g every 24 hours, that was maintained during another 96 hours for the steady state levels achievement in those patients; the blood that was sampling in Set 1 (TDM1) for target attainment. Furthermore, in Set 2 (TDM2), dose was maintained for AKI patients (n=4), or a new steady-state serum level was achieved after 48hrs of vancomycin was adjusted to 0.75g every 12h. Consequently, coverage up to MIC 1mg/L against isolated Gram-positive strains was achieved by negative cultures obtained after dose adjusted in TDM2 for these patients.

In addition, 48hrs of vancomycin therapy started on 22/32 patients with renal function preserved (16/32) or augmented renal clearance by vasopressor requirements in 6/32 of them. Dose prescription was increased to 1g every 8 hours in 6/32 patients at Set 2 (TDM 2) with eradication of Gram-positive strains MIC: 1 mg/L *Staphylococcus spp*., in most strains isolated. Consequently, it was found that the renal function was preserved or augmented in 22/32 patients investigated, and the therapeutic target was achieved at MIC: 0.5 mg/L for all of them; but the target against strains MIC: 1 mg/L was attained only in 50% of patients (16/32) based on the vancomycin serum monitoring in Set 1 (TDM 1). Then, the vancomycin dose regimen was adjusted to 1g every 8 hours for target attainment in 6/32 patients at Set 2 (TDM 2) with eradication of Gram-positive strains MIC: 1 mg/L. Finally, the vancomycin effectiveness was based on serum drug levels and MIC data of Gram-positive pathogens isolated from cultures. Therapeutic target (AUC^{ss}_{0-24}/MIC>400-600) recommended was attained against MIC 1mg/L Gram-positive pathogens isolated.[9]

Piperacillin/Tazobactam: Critically ill major burn patients at the earlier stage of the first septic shock received piperacillin/tazobactam as first choice recommended in hospital against *Enterobacteriaceae* nosocomial Gram-negative pathogens. Then, the antimicrobial therapy with this beta-lactam was given 4.5g q6h administered systemically by pump 3 hrs.-extended infusion at the dose regimen recommended in Set 1 (TDM1), according to the institutional protocol of the hospital for ICU major burn patients; dose adjustment was done in Set 2 (TDM-2), if required to individualize therapy to achieve clinical outcome desired also considering effectiveness and safety.

It was shown that acute renal injury (AKI) occurred in 10/32 patients that received 4.5g q12h in Set 1 (TDM1). On Set 2 (TDM2), the same dose regimen was maintained in 4/10 AKI patients for safety, or yet adjusted to 4.5g q8h for drug effectiveness in another six patients undergoing the continuous Veno-venous hemodialysis filtration procedure (CVVHDF), based on creatinine renal clearance higher than 50mL/min. Piperacillin coverage up to MIC 16 mg/L was attained against susceptible strains, and even against strains of intermediate susceptibility (MIC 32mg/L) despite any strain isolated from cultures in those patients.

Piperacillin coverage by target attainment occurred for all patients with renal function preserved or augmented by vasopressors that received the empirical dose regimen at Set 1 (TDM1), and the target was attained up to MIC 16 mg/L in all of them. In Set 2 (TMD2), the same regimen was maintained, and effectiveness was guaranteed to 22 patients with renal function preserved at dose regimen 4.5g q6h since PK/PD target recommended by Abdull Aziz et al 100% *f*ΔT>100 was achieved up to MIC 16 mg/L susceptible strains.[6]

Cultures of isolated strains investigated during the combined therapy

Therapeutic drug serum monitoring routinely done in the protocol twice a week for patients indicates that TDM was required to guarantee drug efficacy-safety, once the clinical cure occurred in a shorter period by negative cultures for patients undergoing combined therapy. Sites of infection were blood stream (59%), lungs, pneumoniae (PNM) unrelated to mechanical ventilation (20%), wound/bone (15%) and urinary tract (6%). Related to microbiology of isolates by vancomycin therapy, it was shown that majority of Gram-positive pathogens were *Staphylococcus spp* isolated from in septic burn patients were *S. aureus* MIC 0.5-1.0 mg/L (n=20), *Staphylococcus epidermidis,* MIC 1mg/L (n=8), followed by *Streptococcus spp* MIC ≤ 0.5 (n=4). It was shown that the vancomycin therapy guided by cultures and serum levels after dose adjustment done in real time by PK/PD approach permits an earlier clinical intervention to reach the desired outcome with clinical cure of infection in a short period.

In addition, it was isolated Gram-negative strains (n=32), piperacillin susceptible (MIC 0.25 - 16 mg/L) of *Enterobacteriaceae* susceptible strains as *Citrobacter spp* (3), *Enterobacter cloacae* (6), *Escherichia coli* (7), *Klebsiella pneumoniae* (6), *Morganelli morganii* (4), *Proteus mirabilis* (4), *Serratia marcescens* (2). Piperacillin/tazobactam empirical dose regimen 4.5g q6h by 3hrs.-extended infusion in patients with renal function preserved or even augmented by vasopressors was guided by drug serum levels done at TDM 1 and TDM 2, and by cultures to guarantee the target attainment for effectiveness or safety. Clinical and microbiological cures occurred for all of them at dose regimen prescribed in a renal function dependence based on PK-changes. Coverage against Gram-negative *Enterobacteriaceae*, including *K. pneumoniae* strains MIC 0.25 up to 16 mg/L isolated was guaranteed in septic major burns investigated.

Discussion

Vancomycin effectiveness-safety, pharmacokinetics based on serum levels against MIC < 1mg/L

It was shown in Table 2 that significant PK changes related to vasopressor requirements (RFA) occurred by comparison with burn patients with RFP receiving the recommended dose regimen 1g q12h. It was shown increases by twice on elimination rate constant [kel (hr-1)], reduction of biological half-life [t(1/2)β (hrs.)] with a proportional increase on total body clearance [CLt (L/h)], remaining unchanged the apparent volume of distribution [Vd^{ss} (L)] in adult major burns receiving vasopressors. Consequently, target was attained by dose adjustment of 1g q8h that was required in six burn patients receiving vasopressors.

In addition, according to acute kidney injury (AKI), ten patients that initially received vancomycin prescription 1g q12h, required urgent individualization of therapy, and dose was reduced to 1g q24h for safety, Table 2. Consequently, significant PK changes occurred in these AKI patients with significant decreases by twice on elimination rate constant [kel (hr-1)], prolongation of biological half-life [t(1/2)β (hrs.)] and proportional decreases on total body clearance [CLt (L/h)], remaining unchanged the apparent volume of distribution [Vd^{ss} (L)] in adult major burns. Consequently, the target was attained by adjustment eq. 1g q24h required in ten burn patients with an acute kidney injury.

Considering data obtained with septic major burn patients in the previous pharmacokinetic studies done in septic adults, or even in pediatric patients (burned *versus* non-burns), it is well known that vancomycin coverage is impacted by differences in pharmacokinetics mainly due to the changes related to a reduction in biological half-life as a consequence of an increase in total body clearance, mainly expected at the earlier phase of septic shock by the need of vasopressors in adult patients, in spite of volume of distribution unchanged in the majority of those patients.[4,5,9–11] In addition, PK changes that occur in pediatric septic patients are age dependently that affects vancomycin coverage by reduction on biological half-life, as a function of increases on total body clearance between 2 up to 6yrs aged compared with 7-14 years, even without vasopressor agents.[10,11]

Piperacillin effectiveness based on serum monitoring against MIC < 16mg/L

It was shown in Table 2, significant changes related to vasopressor requirements (RFA) that occurred by comparison with RFP patients both receiving the recommended dose regimen 4.5g q6h. It showed significant decreases in the apparent volume of distribution [Vd^{ss} (L)], remaining unchanged the total body clearance [CLt (L/h)], the elimination rate constant [kel (hr-1)] and the biological half-life [t(1/2)β (hrs.)] in those septic burn patients receiving vasopressors. It is important to highlight that the coverage occurred in those patients with vasopressor requirements due to the extended 3hrs-infusion applied also in six burn patients at the earlier period of septic shock.

In addition, according to acute kidney injury (AKI), ten patients that initially received piperacillin/tazobactam, empirical prescription 4.5g q6h, required dose individualized due to acute kidney injury; a new steady state level reached after 96hrs of dose reduced to 4.5g q12h for safety, table 2. Important PK changes occurred in these AKI patients with decreases by trice on elimination rate constant [kel (hr-1)] and proportional prolongation on biological half-life [t(1/2)β (hrs.)]. Significant decreases were related to total body clearance [CLt (L/h)] and the apparent volume of distribution [Vd^{ss} (L)] in those AKI patients. Consequently, the target was attained by adjustment eq. 4.5g q12h required in ten burn patients with an acute kidney injury that occurred at the earlier stage of septic shock.

Additionally, since the continuous Veno-venous hemodialysis filtration (CVVHDF) was installed in 6/10 patients previously with acute kidney injury (AKI), PK changes related to a significant increase in total body clearance [CLt (L/h)] and on the apparent volume of distribution [Vd^{ss} (L)] occurred in those patients. Then, the target was attained by adjustment eq. 4.5g q8h at the earlier stage during the second week of septic shock to guarantee piperacillin/tazobactam effectiveness in those patients undergoing continuous Veno-venous hemodialysis filtration.

Piperacillin pharmacokinetics studies previously reported in septic burn adult patients describe that the total body clearance depends only

on kidney glomerular filtration rate, based on creatinine clearance, since no tubular secretion occurs as described for meropenem, a carbapenem agent, largely prescribed against bacteria's no-Enterobacteriaceae. It is well known that superiority coverage after piperacillin/tazobactam is done by extended 3hrs- infusion or even 4hrs. infusion, more recently recommended, is evident when compared to 0.5 hr.-intermittent infusion. It was reported in the last ten years that the prolonged infusion period impacts positively pharmacokinetics changes, resulting by increases on volume of distribution at the steady state levels, with proportional prolongation of biological half-life resulted by increases on piperacillin serum levels in septic patients with renal function preserved (RFP). Then, drug effectiveness impacted by those PK-changes guaranteed coverage up to MIC 16 mg/L against susceptible strains, reaching also coverage against strains of intermediate susceptibility, MIC 32 mg/L according to CSLI data base. It is important to highlight that vasopressor requirements at the earlier stage of septic shock justify the increases by twice that occurred on piperacillin total body clearance higher than total body clearance of meropenem in critically ill septic adult patients [5–7,12–14]

Conclusion

Combined therapy of septic shock with vancomycin-piperacillin/tazobactam against nosocomial pathogens by PK/PD approach was done twice a week based on cultures, ATBs serum levels to guarantee the target attainment in a short period for all patients. Then, this strategy has been applied in the last ten years in the clinical protocols of our hospital, especially in the ICU of Burned Unit, to guarantee the individualized therapy by antimicrobial coverage, that contributes for combating the mutant´s selection of Enterobacteriaceae and to prevent the development of bacterial resistance resulting in death on ICU by selection of MDR pathogens.

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Authors' contributions

All authors contributed equally to this work based on their specialty. DSG contributed to the study related to ethical approvals at the hospital and the Brazilian Platform for clinical projects, data acquisition, interpretation, and critical review of the manuscript content. SS contributed to the conception and design of the study, acquisition and interpretation of data, statistical analysis and writing of the manuscript with critical review for important intellectual content. EMSJ and JMSJ contributed to clinical data acquisition, interpretation, and critical review of clinical data in the manuscript for important intellectual content. ASGA, GAF, TCO contributed to the critically ill patients care in the ICBU, blood collection of viable samples for serum antibiotics measurements, and blood collection for IL6 studies. TVC and MJS contributed to the revision of manuscript and of all articles included in the manuscript, and especially at the last revision done of references included in the manuscript. PR, NJCD and NMS contributed to the critical revision of data for important intellectual new contents. Nairo Massakaku Sumita (Medicine Doctor) NMS is a new cooperator in our Team that participated in this clinical protocol. PRA and MSS contributed to the discussion of data related to TDM of ATB, and to the critical revision for important intellectual content. All authors read and approved the final manuscript version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Limitations

1) This study included only one center of a Public Tertiary Hospital, Medical School of University of São Paulo, Sao Paulo, Brazil. Reference Burn Center at ICU Burns Unit (4 beds); Ward (20 beds); Emergency admission room and Operating room, (8th floor). Unit was connected to the helipad access route for helicopters (12th floor) of the Main Building. 2) Prolonged hospitalization is expected for severe burns.

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