Clinical toxicology

SIMULTANEOUS QUANTIFICATION OF FIVE β-LACTAM ANTIBIOTICS IN HUMAN PLASMA BY HPLC-DAD: CLINICAL APPLICATION FOR CEFTAZIDIME TREATMENT IN INTENSIVE CARE UNITS

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ABSTRACT

A high-performance liquid chromatography method for the simultaneous determination of five β-lactam antibiotics (cefepime, ceftazidime, cefuroxime, meropenem and piperacilline) in human plasma using ultraviolet detection has been developed to control antimicrobial therapy in intensive care units. Ceftazidime is a time-dependent antibiotic, which presents an optimal efficiency directly correlated with the period during which their plasma concentrations remain above four to five time the minimal inhibition concentration (MIC) of pathogens. Analysis of plasma samples obtained from twenty patients, with or without renal failure, and treated by ceftazidime continuous infusions, has shown the clinical suitability of the method. This retrospective study shown that standard dosages (6g/day ceftazidime continuous infusion) were no always adapted and could be reduced for treatment of patients infected by germs presenting MIC ≤4 µg/mL. For patients with renal failure, ceftazidime plasmatic monitoring has performed more important dosage reductions. This retrospective study proved that antibiotic monitoring is an interesting tool to optimise treatment and to provide significant cost reductions.

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INTRODUCTION

β-lactams are now among the antibiotics most commonly used, especially in intensive care units for the treatment of severed infected patients. These antibiotics are time-dependent, which implies that their activity is directly correlated with the time during which their plasma concentration remains above the minimal inhibition concentration (MIC) for the micro-organism responsible for infection (1). To undergo bactericidal effect, animals models show that cephalosporin plasma concentrations have to remain above the MIC for at least 40% to 70% of the dosing interval, and sometimes up to 100 % of this interval in case of severe infections (2). But, although a time above the MIC superior at 50% of the dosing interval

may be sufficient in immunocompetent patients, concentration four to five times the MIC of the identified pathogen may be needed to obtain the maximal killing rate for immunocompromised patients (3). Higher concentrations seem not to significantly enhance the bactericidal activity (4). Administration of β-lactams by continuous infusion is thereby an useful approach to warrant a stable and efficient antibiotic blood concentration. Therefore, monitoring of plasma β-lactams, combined with determination of the MIC, could be an interesting tool in individualising antimicrobial therapy of severely infected patients.

Various HPLC methods have been published to achieve the analysis of cephalosporins in biological matrices, using several sample preparation procedures, different stationary and mobile phases and various detection modes (5-8). In a previous study, the authors had developed and validated a simple and rapid method for the simultaneous determination of five β-lactam antibiotics (cefepime, ceftazidime, cefuroxime, meropenem and piperacilline) in human plasma using ultraviolet detection (9). This method performed good separation, sensitivity and linearity over a wide concentration range.

Ceftazidime, due to its high degree potency and its very interesting tolerability profile, represents an antibiotic of choice for the treatment of bacterial infections in intensive care units (10). It is a third generation cephalosporin agent, with a broad spectrum of antimicrobial activity against gram-negative microorganisms including *Pseudomonas aeruginosa* (11). This β-lactam presents a half-life close to 1.5 hour and is not significantly metabolized. The antibiotic is excreted unchanged by glomerular filtration, with about 90% of the administered dose being eliminated in the urine within 24 h (12). Consequently, in patients with impaired renal function, the ceftazidime clearance becomes smaller and the half-life increases significantly in correlation with the severity of the renal failure (13). Moreover, as small molecular weight molecules with low protein binding (<20%), ceftazidime is succeptible to be eliminated by continuous hemodiafiltration.

Analysis of plasma samples obtained from twenty patients, with or without renal failure and treated with ceftazidime, has shown the clinical suitability of the method for controlling the adjustment of antimicrobial therapy. All patients received by continuous infusion different doses of ceftazidime calculated in function of weight, MIC and eventual renal failure. The aim of this study was to determine whether blood antibiotic concentrations were well adapted considering the MIC of the identified pathogens.

MATERIAL AND METHOD

Subjects

For this retrospective study, we have selected patients admitted to the medical or surgical intensive care units at the University Hospital of Liège and who have developed infections treated with ceftazidime. The antibiotic treatment, delivered by continuous intravenous infusion of ceftazidime, was evaluated in 10 patients with normal renal function (creatinine clearance: higher than 60 mL/min) and in 10 patients with renal failure (7 patients with creatinine clearance calculated by Cockroft and Gault formula, between 30 and 59 mL/min and 3 patients with creatinine clearance calculated in the same way lower than 30 mL/min) (14). The patients received generally a standard intravenous loading dose of 2 g of ceftazidime over a 30 min period, followed via an automatic pump by a continuous infusion of 2 to 8 g of ceftazidime in 48 mL of serum saline administered over a period of 24 h. Blood samples (2 to 5 per patient) were collected from an in situ venous line in anticoagulant tubes after at least two consecutive doses of ceftazidime in order to ensure steady-states concentrations. Once collected, blood samples were immediately centrifuged, and the plasma was analyzed at the same day or stored at -20°C if analysis was delayed.

Pseudomonas were considered susceptible to ceftazidime if MIC was ≤8 µg/mL, and resistant if MIC was >8 µg/mL, according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing - EUCAST (15).

Method

Ceftazidime was determined by HPLC method with ultraviolet detection, routinely used for several years in our laboratory. The analytical method has been validated for simultaneous quantification of five β-lactam antibiotics in human plasma: piperacilline (penicillin derivate), cefuroxime (second generation cephalosporin), ceftazidime (third generation cephalosporin), cefepime (fourth generation cephalosporin) and meropenem (carbapenem). ceftazidime was generously supplied by GlaxoSmithKline (Erembodegem, Belgium). The plasma sample, after spiked with ceforanide as an internal standard (IS), was submitted to a solid-phase extraction (SPE) prior to HPLC analysis. Chromatographic system consisted of a Waters Alliance 2695 Separation Module, equipped with a quaternary, low-pressure mixing pump, a degassing line and a

thermostated autosampler, connected with a Waters 2996 photodiode array detector (Zellik, Belgium). The separation was achieved on a C8 Symmetry column with a mobile phase consisting of an acetonitrile and phosphate buffer mixture (pH 7.4) in a gradient mode.

The identification of antibiotics was based on the retention time and on the UV spectrum. The retention time of ceftazidime was 10,6 min. and the total run time of analysis was 30 min. As illustrated in Figure 1a, plasma sample spiked with 15 µg/mL of each antibiotic are of high chromatographic quality. For specific quantifications, the wavelength was set at 256 nm for ceftazidime, which correspond to the maximum absorbance wavelength for this β-lactam. Chromatogram of a plasma obtained from the patient 2-M (28,5µg/mL) who received 6g ceftazidime /day by continuous infusion is represented in Figure 1b. The developed technique was linear from 2,5 to 60 µg/mL, with a limit of quantification in plasma experimentally determined at 0,5 µg/mL. This concentration was sufficient to establish correlations between the plasma concentrations and the MICs, generally comprised between 0,5 and 4 µg/mL for the most sensitive germs. This method allowed simultaneous antibiotic quantifications and is not exclusively adapted for patients treated with continuous perfusions. Therefore, plasma samples obtained from patients treated with continuous perfusions were systematically diluted with physiological

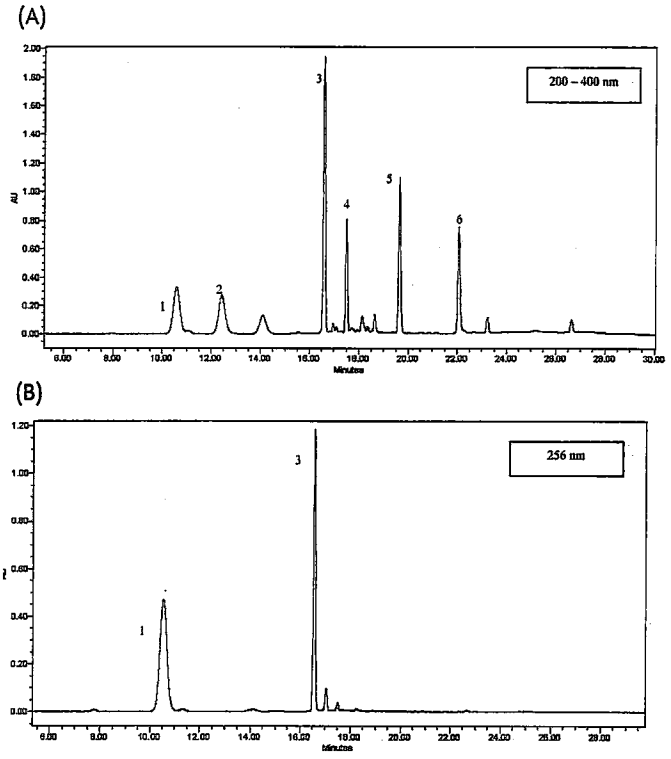

Figure 1: HPLC chromatograms of (A) a plasma spiked with 15 µg/mL of each antibiotic and 20 µg/mL of the internal standard. (1. ceftazidime; 2. cefepime; 3. ceforanide (IS); 4.meropenem: 5. cefuroxime; 6. piperacilline.); (B) a plasma obtained from the patient 2-M (28,5µg/mL) who received 6g ceftazidime /day by continuous infusion. (1. ceftazidime ; 3. ceforanide(IS)).

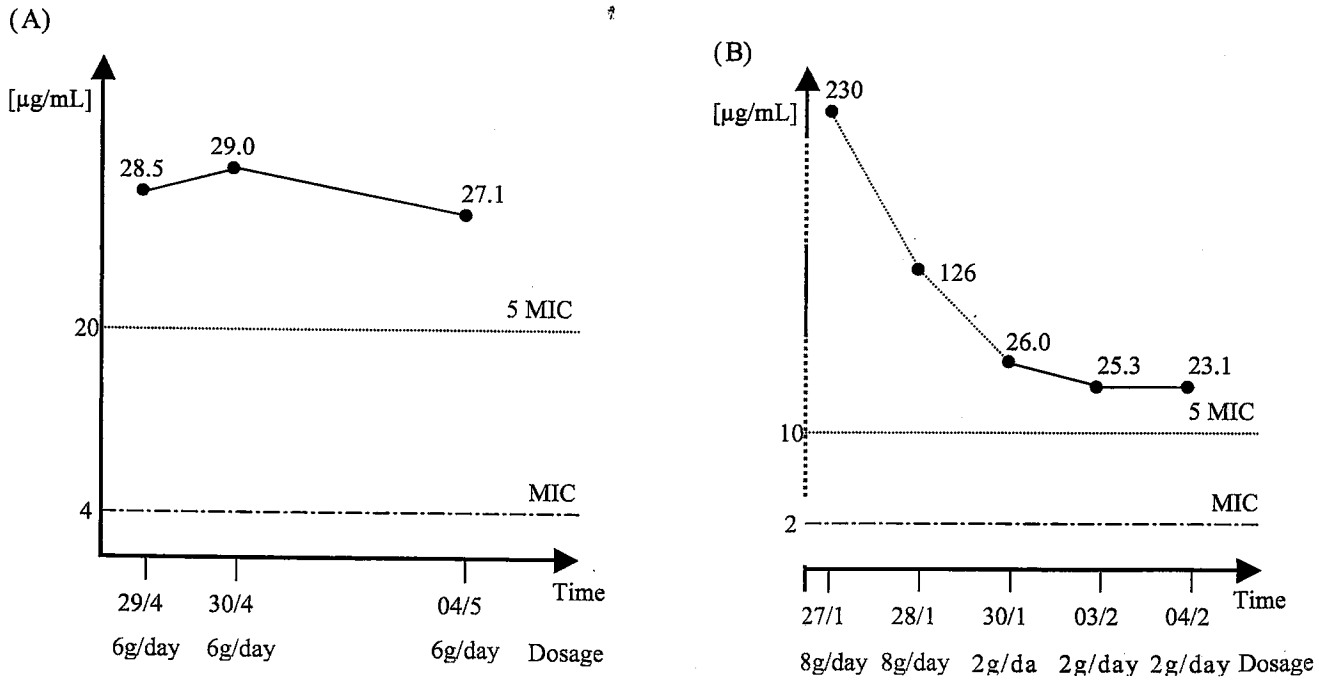

Figure 2: Concentration of ceftazidime in plasma for (A) patient 2-M and (B) patient 20-M.
(A) Patient 2-M: initial loading dose of 2g, followed by a 6g/24h continuous infusion
(B) Patient 20-M: initial loading dose of 2g, followed by a 8g/24h continuous infusion for 2 days before a 2g/24h dosage reduction

serum (NaCl 0.9%) to be sure to obtain values included in the calibration curve. This method is accurate and reproducible (coefficient of variation <10%), allowing, after appropriate dilutions, quantification of plasma levels of ceftazidime from 0,5 to 240 µg/mL without interferences of the other common drugs.

RESULTS

Twenty patients more than 18 years old who had developed severe infections treated by continuous infusion of ceftazidime were retained for this retrospective study. The most common microorganism identified in biological samples (tracheal aspirations, blood, urine) was *Pseudomonas aeruginosa* (n = 18). None of these patients received ceftazidime before the MIC was determined, contained between 1 and 16 µg/mL.

Data obtained for twenty patients have permitted to classify these subjects into three subgroups: first subgroup (called: *Well-adapted treatment*) was constituted of patients who received a well adapted ceftazidime dose as confirmed by therapeutic monitoring (plasmatic concentration = ±5 MIC). For the second subgroup (called: *Adjustment required*), an adjustment of ceftazidime dosage appeared necessary (plasmatic concentration largely >5 MIC). Finally, we have put together in the third subgroup (called: *Adjusted treatment*), the patients who received initially a too high ceftazidime dosage that was successfully adapted after therapeutic monitoring to achieve plasmatic level around 5 MIC. Results for patients with normal renal function and with renal failure are respectively indicated in Table 1 and Table 2. These tables contain in

Table 1: Results obtained for patients with normal renal function.

		Biological results				Antibiotherapy		
Gender	Age	Time	Urea g/L	Creatinine mg/L	CL CR. mL/Min.	MIC	Dose g/24h	Plasmatic Concentration µg/mL
Well-adapted treatment								
1-M	53	14/12	0,53	9,8	84	≤4	6	33,4
		20/12	0,54	4,3	>120	≤4	6	27,9
2-M	64	29/04	0,41	7,3	96	≤4	6	28,5
		30/04	0,32	7,3	97	≤4	6	29,0
		04/05	0,34	5,8	>120	≤4	6	27,1
Adjustment required								
3- F	63	30/10	0,33	4,2	>120	≤1	6	35,6
		31/10	0,25	3,9	>120	≤1	6	29,5
		02/11	0,20	3,8	>120	≤1	6	23,3
4-M	42	28/02	0,21	6,4	>120	≤4	8	35,1
		03/03	-	-	-	≤4	8	42,2
5-M	63	22/02	0,32	6,0	>120	≤2	6	27,3
		23/02	-	-	-	≤2	6	30,6
		25/02	0,20	4,5	>120	≤2	6	26,8
6-M	77	26/10	0,35	6,6	95	≤4	6	33,9
		29/10	0,32	6,0	105	≤4	6	39,9
		30/10	0,42	6,7	94	≤4	6	37,6
		31/10	0,48	5,4	117	≤4	6	35,1
		02/11	0,26	5,0	>120	≤4	6	36,8
7- F	46	13/07	≤0,05	4,3	>120	≤4	6	42,3
		23/07	≤0,05	3,4	>120	≤4	6	41,8
		03/08	0,11	5,4	>120	≤4	6	36,1
8-M	60	22/01	0,39	10,3	86	≤4	8	87,2
		23/01	0,19	5,4	>120	≤4	8	75,5
Adjusted Treatment								
9- F	77	04/07	0,34	6,2	84	≤4	8	63,6
		09/07	0,26	5,3	98	≤4	6	41,3
10- F	52	12/06	0,39	5,6	>120	≤4	8	27,4
		17/06	0,39	5,1	>120	≤4	10	42,4
		20/06	0,38	5,5	>120	≤4	10	44,0
		26/06	0,42	5,5	>120	≤4	10	48,4

Table 2: Results obtained for patients with renal failure.

		Biological results				Antibiotherapy		
Gender	Age	Time	Urea g/L	Creatinine mg/L	CL CR. mL/Min.	MIC	Dose g/24h	Plasmatic Concentration µg/mL
Well-adapted Treatment								
11- F	66	10/11	0,60	32,0	18	≤8	2	39,7
		13/11	0,40	28,5	20	≤8	2	48,8
12-M	78	19/09	0,70	15,2	41	≤16	6	71,7
		20/09	0,63	16,1	39	≤16	6	114,4
		21/09	0,65	17,5	36	≤16	6	100,8
		22/09	0,68	17,4	36	≤16	6	116,9
Adjustment required								
13-F	69	13/11	0,90	9,8	49	≤4	6	86,3
		14/11	1,02	11,5	38	≤4	6	118,8
14-M	75	31/10	0,97	22,2	35	≤1	2	69,3
		02/11	0,59	16,7	46	≤1	2	59,2
15-M	18	13/05	0,86	19,0	54	≤4	6	62,5
		14/05	0,97	19,4	53	≤4	6	60,9
		15/05	0,90	16,9	61	≤4	6	56,4
16-M	78	30/07	0,96	22,8	31	≤2	6	102,6
		03/08	1,49	32,9	22	≤2	6	88,5
		06/08	1,07	25,3	28	≤2	6	84,2
17- F	63	16/07	0,40	3,3	>120	≤2	3	27,0
		17/07	0,92	7,4	61	≤2	3	64,8
		18/07	1,46	10,8	42	≤2	3	158,7
18- F	58	03/08	0,53	9,9	105	≤4	6	116,5
		04/09	0,81	36,4	28	≤4	6	164,6
Adjusted treatment								
19- F	41	18/09	1,33	88,5	8	≤2	2	114,4
		20/09	0,98	78,0	10	≤2	1	46,1
20-M	53	27/01	1,60	30,3	35	≤2	8	230,2
		28/01	1,14	23,6	45	≤2	8	126,8
		30/01	0,50	12,4	85	≤2	2	26,0
		03/02	0,36	10,0	105	≤2	2	25,3
		04/02	0,50	11,3	93	≤2	2	23,1

addition to the plasmatic concentration of antibiotic, several informations: gender, age, dosage, sampling dates, biological results (urea, creatinine, creatinine clearance, MIC).

Subjects with normal renal function

We can observe that all patients with normal renal function received a standard dose of 6g/day ceftazidime by continuous infusion, excepted for the patients 4-M, 8-M, 9-F and 10-F, who received initially a 8g ceftazidime per day. For these dosages, the mean steady-state ceftazidime concentration was 34,2 ± 5,4 µg/mL. Those concentrations were always sufficient (maybe too high for the subjects 6-M and 7-F) to treat efficiently patients infected by germs presenting MIC ≤4 µg/mL (5 MIC = 20 µg/mL), but were certainly too low for MIC ≤8 µg/mL (5 MIC = 40 µg/mL). On the other hand, 6g/day ceftazidime continuous infusions were not adapted to treat infections by germs with MIC lower than 2 µg/mL (5 MIC = 10 µg/mL). Indeed, in this case, an important reduction of dosage could be proposed. The patient number 10-F represented an exception. This woman (height: 160 cm; weight: 128 kg) received a 8g/day ceftazidime continuous infusion to treat a diabetic foot infection, with *Pseudomonas aeruginosa* having a MIC of ≤4 µg/mL. As plasmatic concentration was relatively low for this indication (27,4 µg/mL), physician decided to raise dosage at 10 g/day to obtain at least 40 µg/mL as minimal plasmatic concentration, considering possible underlying bone infection.

Subjects with impaired renal function

Our retrospective study showed that these patients could reach high plasmatic ceftazidime concentrations with low administered dosages. In these patients, higher plasmatic levels are reached and are thus suitable for treatment of infections caused by less sensitive pathogens (patient 12-M: MIC ≤16 µg/mL, 6g/day continuous infusion, plasmatic concentrations above 100 µg/mL). On the other hand, this retrospective study demonstrated that, for patients with renal failure, the reduction of dosages had to be done according to the importance of the renal insufficiency, with the ceftazidime dose generally comprised between 1 and 3 g/day.

The evolution of plasma concentrations over time for patient 2-M, with normal renal function, and for patient 20-M, with acute renal failure, are shown in Figure 2. For the first one, dosage adjustment was not required: ceftazidime plasma concentrations were higher than 5 MIC, without to be high enough to reduce dosage. In this case, therapeutic drug monitoring had shown that initial dosage was well adapted and required no modification. For the second patient, antibiotic plasma concentrations were very important, higher than 100 µg/mL (MIC ≤2 µg/mL), for a 8g/day continuous infusion of ceftazidime. Therefore, a reduction of the dosage, from 8g to 2g/day, was proposed, and has led to ceftazidime plasma concentrations higher than 20 µg/mL, enough to reach antibiotic effectiveness.

For all patients presented in this retrospective study, ceftazidime plasma concentrations determined with the HPLC method were always above the target concentration, setting at 5 MIC. No exception was observed.

DISCUSSION

Experimental studies had demonstrated that ceftazidime has a slow continuous kill characteristic, which was correlated with the time during which serum concentrations exceed the MIC. Indeed, absence of post-antibiotic effect observed with this β-lactam could facilitate the development of antibiotic resistance once antibiotic concentrations fall below this threshold (16). To avoid resistance development, some authors proposed to maintain antibiotic plasmatic concentrations above the MIC for 90 to 100% of the dosing interval. Other studies had demonstrated maximum killing of germs at four to five MIC, with no added effect at higher concentrations (17). Therefore, plasmatic concentrations maintained at 4 to 5 MIC during each dosing period could be retained as therapeutic goal, particularly in cases of severe infected patients (18-19). Thereby, ceftazidime plasmatic concentrations should be always higher than 20 µg/mL to treat a patient infected with pathogens presenting a MIC ≤4 µg/mL. As these concentration levels can't be reached with intermittent dosages, the application of continuous infusions is now generalized to ceftazidime treatments of severed infected patients.

Since the time during which the plasma concentration remains above the MIC is one of the most important parameters for the success of β-lactams, individual determinations of antibiotic concentrations are interesting applications in the treatment of each patient seriously infected. Indeed, these infected patients

may present important changes in haemodynamics and organ function, with modifications of half-lifes, volumes of distribution or clearances (20). The knowledge of the pharmacokinetic profile of antimicrobial agents in this subpopulation is thus crucial to adjust correctly the dosage. The therapeutic failure may result from inadequate administered treatment, which can nevertheless be corrected with the therapeutic drug monitoring.

The aim of our retrospective study was to analyse in two groups, with or without renal failure, correlations between ceftazidime dosages and ceftazidime plasmatic concentrations, and determined if adjustment of dosage were required, considering MIC of microorganisms inducing infection. For all patients, ceftazidime plasma concentrations determined with the HPLC method were above 5 MIC. The therapeutic drug monitoring shown that dosages administered to patients in this retrospective study were often too high. For patients with normal renal function, this retrospective study shown that 6g/day ceftazidime continuous infusions were always sufficient to treat infections with germs presenting MIC ≤4µg/mL, with a mean steady-state ceftazidime concentration at 34,2 µg/mL. In effect, ceftazidime concentrations, determined by HPLC analysis, were always above the threshold limit set at 20 µg/mL (MIC ≤4 µg/mL). For a lot of these patients, reduction of dosages to 3 - 4g/day could certainly be proposed without lack of efficiency. For patients infected with very sensitive bugs (MIC ≤1µg/mL), lower dosages than 6g/day should always be considered, resulting in important reduction of antibiotic treatment cost. On the other hand, plasma concentrations observed after 6g/day ceftazidime continuous infusions are always to low for the treatment of normal renal patients infected with germs presenting MIC ≥8 µg/mL, which is in agreement with the sensitive breakpoints defined by the EUCAST (15). Previous studies with normal renal populations presented same observations (16). For renal insufficient patients, our retrospective study showed that it wasn't easy to predict plasmatic ceftazidime concentrations. The adjustment of the dosage, generally comprised between 1 to 3g/day, had to be done according to the determined plasmatic concentration and the importance of the renal insufficiency.

In this case also, we have obtained an important reduction of antibiotic treatment cost. Our study shown that too high ceftazidime dosages were always administered if we considered 5 MIC level as optimal efficiency plasmatic concentration. In almost all cases reported, therapeutic drug monitoring proposed a dosage reduction, sometimes very significant.

Optimisation of treatment with plasmatic ceftazidime determination could reduce the antibiotherapy cost. Adjustment of ceftazidime infusion from 6 to 4 grams per day allows approximately an economy of 250 euros per week, which could represent an important amount on looking at the large prescription of ceftazidime in intensive care units. These observations have to be confirmed with a perspective study including the clinical efficacy of the antibiotic treatments.

CONCLUSION

Analysis of plasma samples obtained from twenty patients treated by ceftazidime continuous infusion has shown the clinical suitability of the method for controlling the antimicrobial therapy. This application could propose to physicians a highly performing approach of the antibiotherapy in intensive care units. Our retrospective study proved that ceftazidime dosages were often too high, which caused important and useless expenses. Therefore, ceftazidime plasmatic monitoring is an interesting tool to optimise antibiotic treatment and to provide significant pharmacoeconomic advantages.

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