Quantification and explanation of the variability of first dose amikacin concentrations in critically ill patients admitted at the emergency department: a population pharmacokinetic analysis.

Authors:

Sabrina De Winter*¹, Reinier van Hest*², Erwin Dreesen³, Pieter Annaert^{4,5}, Joost Wauters⁶, Wouter Meersseman⁶, Nele Van den Eede⁷, Stefanie Desmet⁷, Sandra Verelst⁸, Peter Vanbrabant⁶, Willy Peetermans⁶, Isabel Spriet^{1,9}

*Shared first author

Affiliations

1 Department of Pharmacy, University Hospitals Leuven, Leuven, Belgium

2 Department of Hospital Pharmacy & Clinical Pharmacology, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands

3 Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium. Department of Pharmacy, Uppsala University, Uppsala, Sweden. ORCID-ID: 0000-0002-0785-2930

4 Department of Pharmaceutical and Pharmacological Sciences, Drug delivery and Disposition, KU Leuven, Leuven, Belgium. ORCID-ID: 0000-0003-3525-7351

5 BioNotus, Galileilaan 15, 2845 Niel (Belgium)

6 Department of Internal Medicine, University Hospitals Leuven, Belgium

7 Laboratory of Clinical Bacteriology and Mycology, University Hospitals Leuven, Belgium

8 Department of Emergency Medicine, University Hospitals Leuven, Belgium

9 Department of Pharmacy, University Hospitals Leuven and Department of Pharmaceutical and Pharmacological Sciences, University of Leuven, Leuven, Belgium. ORCID-ID: 0000-0001-6342-0676

Abstract

Background There may be differences in determinants defining amikacin exposure in emergency department (ED) versus intensive care (ICU) patients, in whom amikacin peak concentration is highly variable between patients. Moreover, fluid resuscitation and vasopressors have just been initiated at the ED. Nevertheless, no population pharmacokinetic modelling data of amikacin are available for ED patients.

Objective The aim of this study was to quantify interindividual variability (IIV) in the pharmacokinetics of amikacin in patients admitted at the ED and to identify which patient characteristics explain this IIV.

Methods Patients presenting at the ED with severe sepsis or septic shock were randomly assigned to receive amikacin 25 mg/kg or 15 mg/kg intravenously. Blood samples were collected at 1, 6 and 24 hours after the onset of the first amikacin infusion. Data were analyzed using non-linear mixed-effects modelling.

Results A two-compartment population pharmacokinetic model was developed based on 279 amikacin concentrations from 97 patients. IIV in clearance (CL) and central distribution volume (V₁) were 71% respectively 26%. Body mass index (BMI), serum total protein levels, serum sodium levels and fluid balance 24 hours after amikacin administration explained 30% of the IIV in V₁, leaving 18% IIV unexplained. BMI and creatinine clearance according to the Chronic Kidney Disease – Epidemiology Collaboration equation (CKD-EPI) 24 hours after amikacin administration explained 46% of IIV in CL, and 39% remained unexplained. **Conclusion** IIV in amikacin pharmacokinetics in ED patients is large. In patients with low serum sodium, low total protein levels, high fluid balance higher doses may be considered.

Key points:

- In patients with severe sepsis or septic shock admitted at the emergency department, the volume of distribution of amikacin is increased and the amikacin clearance are in line to what has been documented before for patients admitted in the intensive care units.
- Taking into account the EUCAST susceptibility breakpoints for *Enterobacteriaceae* and *Pseudomonas aeruginosa*, the first amikacin dose of 25 mg/kg should be given to obtain the amikacin level of 64 mg/L in patients with severe sepsis or septic shock admitted at the emergency department.
- For patients with low serum total protein levels, low serum sodium levels, or positive fluid balance even a higher first dose than 25 mg/kg is recommended to avoid treatment failure.

Main Text

1. Introduction

In patients with severe sepsis and septic shock, early and adequate administration of antimicrobials improves patients' survival [1,2]. Empiric combination therapy with aminoglycosides is often applied and recommended in severe sepsis and septic shock guidelines to broaden the coverage of Gram-negative bacteria [2,3]. Aminoglycosides are concentrationdependent antimicrobials. The pharmacokinetics and pharmacodynamics target is defined as a peak concentration which should exceed at least 8 times the minimal inhibitory concentration (MIC) of the infecting pathogen ($C_{max}/MIC \geq 8$). Therapeutic drug monitoring with peak and trough monitoring is applied to ensure target attainment and avoid renal insufficiency, respectively [4].

However, several studies demonstrated a decreased C_{max} of aminoglycosides in critically ill patients admitted at the intensive care unit (ICU) when using a standard once-daily dosing regimen of amikacin 15 mg/kg or gentamicin 3 mg/kg [5,6]. Antimicrobial underdosing, leading to low plasma exposure, has been associated with decreased clinical outcome, which supports the administration of higher first doses, up to 25 mg/kg for amikacin and up to 7 mg/kg for gentamicin in patients admitted at the ICU [7,8]. However, even then, the pharmacokinetics and pharmacodynamics target ($C_{max}/MIC \geq 8$) is not obtained in 25% of patients [5,9-11]. The volume of distribution (V_d) of aminoglycosides in this patient population is increased up to two times, especially in the early hyperdynamic phase of sepsis, and a wide interindividual variability (IIV) in the V_d has been reported in ICU patients [9,12-20]. There may be a difference in determinants defining amikacin exposure in ED vs. ICU patients. At the time the first dose of antimicrobials is administered in septic patients admitted at the ED, fluid resuscitation has just been initiated. Moreover, the extent to which the endothelium is damaged as a result of sepsis in comparison with ICU patients is unclear. In contrast to patients hospitalized at the ICU [21], edema formation due to capillary leak is hardly seen in ED patients. In addition, patients admitted at the ED from home or even from the hospital ward are often in a dehydrated state which might contribute to a decrease in the V_d [22]. In contrast, ICU patients tend to have a positive fluid balance [21]. Nevertheless, no data on population pharmacokinetic modelling of amikacin are available for patients admitted with severe sepsis or septic shock at the emergency department (ED), despite its frequent use in this setting [23]. Recently, we published the results of a randomized controlled trial evaluating the impact of a usual vs. higher dose of amikacin in septic ED patients on the pharmacokinetics and pharmacodynamics target-attainment [24]. When using actual documented MICs as denominator, the exposure in the individual included patients was sufficient, also when using a standard dose of 15 mg/kg. However, when using the EUCAST

(European Committee on Antimicrobial Susceptibility Testing) susceptibility breakpoints for *Enterobacteriaceae* and *Pseudomonas aeruginosa*, the target was only attained in 40% and 76% of included patients, when they were assigned to the 15 vs. 25 mg/kg, respectively [24]. In this study, a population pharmacokinetic model for amikacin in ED patients with severe sepsis or septic shock was developed. The aim of this study was to quantify IIV in the pharmacokinetics of the first dose amikacin in ED patients, and to identify which patient characteristics explain this IIV as well as to evaluate the impact of these patient characteristics on probability of target attainment (PTA).

2. Patients and methods

2.1 Patients

Adults (≥ 18 years) with severe sepsis or septic shock according to standard criteria admitted at the ED of the University Hospitals Leuven between 8 am and 5 pm on weekdays, were prospectively included from January 2013 to August 2014 [25]. This timeframe was chosen in function of availability of the research team. Written informed consent was obtained from each patient or relative. Patients with (1) age < 18 years, (2) pregnancy, (3) burns, (4) amikacin treatment 2 weeks prior to inclusion, (5) known allergy to aminoglycosides, (6) a Do Not Resuscitate code, or (7) dialysis within 24 hours after admission were excluded. Patients were randomly assigned by envelope to a single dose of 15 mg/kg vs. 25 mg/kg, using total body weight (TBW) for dose calculation. The dose was limited to 1.2 g and 2 g in the 15 mg/kg vs. 25 mg/kg group for patients with a TBW higher than 80 kg. Amikacin (Amukin®Bristol-Myers Squibb) was given intravenously as a 30-minute infusion. At the discretion of the treating clinician, other antibiotics were prescribed in combination with amikacin. Blood samples were collected in lithium-heparin tubes at 1, 6 and 24 hours after the onset of the first amikacin infusion.

2.2 Bioanalytical Assay

The samples were immediately analysed in our hospital using a validated homogeneous turbidimetric immunoassay method [26]. A CE-IVD labeled immunoassay (AMIK2, Roche Diagnostics GmbH) performed on Cobas system (Roche Diagnostics GmbH) was used. The measuring range is 0.8-40 mg/L. The lower limit of quantification is 0.8 mg/L. Concentrations below this limit were not considered in the analyses. For concentrations above the upper limit of quantification, samples were manually diluted and were reassayed.

2.3 Population pharmacokinetic data analysis

2.3.1 Model development

Amikacin concentration-time data were analysed using nonlinear mixed-effects modelling (NONMEM v.7.3.0, ICON Development Solutions, Gaithersburg, Maryland, USA) [27]. Pirana 2.9.4 was used as an interface for NONMEM, Xpose and R. The first-order conditional estimation (FOCE) method with interaction was used throughout the data analysis. A three-step approach was attempted during the modelling process.

During the first step, a compartmental population pharmacokinetic model was established, quantifying amikacin Vd and clearance (CL). The ADVAN 1 subroutine was used for 1 compartmental models and the ADVAN 3 subroutine for 2 compartmental models, in which the following parameters were estimated: central and peripheral Vd (V1 and V2, respectively), CL and intercompartmental clearances (Q). Moreover, IIV was estimated for the pharmacokinetic parameters assuming a log-normal distribution. In addition, residual variability was estimated by testing additive, proportional and combined error models. Whether addition of a parameter to the model provided a better fit over the reduced model (1 degree of freedom) was evaluated by the objective function value (OFV), where a drop of >3.84 units

corresponds to a significance level of a p-value <0.05 , the precision (relative standard error (RSE)) with which parameters were estimated, the magnitude of residual variability, shrinkage of random-effects parameters and goodness of fit plots.

During the second step, the following patient-related covariates were tested for association with amikacin Vd (V1 and/or V2), CL and Q: sex, age, total body weight (TBW), ideal body weight (IBW), body mass index (BMI), body surface area (BSA), dosing group, indication amikacin (severe sepsis vs. septic shock) at inclusion, body temperature at inclusion, mean arterial pressure (MAP) at inclusion, Acute Physiology And Chronic Health Evaluation (APACHE) II upon admission, Sequential Organ Failure Assessment (SOFA) upon admission, use of vasopressors at inclusion, dose of noradrenaline at inclusion, mechanically ventilated at inclusion, white blood cell count at inclusion, C-reactive protein at inclusion, serum sodium at inclusion and at 24 h, serum albumin at inclusion, serum total protein at inclusion, hematocrit at inclusion, platelet count at inclusion, urea at inclusion, prothrombin time (PT) at inclusion, serum creatinine at inclusion and at 24 h, estimated creatinine clearance by Cockcroft-Gault (eCL_{CG}) at inclusion and at 24 h, estimated glomerular filtration rate according to the Chronic Kidney disease – epidemiology (CKD-EPI) at inclusion and at 24 h, fluid therapy during minus 6 h until inclusion, fluid therapy first 1 hour after inclusion, fluid therapy first 24 hours after inclusion, diuresis during first 24 hours after inclusion and fluid balance during first 24 hours after inclusion. Fluid therapy is defined as the sum of the administration of fluid, intravenous infusions and medication volumes and fluid balance is the difference between fluid therapy and diuresis.

Handling of missing data was carried out as follows: 1) if less than 10% of data was missing: the median value was imputed; 2) if 10% or more, but less than 50%, was missing: concentration-time data from patients with missing covariate data were ignored in estimating the association between the pharmacokinetic parameter and the concerning covariate, as described previously [28]; 3) if 50% or more was missing: the covariate was excluded. Given the a priori known large effect of renal function on amikacin CL, all available measures of renal function (serum creatinine at inclusion and at 24 h, eCL_{CG} at inclusion and at 24 h, CKD-EPI at inclusion and at 24 h) were first tested in a univariate way and the measure that had the statistically strongest association was included in the basic model. Subsequently, since amikacin dose is based on body weight, a correlation was introduced between body size and Vd and CL. For that reason, all available measures of body size recorded on ED admission (TBW, IBW, BSA, BMI) were tested by adding them separately to the model with the selected association between renal function and CL in it. The measure of body size that had the statistically strongest association was included.

Next, all other covariates were tested by adding them separately to the basic model with associations in it between Vd and the selected measure of body size, and between CL and the selected measures of body size and renal function. The following criteria were used for covariate selection: 1) biological plausibility of a covariate-pharmacokinetic parameter association; 2) statistical significance of the association between the covariate and the pharmacokinetic parameter using a p-value cut-off of 0.05, corresponding with a decrease in OFV of >3.84 points; 3) a reduction in IIV and/or residual variability, and 4) improvement of the goodness of fit plots.

Finally, a stepwise forward addition and backward elimination procedure was performed with all statistically significant covariates next to the associations between Vd and the selected measure of body size, and between CL and the selected measures of body size and renal function. When addition, respectively exclusion of a covariate-pharmacokinetic parameter

association resulted in a decrease, respectively an increase of the OFV of >10.8 ($p < 0.001$, one degree of freedom), the association was selected for the final model prior to validation. For more detailed information on how covariates are modeled, see supplementary material (file 1).

2.3.2 Model evaluation

The robustness of the final model was tested using a bootstrap analysis, in which the dataset was resampled with replacement and fitted to the model 1000 times. In addition, the model's capacity to predict the range of observed concentrations was tested by means of a prediction corrected visual predictive check (VPC) with 1000 simulations [29]. The bootstrap as well as the VPC analyses were performed using Perl-speaks-NONMEM version 3.5.3 software (PsN, Uppsala, Sweden).

2.3.3 Pharmacokinetic model simulations

Monte Carlo simulations were performed to evaluate the impact of the covariates retained in the final model on amikacin peak concentrations and on target-attainment after a single administration of 15 mg/kg or 25 mg/kg amikacin. Amikacin C_{max} was defined as the concentration 1 h after start of a 30 minute infusion. The target was defined as the peak/MIC > 8 resulting in a peak of 64 mg/L for a MIC of 8 mg/L which is the EUCAST susceptibility breakpoints for *Enterobacteriaceae* and *Pseudomonas aeruginosa* [30]. The PTA was subsequently calculated for this target. Simulations with up to 1000 patients per category were based on the final model. The patients in each category had all median characteristics of the population, including a total body weight of 70 kg, except for the indicated covariate, which was set to be the 5th or 95th percentile of the population of the concerning covariate. The retained covariates in the final model were not correlated with each other (see supplementary figure 1).

3. Results

Ninety-seven patients with severe sepsis or septic shock were included in the study. Patients' baseline characteristics are shown in Table 1. The amikacin starting dose was 15 mg/kg in 48 patients and 25 mg/kg in 49 patients. Of all scheduled samples, 4.1 percent (12/291) were missing; eleven for clinical reasons (i.e. decease, excessive blood loss) and one for analytical reason, leaving 279 amikacin blood samples collected within 24 hours of inclusion. Individual concentration-time profiles illustrated a large variability in amikacin exposure (ranges of 32.6-137.6 mg/L vs. 0.8-52 mg/L for the peak and trough levels, respectively).

3.1 Population pharmacokinetic model

During the first step of the data analysis, the untransformed amikacin concentration-time data best fitted a two-compartment model with first-order elimination. IIV was estimated for V₁ and CL, including their correlation. IIV in V₁ and CL were 26% and 71%, respectively. Parameter estimates of this structural model are presented in Table 2. During the second step, CKD-EPI at 24 h was the measure of renal function that had the strongest associations with amikacin CL (OFV drop: 89 units), and BMI was the measure of body size that had the statistically strongest association with V₁ and CL (OFV drop: 6.3 and 15 units respectively). The other covariates were added separately to the model with the associations between CKD-EPI at 24 h and CL and between BMI and V₁ and CL. The covariates SOFA score upon admission, body temperature at inclusion, serum albumin at inclusion, hematocrit at inclusion, total serum protein at inclusion, serum sodium at inclusion and fluid balance for the first 24 hours after inclusion were statistically significantly associated with amikacin V₁ ($p < 0.05$). SOFA score upon admission, severe sepsis or septic shock and MAP at inclusion demonstrated statistically significant ($p < 0.05$) associations with amikacin V₂.

The presence of severe sepsis vs. septic shock, MAP at inclusion, fluid balance for the first 24

hours, SOFA score upon admission, white blood cell count at inclusion, and PT at inclusion showed statistically significant ($p < 0.05$) associations with amikacin CL. Mean arterial blood pressure (MAP) at inclusion showed a statistically significant ($p < 0.05$) univariate correlation with amikacin Q.

Subsequently, a stepwise forward addition and backward elimination procedure was performed, which resulted in inclusion of the following associations in the final model (prior to validation): a higher BMI, a lower serum total protein concentrations at inclusion, a lower serum sodium concentration at inclusion and a positive fluid balance measured during the first 24 hours after inclusion are associated with a larger central Vd. A higher BMI and a higher CKD-EPI at 24 h were associated with a higher amikacin clearance.

The parameter estimates of this final model are summarized in Table 2.

BMI, serum total protein concentrations, serum sodium concentration, and fluid balance measured during the first 24 hours after amikacin administration explained 30% of the IIV in amikacin V1, and 18% IIV remained unexplained.

BMI and CKD-EPI at 24 h explained 46% of IIV in amikacin CL, and 39% of the IIV remained unexplained.

Table 2 shows that bootstrap median values closely agree with the population estimates of the final model. Figures 1A and 1B illustrate the VPC plots of the final model for the 15 mg/kg and the 25 mg/kg dosing group. Both VPC plots demonstrated an adequate fit of the model, with only a slight underprediction in the 15 mg/kg dosing group of the 5th percentile at 6 h and a slight overprediction in the 25 mg/kg dosing group of the 95th percentile at the end of the dosing interval. The percentage of observations outside the 95% CI is 6.9% (10 of 144 data points) in the 15 mg/kg dosing group and 13.4% (19 of 142 data points) in the 25 mg/kg dosing group.

3.2 Pharmacokinetic simulations

Target attainment in function of the retained covariates was simulated (Figure 2). The boxplots per category are displayed in Figure 2A and 2B for the 15 mg/kg and 25 mg/kg dosing group, respectively. We used the following categories in the simulations: 1) median patient, 2) serum total protein, 5th percentile (i.e. 44 g/L), 3) serum total protein, 95th percentile (i.e. 81 g/L), 4) serum sodium, 5th percentile (i.e. 126 mmol/L), 5) serum sodium, 95th percentile (i.e. 147 mmol/L), 6) fluid balance first 24 h, 5th percentile (i.e. 416 mL), 7) fluid balance first 24 h, 95th percentile (i.e. 8671 mL), 8) CKD-EPI 24 h, 5th percentile (i.e. 10 mL/min), 9) CKD-EPI 24 h, 95th percentile (i.e. 117 mL/min).

BMI was not included in the simulations because a different BMI is caused by a different body weight which means a different dose. As a result, it is not possible to show in simulations how much variability is explained by BMI, as can be done for the other covariates because the same dose must be simulated in each patient.

In case of a peak target of 64 mg/L, assuming an MIC of 8, PTA was low for all categories in the 15 mg/kg dosing group. In the 25 mg/kg dosing group, a 5th percentile serum total protein (i.e. 44 g/L), a 5th percentile serum sodium (i.e. 126 mmol/L), a 95th percentile fluid balance first 24 h (i.e. 8671 mL) and a 95th percentile CKD-EPI 24 h (i.e. 117 mL/min) had an PTA between 84 and 95%. A patient with all median values of the included covariates had an PTA of 99% in the simulations in the 25 mg/kg dosing group.

4. Discussion

Our analysis showed that the population pharmacokinetic estimates of first dose amikacin demonstrate considerable IIV. For CL, almost half of the IIV could be explained by BMI and CKD-EPI at 24 h. Almost one third of the IIV in V1 could be explained by BMI, serum total

protein concentration, serum sodium concentration, and fluid balance measured during the first 24 hours. The estimated pharmacokinetic parameter in our population of ED patients admitted with severe sepsis or septic shock are similar to earlier reported values from pharmacokinetic models for amikacin and gentamicin for septic patients admitted at the ICU [16,21,31-33] (see supplementary table 1). Also, the identified covariates influencing Vd such as serum total protein and fluid balance are in line with the results previously reported in literature [10,16,32]. Innovative in this study is the importance of serum sodium concentration on Vd.

A negative correlation was found between serum sodium and V1. The addition of serum sodium to the final model with covariates on central Vd resulted in a statistically significant improvement of the fit ($p < 0.001$) and a decrease in the estimate for IIV in V1 from 21% to 18%. Serum sodium concentrations also significantly correlated with amikacin Cmax according to our multivariable analysis in our previously published study, analysing individual pharmacokinetic profiles [24]. Since a positive correlation between serum sodium concentrations and serum urea or serum creatinine concentrations was found, we hypothesize that higher serum sodium concentrations in ED patients are predominantly found in dehydrated patients, which might presumably leads to a lower central amikacin Vd [24].

Furthermore, a lower serum total protein at inclusion was found to be associated with a larger Vd. Previous studies also demonstrated that hypoalbuminemia was independently associated with an increased Vd [16,32]. Loss of albumin may be a marker for endothelial leakage originated by sepsis or an increased intravascular volume due to fluid resuscitation [34]. However, in our study, serum albumin was not a statistically significant covariate in contrast to serum total protein (correlation coefficient=0.671, see supplementary figure 2 for correlation plot). This might be explained by the fact that the IIV in albumin concentrations at inclusion (i.e. mean $\pm$ SD = 32.1 ± 6.3 g/L and median (range) = 32.7 (14.9 – 45.1) g/L) in our data was relatively small as opposed to serum total protein concentrations (i.e. mean $\pm$ SD= 64.6 ± 11.2 g/L and median (range) = 66 (26 - 90) g/L). In patients hospitalized at the ICU, the serum albumin concentrations are generally lower; serum albumin concentrations of 24 ± 6 g/L and 21.5 [10-36] g/L were previously reported by Lugo et al. as mean $\pm$ SD, and by Hodiamont et al. as median (range) [16,32], but data on serum total protein concentrations were however not available.

In addition, a positive fluid balance was significantly associated with a larger Vd which seems plausible for a hydrophilic drug like amikacin. Moreover, de Montmollin et al. showed in their multivariable analysis that the 24 h fluid balance *before* inclusion was significantly associated with subtherapeutic amikacin Cmax in ICU patients [10]. Since fluid intake and diuresis before ED admission were lacking, 24 h fluid balance *after starting amikacin* was included in our analysis.

Body weight, BSA, the ratio of partial arterial oxygen pressure (PaO₂) to fractional inspired oxygen (FiO₂) (i.e. PaO₂/FiO₂ ratio), oxygen extraction ratio (defined as the ratio of oxygen consumption to oxygen delivery) and APACHE II score have also been identified in literature as covariates explaining the IIV of Vd [16,17,21,33,35]. We tested the impact of APACHE II on Vd, but this covariate was not retained. It would be interesting to collect alternative markers for capillary leak, dehydration and fluid resuscitation in order to better understand their impact on Vd.

Another interesting observation is that there is almost 60% correlation between CL and V1. Probably, fluid load, as a result of fluid resuscitation in patients with sepsis and shock, is responsible for an increased V1 (i.e. fluid balance is associated with IIV in V1) but probably as

well with enhanced amikacin clearance, as it is expected that fluid administration and increased cardiac output might lead to at least temporary augmented renal clearance.

BMI and CKD-EPI estimated glomerular filtration explain 46% of the IIV in amikacin CL. Delattre et al. highlighted the significant influence of eCL_{CG} on the clearance of amikacin in critically ill septic ICU patients during the early phase of the disease process [31]. Boidin et al. also found out that eCL_{CG} was a more influential covariate on amikacin clearance than the glomerular filtration rate estimated by the equation from CKD-EPI [33]. Hodiamont et al. identified that creatinine clearance calculated by 6 h timed urinary collection resulted in an acceptable estimation of gentamicin CL, whilst serum creatinine concentrations and eCL_{CG} overestimated gentamicin CL, presumably due to the absence of active secretion for gentamicin [32]. We did not correct the CKD-EPI equation based on the patient's actual body surface area. A recent study evaluated the bias between kidney function estimated by 56 formulas including CKD-EPI, and measured creatinine clearance (by urinary collection) in overweight and obese patients. The authors identified that estimating formulas fail to reflect real renal function in overweight and obese patients, and that adjusting for BSA leads to relevant underestimation of measured GFR. The impact of it on our results might be limited as none of the included patient has a BMI over 40 kg/m² [36]. Moreover, we prefer to use parameters that are readily available to the physician. By default in our hospital, the laboratory provides an estimated GFR based on the CKD-EPI formula corrected for a BSA of 1.73 m². In practice, the emergency physician does not convert the estimated GFR to the patient's actual BSA. Next to the impact of BMI and CKD-EPI, 39% of the IIV in CL remains unexplained. Literature shows that positive end-expiratory pressure and the use of catecholamines have an effect on the IIV of CL [16,17] but this could not be confirmed in our study.

Simulations were performed to calculate the PTA according to the EUCAST susceptibility breakpoints for *Enterobacteriaceae* and *Pseudomonas aeruginosa*, taking into account the median characteristics of the population and the 5th or 95th percentile values of the retained covariates (Figure 2). A first dose of 25 mg/kg should be given in all patients admitted at the ED with sepsis or septic shock in order to warrant a PTA of > 90 %. The first dose of 15 mg/kg should thus be completely abandoned at the ED. In patients with high fluid load, characterized by low serum total protein, low serum sodium or maybe also when presenting with augmented renal clearance, even higher first doses up to 30 mg/kg or more, can be considered, as in these situations, PTA decreasing [33,37,38].

To our knowledge, this is the first population pharmacokinetic model developed for first dose amikacin based on data of patients admitted at the ED with severe sepsis and septic shock. This model was based on 279 amikacin concentrations measured in 97 septic patients during the first 24 hours of antimicrobial therapy. The strength of this prospective study is the accuracy of timing of amikacin administration and sample collection under strictly controlled conditions as part of a randomized controlled trial. Only 4% of planned samples were missing, contributing to an accurate estimation of pharmacokinetic parameters. No amikacin concentrations have been determined between 6 and 24 hours, which means that the estimates for the second compartment may be somewhat uncertain (see the relatively high RSE (%) on V₂ of 36%). This is a limitation, but nevertheless, it was better to use a 2 compartmental model.

Conclusion

IIV in amikacin pharmacokinetics in ED patients is large and patient characteristics associated with it, like renal function, serum total protein concentrations, serum sodium concentration and fluid balance, are in line with what has been documented for ICU patients. These covariates can

explain almost half of the IIV in CL, and almost one third of the IIV in V1. Taking into account the EUCAST susceptibility breakpoints for *Enterobacteriaceae* and *Pseudomonas aeruginosa*, the first dose of 25 mg/kg should be given to obtain the amikacin level of 64 mg/L in the vast majority of patients. In patients with low serum sodium, low total protein levels or high fluid balance, even higher first doses may be considered.

Ethics declarations

Funding: ED is a postdoctoral research fellow of the Research Foundation – Flanders (FWO), Belgium (grant number 12X9420N). The other authors declare no sources of funding were used to assist with the preparation of the results of this original research.

Conflict of interest: ED received consultancy fees from argenx and Janssen (all honoraria/fees paid to the University). The other authors declare no conflicts of interest that are relevant to the content of this original research.

Ethics Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study received institutional approval from the Clinical Trial Center of University Hospitals Leuven (B32220109909).

Consent to participate: Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Consent for publication: Not applicable

Availability of data and material: The authors confirm that the data supporting the findings of this study are available upon request.

Code availability (software application or custom code): The authors confirm that the code is available upon request.

Research reporting

Clinical Trial Registration: ClinicalTrials.gov ID: NCT02365272

Authors' contribution

SDW, RvH and IS designed the trial. ED, PA, JW, WM, SV, PV and WP participated in its design. SDW conducted the study and performed the database management. JW, WM, SV and PV participated in the recruitment of patients. NVdE and SD performed the laboratory analyses. RvH performed the population pharmacokinetic analysis. SDW and RvH performed statistical analyses. SDW, RvH, ED and IS drafted the article and all authors participated in its critical revision.

References

1. Gaieski DF, Mikkelsen ME, Band RA, et al. Impact of time to antibiotics on survival in patients with severe sepsis or septic shock in whom early goal-directed therapy was initiated in the emergency department. *Crit Care Med.* 2010;38:1045-53.
2. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med.*

2013;39:165-228.

3. Elbaz M, Zadka H, Weiss-Meilik A, et al. Effectiveness and safety of an institutional aminoglycoside-based regimen as empirical treatment of patients with pyelonephritis. *J Antimicrob Chemother.* 2020;75:2307-13.
4. Spriet I, van Hest RM, Peetermans WE, et al. Comment on: Effectiveness and safety of an institutional aminoglycoside-based regimen as empirical treatment of patients with pyelonephritis. *J Antimicrob Chemother.* 2020;75:3696-7.
5. Gálvez R, Luengo C, Cornejo R, et al. Higher than recommended amikacin loading doses achieve pharmacokinetic targets without associated toxicity. *Int J Antimicrob Agents.* 2011;38:146-51.
6. Roger C, Nucci B, Molinari N, et al. Standard dosing of amikacin and gentamicin in critically ill patients results in variable and subtherapeutic concentrations. *Int J Antimicrob Agents.* 2015;46:21-7.
7. Beaucaire G, Leroy O, Beuscart C, et al. Clinical and bacteriological efficacy, and practical aspects of amikacin given once daily for severe infections. *J Antimicrob Chemother.* 1991;27:91-103.
8. Moore RD, Smith CR, Lietma PS. Association of aminoglycoside plasma level with therapeutic outcome in Gram-negative pneumonia. *Am J Med.* 1984;77:657-62.
9. Taccone FS, Laterre PF, Spapen H, et al. Revisiting the loading dose of amikacin for patients with severe sepsis and septic shock. *Crit Care.* 2010;14:R53.
10. de Montmollin E, Bouadma L, Gault N, et al. Predictors of insufficient amikacin peak concentration in critically ill patients receiving a 25 mg/kg total body weight regimen. *Intensive Care Med.* 2014;40:998-1005.
11. Rea RS, Capitano B, Bies R, et al. Suboptimal aminoglycoside dosing in critically ill patients. *Ther Drug Monit.* 2008;30:674-81.
12. Marik PE. Aminoglycoside volume of distribution and illness severity in critically ill patients. *Anaesth Intensive Care.* 1993;21:172-73.
13. Triginer C, Izquierdo I, Fernández R, et al. Gentamicin volume of distribution in critically ill septic patients. *Intensive Care Med.* 1990;16:303-6.
14. Oparaoji EC, Cornwell EE 3rd, Hekmat E, et al. Aminoglycoside volume of distribution in postoperative patients with septic shock. *Clin Pharm.* 1993;12:131-4.
15. Buijk SE, Mouton JW, Gyssens IC, et al. Experience with a once-daily dosing program of aminoglycosides in critically ill patients. *Intensive Care Med.* 2002;28:936-42.
16. Lugo G, Castañeda-Hernández G. Relationship between hemodynamic and vital support measures and pharmacokinetic variability of amikacin in critically ill patients with sepsis. *Crit Care Med.* 1997;25:806-11.
17. Lugo-Goytia G, Castañeda-Hernández G. Bayesian approach to control of amikacin serum concentrations in critically ill patients with sepsis. *Ann Pharmacother.* 2000;34:1389-94.
18. Tang GJ, Tang JJ, Lin BS, et al. Factors affecting gentamicin pharmacokinetics in septic patients. *Acta Anaesthesiol Scand.* 1999;43:726-30.
19. Hassan E, Ober JD. Predicted and measured aminoglycoside pharmacokinetic parameters in critically ill patients. *Antimicrob Agents Chemother.* 1987;31:1855-8.
20. Beckhouse MJ, Whyte IM, Byth PL, et al. Altered aminoglycoside pharmacokinetics in the critically ill. *Anaesth Intensive Care.* 1988;16:418-22.
21. Pea F. Plasma pharmacokinetics of antimicrobial agents in critically ill patients. *Curr Clin Pharmacol.* 2013;8:5-12.
22. Khoujah D, Martinelli AN, Winters ME. Resuscitating the Critically Ill Geriatric Emergency Department Patient. *Emerg Med Clin North Am.* 2019;37:569-81.
23. Marsot A, Guilhaumou R, Riff C, et al. Amikacin in Critically Ill Patients: A Review of Population Pharmacokinetic Studies. *Clin Pharmacokinet.* 2017;56:127-38.

24. De Winter S, Wauters J, Meersseman W, et al. Higher versus standard amikacin single dose in emergency department patients with severe sepsis and shock: a randomized controlled trial. *Int J Antimicrob Agents*. 2018;51:562-70.
25. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup. Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock, 2012. *Intensive Care Med*. 2013;39:165-228.
26. von Eckardstein A, Roth HJ, Jones G, et al. cobas 8000 Modular analyzer series evaluated under routine-like conditions at 14 sites in Australia, Europe, and the United States. *J Lab Autom*. 2013;18:306-27.
27. Beal S, Sheiner LB, Boeckmann A, et al. NONMEM users guides. Ellicott City (MD): Icon Development Solutions;2011.
28. van Hest RM, van Gelder T, Bouw R, et al. Time-dependent clearance of mycophenolic acid in renal transplant recipients. *Br J Clin Pharmacol*. 2007;63:741-52.
29. Holford N. The visual predictive check-superiority to standard diagnostic (Rorschach) plots. In: Abstracts of the annual meeting of the Population Approach Group in Europe. Pamplona, Spain. Poster 105. 2005. <http://www.pagemeeting.org/page/page2005/Posters2005.pdf> Accessed 25 February 2021.
30. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters; v. 9.0, valid from 2019-01-01. http://www.eucast.org/clinical_breakpoints/. Accessed 25 February 2021.
31. Delattre I, Musuamba FT, Nyberg J, et al. Population pharmacokinetic modeling and optimal sampling strategy for Bayesian estimation of amikacin exposure in critically ill septic patients. *Ther Drug Monit*. 2010;32:749-56.
32. Hodiamont CJ, Juffermans NP, Bouman CSC, et al. Determinants of gentamicin concentrations in critically ill patients: a population pharmacokinetic analysis. *Int J Antimicrob Agents*. 2017;49:204-11.
33. Boidin C, Bourguignon L, Cohen S, et al. Amikacin Initial Dose in Critically Ill Patients: a Nonparametric Approach To Optimize A Priori Pharmacokinetic/Pharmacodynamic Target Attainments in Individual Patients. *Antimicrob Agents Chemother*. 2019. <https://doi.org/10.1128/AAC.00993-19>.
34. Chelazzi C, Villa G, Mancinelli P, et al. Glycocalyx and sepsis-induced alterations in vascular permeability. *Crit Care*. 2015;19:26.
35. Burdet C, Pajot O, Couffignal C, et al. Population pharmacokinetics of single-dose amikacin in critically ill patients with suspected ventilator-associated pneumonia. *Eur J Clin Pharmacol*. 2015;71:75-83.
36. López-Martínez M, Luis-Lima S, Morales E, et al. The estimation of GFR and the adjustment for BSA in overweight and obesity: a dreadful combination of two errors. *Int J Obes (Lond)*. 2020;44:1129-40.
37. Matthaiou DK, De Waele J, Dimopoulos G. What is new in the use of aminoglycosides in critically ill patients? *Intensive Care Med*. 2014;40:1553-5.
38. Marsot A, Hraiech S, Cassir N, et al. Aminoglycosides in critically ill patients: which dosing regimens for which pathogens? 2020;56:106124.

Figure 1

The prediction-corrected visual predictive checks of the two-compartmental model of amikacin: (A) Final Model of 15 mg/kg dosing group and (B) Final Model of the 25 mg/kg dosing group.

Figure 1A

Prediction-corrected Visual Predictive Check for the 15 mg/kg dosing group. The solid black line is the observed median and the dashed lines are the 5th and 95th percentiles of the observed data. The red shaded area is the 95% CI of the model-predicted median and the blue shaded areas are the 95% CI of the model predicted 5th and 95th percentiles. The open circles are the concentrations as observed. The black solid and dashed lines run within their respective shaded areas, thereby demonstrating adequate fit of the model, with the exception of the 5th percentile which shows a slight underestimation of concentrations at t=6 h.

Figure 1B

Prediction-corrected Visual Predictive Check for the 25 mg/kg dosing group. The solid black line is the observed median and the dashed lines are the 5th and 95th percentiles of the observed data. The red shaded area is the 95% CI of the model-predicted median and the blue shaded areas are the 95% CI of the model predicted 5th and 95th percentiles. The open circles are the concentrations as observed. The black solid and dashed lines run within their respective shaded areas, thereby demonstrating adequate fit of the model, with the exception of the 95th percentile at t=24 h which shows a slight overestimation.

Figure 2

The boxplots of the simulations evaluating the effect of the covariates on the peak target 64 mg/L for the 15 mg/kg (Figure 2A) and 25 mg/kg (Figure 2B) dosing group, respectively.

Figure 2A

Shown are simulation based on the final model of 1000 patients per category/boxplot. The patients in each category have all the median characteristics of the population, including a total bodyweight of 70 kg, except for the indicated variable, which then is the 5th or 95th percentile of the population of that variable. The boxes represent the 25th to 75th percentile with the black horizontal line being the median, the whiskers represent the minimum and maximum simulated maximum concentration (C_{max}). The dashed line at 64 mg/L represents the peak target of 64 mg/L assuming an MIC of 8 mg/L. PTA = probability of target attainment (%).

Figure 2B

Shown are simulation based on the final model of 1000 patients per category/boxplot. The patients in each category have all the median characteristics of the population, including a total bodyweight of 70 kg, except for the indicated variable, which then is the 5th or 95th percentile of the population of that variable. The boxes represent the 25th to 75th percentile with the black horizontal line being the median, the whiskers represent the minimum and maximum simulated maximum concentration (C_{max}). The dashed line at 64 mg/L represents the peak target of 64 mg/L assuming an MIC of 8 mg/L. PTA = probability of target attainment (%). The PTA for 4 covariate effects is reported to be 100 % while the minimum values lies below 64 mg/L. This is the result of rounding the PTA to whole percentages. So maximum 5 values are below 64 mg/L.

Table 1:
Baseline characteristics of critically ill patients receiving first dose of amikacin (n=97)

Characteristic	Value ^a
Patients' demographics	
Male [n (%)]	62 (64 %)
Age, years	73 (62 – 81)
Total body weight, kg	70 (60 – 85)
Ideal body weight, kg	63 (57 – 70)
Body mass index, kg/m ²	25 (21 – 28)
Body surface area, m ²	1,82 (1,67 – 1,95)
Preadmission renal insufficiency (eGFR<60ml/min) [n (%)]	26 (30%) (n=86)
Dosing group: [n (%)]	
15 mg/kg	48 (49%)
25 mg/kg	49 (51%)
Clinical and hemodynamic parameters	
Indication of amikacin: severe sepsis/septic shock [n (%)]	72 (74%) / 25 (26%)
Body temperature at inclusion, °C	37.8 (36.7 – 38.5)
Mean Arterial Pressure at inclusion, mm Hg	66 (59 – 74)
APACHE II score	22 (18 – 27)
SOFA score	6 (4 - 8)
Use of vasopressors at inclusion, [n (%)]	17 (17.5)
Dose of noradrenaline at inclusion if noradrenaline was started at inclusion, mcg/kg/min	0.1 (0.05 – 0.25) (n = 17)
Mechanically ventilated at inclusion [n (%)]	16 (16%)
Biochemical parameters ^b	
White blood cell count, x10 ⁹ /L	12.3 (8.0 – 19.9)
C-reactive protein, mg/L	142 (48 - 263)
Serum sodium, mmol/L	138.4 (134.1 – 141.1)
Serum sodium at 24 h after inclusion, mmol/L	139.4 (136.5 – 142.4)
Serum albumin, g/L	32.7 (28.3 – 36.6)

Serum total protein, g/L	66 (56 – 72.5)
Hematocrit, %	0.359 (0.307 – 0.422)
Platelet count, $\times 10^9/\text{L}$	213 (146 – 274)
Prothrombin time, %	60 (39 – 75.9)
Serum creatinine, mg/dL	1.48 (1.04 – 2.21)
Urea, mg/dL	65 (45.5 – 90.5)
eCrCl Cockcroft-Gault, mL/min	38.4 (26.5 – 62.9)
eGFR CKD-EPI, mL/min/1.73 m ²	43 (25 – 68.5)
Serum creatinine at 24 h after inclusion, mg/dL	1.27 (0.76 – 1.96)
eCrCl Cockcroft-Gault at 24 h after inclusion, mL/min	52.5 (31.2 – 75.1)
eGFR CKD-EPI at 24 h after inclusion, mL/min/1.73 m ²	52.5 (26 – 85)
Fluid therapy	
Fluid IN minus 6 h until inclusion, mL	1050 (530 - 1685)
Fluid IN inclusion until 1 h later, mL	300 (170 – 603)
Fluid IN inclusion until 24 h later, mL	4040 (3009 – 5299)
Diuresis inclusion until 24 h later, mL	1430 (910 – 2003) (n=85)
Fluid Balance first 24 h after inclusion, mL	2780 (1406 – 3824)

eCrCl, estimated creatinine clearance by the Cockcroft-Gault formula and eGFR, estimated glomerular filtration rate according to the Chronic Kidney Disease - Epidemiology (CKDEPI) Collaboration equation, respectively, Fluid IN is the sum of administration of fluid, intravenous infusions and medication volumes.

^a Data are median (interquartile range) unless otherwise stated.

^b At inclusion unless otherwise stated.

Table 2: Parameter estimates of the structural model

Parameter	Basic model	Final model (RSE%) ovariates	Bootstrap estimates (95% confidence interval) of final model with covariates ^a
<u>Population parameters</u>			
CL (L/h)	1.8 (8.0)	1.8 (6.5)	1.8 (1.6 - 2.0)
V1 (L)	17 (3.3)	16 (3.2)	16 (15 - 17)
Q (L/h)	0.75 (7.9)	0.77 (11)	0.79 (0.66 - 1.6)
V2 (L)	8.6 (41)	13 (36)	12 (5.3 - 23)
Interindividual variability ^b (%CV)			
CL (%) [eta-shrinkage%]	71 (21) [4.5]	39 (31) [7.8]	36 (26 – 50)
V1 (%) [eta-shrinkage%]	26 (34) [19]	18 (50) [26]	17 (8.9 – 25)
Correlation coefficient IIV CL and IIV V1 (%)	21 (60)	58 (34)	59 (55 – 58)
<u>Residual unexplained variability</u>			
Proportional residual variability (%)	19 (12)	19 (12)	18 (14 – 22)

<u>Covariate associations</u>			
CKD-EPI at 24 h with CL	-	0.86 (12)	0.86 (0.65 - 1.1)
BMI with CL	-	0.86 (21)	0.86 (0.53 - 1.2)
BMI with V1	-	0.42 (40)	0.42 (0.08 - 0.75)
Serum total protein with V1	-	-0.53 (29)	-0.52 (-0.90 - -0.18)
Serum sodium with V1	-	-2.1 (28)	-2.1 (-3.4 - -0.91)
Fluid balance first 24 h with V1	-	0.00005 (25)	0.0005 (0.00003 - 0.00007)
Correction factor when fluid balance first 24 h is not available	-	0.90 (7.9)	0.89 (0.76 - 1.1)

BMI: body mass index, CV: coefficient of variation, CKD-EPI: glomerular filtration estimated according to the Chronic Kidney Disease - Epidemiology Collaboration equation, CL: amikacin clearance, Q: amikacin intercompartmental clearance, RSE: relative standard error, V1: amikacin central volume of distribution, V2: amikacin peripheral volume of distribution. ^a972 of 1000 bootstrap runs minimised successfully
^bthe CV(%) representing the interindividual variability was calculated by $\sqrt{\exp(\omega^2)-1}$