Population pharmacokinetics and pharmacodynamics of levofloxacin in acutely hospitalized older patients with various degrees of renal function: the difficult balance between efficacy and safety

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Abstract

A retrospective study was conducted in a large sample of acutely hospitalized older patients who underwent therapeutic drug monitoring during levofloxacin treatment. The aim was to assess the population pharmacokinetics (popPK) and pharmacodynamics of levofloxacin among older patients.

PopPK and Monte Carlo simulation were performed for defining the permissible doses in older patients according to various degrees of renal function. CART analysis was used to detect the cut-off AUC_{24h}/MIC ratio that best correlated with clinical outcome. Probability of target attainment (PTA) of this value was calculated against different pathogens. 168 patients were included, and 330 trough and 239 peak concentrations were used for the popPK analysis. Creatinine clearance was the only covariate that improved the model fit (Levofloxacin $CL=0.399+0.051 \cdot CrCL_{CKD\text{-}EPI}$). Drug doses ranged between 500 mg every 48h and 500 mg every 12h in relation to different renal function. The identified cut-off AUC_{24h}/MIC ratio (≥ 95.7) was the only covariate that correlated with favorable clinical outcome at multivariate regression analysis (OR 20.85; 95% CI 1.56–186.73). PTAs were optimal (>80%) against *E. coli* and *H. influenzae*, borderline against *S. aureus*, and suboptimal against *P. aeruginosa*. Levofloxacin doses defined in our study may be effective for the treatment of infections due to bacterial pathogens with an MIC ≤ 0.5 mg/L in older patients with various degrees of renal function, while minimizing the toxicity risk. Conversely, the addition of another active antimicrobial should be considered whenever treating infections caused by less susceptible pathogens.

Key words: fluoroquinolones, personalized therapy, safety, efficacy, population pharmacokinetics

Introduction

Levofloxacin is a fluoroquinolone antibiotic with one of the broadest spectra of activity, encompassing both Gram-negative and Gram-positive organisms, atypical and anaerobic bacteria (1). Accordingly, it has been used for many years for the treatment of a variety of infections, such as community-acquired pneumonia, skin and soft tissues infections, urinary tract infections, acute exacerbation of chronic bronchitis and sinusitis (2, 3).

Levofloxacin is a moderately lipophilic drug, which is mainly renally eliminated as an unchanged moiety. A linear relationship between drug clearance (CL) and creatinine clearance (CrCL) has been demonstrated (4). From a pharmacodynamic point of view, it has been shown that the most relevant predictor of fluoroquinolone efficacy in clinical settings is the 24-hour area under the concentration-time curve (AUC_{24h})/minimum inhibitory concentration (MIC) ratio. Different AUC_{24h}/MIC ratios have been proposed as optimal targets according to the invading pathogen. Although an AUC_{24h}/MIC ratio of 25-30 may suffice for infections due to *S. pneumoniae* (5), values of 100-125 have been recommended for efficacy against those due to Gram-negative pathogens (6, 7). Interestingly, an AUC_{24h}/MIC target of ≥ 87 was associated with microbiological eradication of both Gram-positives and Gram-negatives among 47 patients who were treated with levofloxacin for nosocomial pneumonia (8). However, it should be noticed that in this study levofloxacin was combined with other agents in those patients infected with *Pseudomonas aeruginosa* (ceftazidime or piperacillin/tazobactam) or with methicillin-resistant *Staphylococcus aureus* (vancomycin) (8). Similarly, combination therapy was also present in the retrospective analysis by Schentag et al. (7).

Fluoroquinolones are among the most frequently used antimicrobials for the treatment of community acquired infections, which account for a significant amount of emergency visits and hospitalizations among older adults. Older patients may be at increased risk of adverse drug reactions (ADRs), mainly because of the pathophysiological changes associated with ageing processes and/or of polypharmacy (9). High frequency of tendinopathy and of tendon ruptures in

older patients were associated with ageing, impairment of renal function and corticosteroid co-administration (10, 11).

Accordingly, since levofloxacin toxicity is dose dependent (12), from a safety perspective, dosage adjustments in older patients with varying degrees of renal impairment should be warranted in order to avoid drug-related toxicity (13, 14).

The primary aim of this study was to describe the population pharmacokinetics and pharmacodynamics of high dose levofloxacin in a large sample of acutely hospitalized older patients in order to estimate the permissible doses that would grant safe and effective exposure in older patients with various degrees of renal function.

Materials and Methods

Study design

This was a retrospective study conducted between May 2007 and December 2012 among older patients aged ≥ 65 years, who were admitted at the 1st Division of Internal Medicine of the Santa Maria della Misericordia University Hospital of Udine, Udine, Italy, and who underwent therapeutic drug monitoring (TDM) of levofloxacin at the Institute of Clinical Pharmacology of the same hospital. The study was approved by the Regional Ethics Committee. Informed written consent was waived according to the retrospective and observational nature of the study.

Patients received levofloxacin because of documented or suspected bacterial infection. The use of additional antimicrobial agents was permitted and at the discretion of treating physician (ceftazidime, piperacillin/tazobactam or meropenem for suspected and/or proven Gram-negative infections; vancomycin or teicoplanin for suspected and/or proven MRSA infections).

The dosage of levofloxacin was initially chosen by the attending physician and subsequently adjusted on the basis of TDM-guided clinical pharmacological advices that were made promptly available in the hospital intranet. TDM of levofloxacin is routinely performed at our hospital, with target concentrations of 1-3 mg/L for trough and of 6-9 mg/L for peak (which was collected 2 hours after oral administration or 1.5 hours after i.v. administration), respectively. These concentrations correspond to AUC_{24h} values between 50 and 160 mg·h/L, that are the range of exposures normally observed with the standard high dose of 500 mg every 12h (that is licensed in Italy) in subjects with normal renal function (7, 15-17). This TDM-guided approach, by maintaining exposure within the expected normal range, is finalized to prevent theoretical overexposure (arbitrarily defined as AUC_{24h} > 160 mg·h/L) and may concur to minimize the risk of exposure dependent toxicity in older patients, definitely the population at greater risk of toxicity during levofloxacin therapy (11).

The following demographic and clinical data were retrieved from each patient's medical record: age, gender, weight, height, type and site of infection, bacterial clinical isolate (whenever available) with MIC of levofloxacin, underlying disease(s), serum creatinine, levofloxacin dose,

route of administration and TDM data, and co-treatment with any other drug. Baseline and end of therapy C-reactive protein (CRP) were also collected. Creatinine clearance was estimated by means of the Chronic Kidney Disease Epidemiology (CKD-EPI) formula ($CrCL_{CKD\text{-}EPI}$) (18).

Blood samples for TDM were collected at least 48 hours from starting levofloxacin. Levofloxacin concentrations were analyzed by means of a validated high performance liquid chromatography (HPLC) method with UV detection, as previously described (4). Precision and accuracy were assessed by performing replicate analysis of quality control samples against calibration standards. Intra- and inter-assay coefficients of variation were always less than 10%. The lower limit of detection was 0.1 mg/L.

Assessment of clinical outcome

Clinical outcomes were defined as cured, improved, unchanged or failed according to treatment response assessed at end of therapy by the attending physician. A patient was classified as cured if signs and symptoms of infection disappeared at the end of therapy, as improved in case of partial clinical response associated with significant decrease in CRP values from baseline, as unchanged or failed in case of absence of clinical response at the end of therapy. Patients cured and improved were considered to have a successful clinical outcome.

Population pharmacokinetic modeling

One and two-compartment models were developed and fitted using the non-parametric adaptive grid (NPAG) approach included in the Pmetrics package for R (Los Angeles, CA, USA) (19). The base-weighting scheme was developed by use of a polynomial function that relates drug concentration to the standard deviation (SD) of the observations, using the between-day assay variability data. Maximum a posteriori probability (MAP)-Bayesian parameter estimates for levofloxacin were determined for each patient in the dataset, and were used for describing the pharmacokinetic parameters (ka, kcp, kpc, CL, Vd, F_{os}, T_{lag}) for each patient in the population.

Firstly, we developed a basic model without covariates by using the building dataset, which was parameterized only for clearance (CL) and for volume of distribution (Vd). Subsequently, we tested covariates that were deemed clinically relevant. Only those covariates that significantly increased the log-likelihood value of the covariate model (i.e. twice the difference in log-likelihood value for the covariate versus the base model with the appropriate degrees of freedom assessed against a $\chi 2$ distribution) were retained for further analysis.

The model performance was further evaluated by assessing the goodness-of-fit of the observed-predicted plot, the coefficient of determination of the linear regression of the observed-predicted values and the OFV (Objective Function Value) of each run. Additionally, also a visual predictive check (VPC) and normalized prediction distribution errors (NPDEs) were determined. The VPC compares the observed concentrations overlaid with model-predicted concentration-time profiles; 95% of observed concentrations should reside within the 95% confidence interval (CI) derived from model predictions. NPDEs provide a quantitative assessment of the final model and are considered a better evaluation tool than a plot of weighted residuals, especially when dealing with models with covariates (20). NPDEs should be normally distributed when the model is appropriately fitted.

Monte Carlo simulation for estimation of levofloxacin doses predicting optimal target drug exposure in older patients with various degrees of renal function

One thousand-subject Monte Carlo simulations were conducted using Pmetrics to estimate the AUC_{24h} achievable with various candidate regimens of levofloxacin (125 mg every 48h, 250 mg every 48h, 250 mg daily, 500 mg every 48h, 750 mg every 48h, 500 mg daily, 750 mg daily and 500 mg every 12h) for different levels of renal function (0-19, 20-39, 40-59, 60-79 and > 80 mL/min/1.73 m^2).

In order to define the permissible levofloxacin doses in the study population, we considered as desirable in this population the achievement of the exposure range that was observed in healthy

163 volunteers with normal renal function with the standard high dose of 500 mg every 12h (AUC_{24h} of
164 50-160 mg·h/L) (14-16). Consistently, AUC_{24h} < 50 mg·h/L was defined as underexposure, AUC_{24h}
165 between 50 and 160 mg·h/L was defined as optimal target exposure, and AUC_{24h} > 160 mg·h/L was
166 defined as overexposure. Permissible doses were defined as those producing a less than 10% of
167 probability of causing both drug underexposure and overexposure in each class of renal function.
168 The identified levofloxacin doses were considered sufficiently safe for clinical use in this
169 population, and were subsequently tested in the pharmacokinetic/pharmacodynamic analysis.
170
171 ***Pharmacokinetic/pharmacodynamic (PK/PD) analysis***
172 AUC_{24h}/MIC ratios were calculated for all of those patients who had bacterial clinical
173 isolates yielded and tested for levofloxacin susceptibility. Considering that levofloxacin is
174 approximately 30% plasma protein bound, all the pharmacodynamic targets were multiplied by
175 factor 0.7 in order to obtain the free targets ($fAUC_{24h}$/MIC), which were than included in the PK/PD
176 analysis.
177 Logistic regression analysis was used to explore the relationship between drug exposure and
178 other clinical factors on the probability of clinical outcome. For those patients who had
179 antimicrobial combination therapy, we created a dichotomous categorical variable. Covariates
180 resulting with a P < 0.20 at the univariate analysis were deemed of potential clinical relevance and
181 then included in the multivariate model on the basis of a forwards stepwise approach.
182 Classification and regression tree (CART) analysis was used to develop a prediction model
183 for detecting the cut-off value of AUC_{24h}/MIC ratio that best correlates with favorable clinical
184 outcome in the study population. Subsequently, the validity of the identified cut-off value was
185 tested by means of receiver operating characteristic (ROC) analysis.
186
187 ***Probability of target attainment (PTA) and cumulative fraction of response (CFR) at the cut-off***
188 ***AUC_{24h}/MIC ratio associated with favorable clinical outcome***

We estimated the probability of target attainment (PTA) of the identified cut-off value of AUC_{24h}/MIC ratio in relation to the various levofloxacin doses. The cumulative fraction of response (CFR)(21) was then assessed against those bacterial species that were more frequently isolated in the study population. Optimal CFR was defined as ≥ 80 % of subjects within the desired AUC_{24h}/MIC range.

Statistical analysis

The Kolmogorov–Smirnov test was used to assess whether data were normally or non-normally distributed. Accordingly, the mean+SD or median with IQR were used in the descriptive statistics. Categorical variables were compared by the χ^2 test or Fisher's exact test, while continuous variables were compared using the Student's t-test or Mann–Whitney test. A P value < 0.05 was required to achieve statistical significance. All statistical analysis were performed using Systat version 13 (Systat Software, Inc., USA).

Results

Patients characteristics

One-hundred and sixty height acutely hospitalized older patients were included in this study. Demographic and clinical data are summarized in Table 1. The majority of patients were males (103/168, 61.3%), and the median (IQR) age of the study population was of 81 years (76 - 88). Community acquired pneumonia, urinary tract infections and acute exacerbation of chronic bronchitis accounted for most of the bacterial infections requiring levofloxacin treatments (118/168, 70.2%). Levofloxacin was administered mainly orally (145/168, 86.3%) for a median length of treatment of 10 days. Favorable clinical outcome was reported in 73.2% of cases (123/168).

Population pharmacokinetic analysis

A total of 569 levofloxacin plasma concentrations (330 trough and 239 peak concentrations) were included in the population analysis. A two-compartment linear model, with first-order input (for orally administered doses) and first-order clearance from the central compartment, best described levofloxacin concentrations. Compartments were connected by first order inter-compartmental rate constants.

The only covariate that improved the model fit was $CrCL_{CKD\text{-}EPI}$ (OFV reduction from 2125 to 2086; $p < 0.01$). The final model for clearance was as follows:

$$\text{Levofloxacin CL} = 0.399 + 0.051 \cdot CrCL_{CKD\text{-}EPI}$$

where: CL is the value of levofloxacin clearance and $CrCL_{CKD\text{-}EPI}$ is the estimated creatinine clearance by means of the CKD-EPI formula.

Fig. 1 shows the diagnostic plots for the final covariate model. After MAP-Bayesian estimation, the observed versus predicted plot had an intercept and slope that were close to zero and 1, respectively [Observed = 0.146 + 0.973·Predicted ($r^2 = 0.905$; $p < 0.01$)]. Bias and precision were acceptable (0.064 mg/L for bias and 1.64 mg/L for precision).

The mean (± SD) and the median pharmacokinetic parameter estimates for the final covariate model are shown in Table 2. The distribution of the observed concentrations was consistent with that of the predicted concentrations, as suggested by the VPC plot (Fig. 2). The normal distribution of NPDEs ($p = 0.115$ at the Shapiro-Wilk for normality test) confirmed the adequacy of the model for dosing simulations.

Monte Carlo simulation for estimation of levofloxacin doses predicting optimal target drug exposure in older patients with various degrees of renal function

Table 3 shows the distributions of probabilities of simulated patients having underexposure, optimal target exposure and overexposure with the various permissible doses of levofloxacin. The regimens that were associated with the highest proportion of optimal target exposure and lowest risk of under and/or overexposure were as follows: 500 mg every 48 h for $CrCL_{CKD\text{-}EPI} < 20$ ml/min/1.73 m^2; 750 mg every 48 h for $CrCL_{CKD\text{-}EPI}$ of 20-39 ml/min/1.73 m^2; 500 mg every 24 h for $CrCL_{CKD\text{-}EPI}$ of 40-59 ml/min/1.73 m^2; 750 mg every 24 h for $CrCL_{CKD\text{-}EPI}$ of 60-79 ml/min/1.73 m^2 and 500 mg every 12 h for $CrCL_{CKD\text{-}EPI}$ of > 80 ml/min/1.73 m^2. Nevertheless, > 20% risk of underexposure could be expected when using 500 mg every 24 h or 750 mg every 24 h in patients with $CrCL_{CKD\text{-}EPI}$ of 40-59 and 60-79 ml/min/1.73 m^2, respectively. Similarly, > 10% risk of overexposure could be observed when using 500 mg every 48 h or 500 mg every 12 h in patients with $CrCL_{CKD\text{-}EPI}$ of < 20 and > 80 ml/min/1.73 m^2, respectively.

PK/PD analysis

Forty-nine patients had documented bacterial infections, but only 41 out of them (83.7%) were eligible for the PK/PD analysis (4 had to be excluded because of infections caused by levofloxacin-resistant pathogens, 3 because of death for other causes and 1 because of stopping therapy for adverse events). Most of the eligible patients received levofloxacin as monotherapy (56.1%) and had favorable clinical outcome (75.6%).

Blood and urine accounted for most of the primary source of infection (80.5 %). The bacteria most frequently yielded were *E. coli*, *S. aureus* and *P. aeruginosa*, which accounted overall for 65.1% (28/43) of isolates (Table 4).

The cut-off value of total AUC_{24h}/MIC ratio identified as valuable predictor of favorable clinical outcome at CART analysis was of ≥ 95.7. Among the five patients whose AUC_{24h}/MIC ratios were below this breakpoint, in only one case (1/5, 20%) a positive clinical outcome was observed. Conversely, of the thirt-six patients having AUC_{24h}/MIC ratios ≥ 95.7, a positive clinical outcome was observed in thirty (30/36, 83.3%) cases. The area under the ROC curve for this cut-off value was high (0.79).

Among the various covariates that were tested at the univariate analysis for potential relationship with favorable clinical outcome (age, gender, weight, $CrCL_{CKD-EPI,}$ route of levofloxacin administration, AUC_{24h}/MIC ratio ≥ 95.7, length of levofloxacin treatment, co-treatment with other antimicrobials), only weight ($p = 0.117$, log-likelihood = -21.399) and AUC_{24h}/MIC ratio ≥ 95.7 ($p < 0.05$, log-likelihood = -19.328) were predictive of a favorable clinical outcome. At the multivariate logistic regression analysis, only AUC_{24h}/MIC ratio ≥ 95.7 was definitely associated with favorable clinical outcome (OR 20.85: 95% CI 1.56 – 186.73, $p < 0.05$, log-likelihood = -16.828).

PTA and CFR at the cut off AUC_{24h}/MIC ratio associated with favorable clinical outcome

Fig. 3 shows the probability of achieving the AUC_{24h}/MIC ratio cut-off value of ≥ 95.7 with the various permissible doses of levofloxacin. The analysis showed that the permissible levofloxacin doses may achieve optimal PTAs only against those pathogens with an MIC for levofloxacin of ≤ 0.5 mg/L.

Table 5 summarizes the levofloxacin doses that resulted effective AUC_{24h} s in older patients in relation to different degrees of susceptibility of the pathogens to levofloxacin.

279 Table 6 shows the CFR of the permissible doses of levofloxacin against the bacterial
280 pathogens that were most frequently yielded in our study population (*E. coli*. *S. aureus*, *H.*
281 *influenzae* and *P. aeruginosa*). Although optimal CFR were always achieved against *S. aureus*, *H.*
282 *influenzae* and *E.coli*, this was never the case against *P. aeruginosa*.
283

Discussion

In this study we addressed the issue of dosing optimization with levofloxacin in acutely hospitalized older patients, among whom the attainment of optimal pharmacodynamic targets of efficacy with fluoroquinolones should be balanced against safety concerns.

Population pharmacokinetic modeling provided robust estimates of the pharmacokinetic parameters in our population. The final model explained almost 91% of the variability of drug concentrations over time, with acceptable bias and precision. The pharmacokinetic estimates of levofloxacin in the study population are quite different from those previously described in other cohorts. The mean CL of levofloxacin in our population was consistently lower (2.53 L/h) than that observed among healthy volunteers (16), adult patients with normal renal function (8, 22, 23), and elderly patients with CAP (24). Of note, this is in agreement with the fact that most of our patients, differently from those of the other studies, were very old (mean age 81.2 years) and had impaired renal function (median $CrCL_{CKD\text{-}EPI}$ of 30.4 mL/min/1.73 m^2).

The fact that $CrCL_{CKD\text{-}EPI}$ was the only covariate that improved model fit is similar to previous findings in elderly patients (25). This suggests that estimation of renal function by means of this formula should be considered mandatory in older patients for calculating appropriate dose adjustments of levofloxacin in order to avoid drug overexposure. Interestingly, our Monte Carlo simulations provided a detailed stratification of dose adjustments of levofloxacin in relation to different levels of renal function in older patients. It is worth noting that in patients with severe renal impairment ($CrCL_{CKD\text{-}EPI}$ < 40 mL/min/1.73 m^2), levofloxacin dosage must be more than halved in order to avoid overexposure.

Our approach, by targeting in all of the patients drug exposure within a desired range similar to that observed in subjects with normal renal function, may minimize the risk of exposure-dependent toxicity among older patients. This is in agreement with a recent Japanese study showing that adjustments of levofloxacin dose in relation to the degree of renal function may help in decreasing the incidence of adverse events in elderly patients (14). In this regard, it is worth

mentioning that among our study population no patients suffered from tendinopathy or had to stop therapy because of chondrotoxicity (data not shown).

The opportunity of defining permissible doses of levofloxacin in older patients is furtherly strengthened by the findings of two recent reviews showing that levofloxacin is the fluoroquinolone associated with the highest risk of causing tendon damages (10, 12). This may furtherly strengthen the valuable role that a real-time TDM-guided approach of levofloxacin dosage adjustments may have in preventing drug-related toxicity in older patients.

Our approach still ensured patients a high probability of having favorable clinical outcome. The relatively high cut-off value of AUC_{24h}/MIC ratio identified by CART analysis as a valuable predictor of clinical efficacy among our study population (≥ 95.7) was similar to that reported previously by Drusano et al. among patients with nosocomial pneumonia (8). This might be explained by the fact that most of the bacterial clinical isolates included in our analysis, similarly to what occurred in the Drusano's one, were Gram-negative pathogens, which were shown to require much higher pharmacodynamic thresholds than Gram-positives.

Importantly, our pharmacodynamic analyses suggested that pathogens with an MIC ≤ 0.5 mg/L are adequately treated. However, even if this value is lower than the EUCAST clinical breakpoint of susceptibility of levofloxacin against Gram-negative and Gram-positive pathogens which is set to 1 mg/L, it corresponds to that of USCAST for *S. aureus* and *E. coli* (26). In both cases, this poses potential concerns about the efficacy of levofloxacin monotherapy in some settings. Results similar to ours were reported in a population pharmacokinetic analysis of 38 adults Korean patients. In that study a levofloxacin regimen of 250 and 500 mg once daily in patients with CrCL of 20-50 and > 50 mL/min, respectively, resulted in AUC_{24h}/MIC ratio > 100 only against pathogens with an MIC up to and including 0.5 mg/L (23). Conversely, in another study it was shown that dosing regimens of 125, 250, and 500 mg once daily were predicted to ensure PTA > 90% against pathogens with an MIC up to 2 mg/L in patients with CrCL < 20, 20-50 and > 50 mL/min respectively (27). Besides, it is worth mentioning that our study is unique in that PTAs

were estimated for various doses of levofloxacin that were different in relation to various degrees of renal function. This step, in our opinion, should be considered mandatory nowadays in order to prevent exposure-related toxicity with levofloxacin in older patients (12).

When looking at species-specific CFR, optimal CFR in older patients may be predicted in relation to the permissible doses against *E. coli* and *H. influenzae*, whereas borderline CFR may be achieved against *S. aureus*. This offers the opportunity to speculate that levofloxacin may still represent a valuable therapeutic weapon in older patients for the treatment of urinary tract infections, which are frequently caused by *E. coli*. Similarly, levofloxacin may be valuable in the treatment of hematogenous discitis, which may be frequently caused by methicillin-susceptible *S. aureus*. Conversely, only suboptimal CFR were observed against *P. aeruginosa*, and this means that nowadays levofloxacin should not be considered as effective anti-pseudomonal monotherapy.

This study has several limitations. The retrospective design, the lack of evaluation of microbiological eradication in assessing clinical outcome and the use of combination antimicrobial therapy are all relevant considerations. As far as the population analysis is concerned, we recognize that estimate of *ka* might not be robustly enough, due to the limited variability in sampling time of peak concentrations. Additionally, we recognize that our definition of overexposure is arbitrary, but we strongly believe that this approach may be helpful in containing the risk of exposure-dependent toxicity with levofloxacin. Finally, we acknowledge that our PK/PD analysis was based mainly on Gram-negatives pathogens, and this could mean that the identified cut-off AUC_{24h}/MIC target is probably too high for *S. pneumoniae*, a pathogen for which an AUC_{24h}/MIC > 30 is commonly accepted as pharmacodynamic target of efficacy. Nevertheless, the large patient sample size and the heterogeneity of patients' diagnosis could strengthen the generalizability of our results.

In conclusion, our study is unique in that it defined for the first time the permissible doses of levofloxacin that should be administered in older patients with various degrees of renal function in order to minimize the risk of exposure-dependent toxicity. Additionally, it highlights that these

doses might be effective only when treating infections due to bacterial pathogens with an MIC ≤ 0.5 mg/L, which could have implications for *in vivo* susceptibility clinical breakpoints.

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We declare that we have no conflicts of interest related to this work.

References

1. **Blondeau JM.** 1999. Expanded activity and utility of the new fluoroquinolones: a review. Clin Ther **21:**3-40; discussion 41-42.
2. **Anderson VR, Perry CM.** 2008. Levofloxacin : a review of its use as a high-dose, short-course treatment for bacterial infection. Drugs **68:**535-565.
3. **Noreddin AM, Elkhatib WF.** 2010. Levofloxacin in the treatment of community-acquired pneumonia. Expert Rev Anti Infect Ther **8:**505-514.
4. **Pea F, Di Qual E, Cusenza A, Brollo L, Baldassarre M, Furlanut M.** 2003. Pharmacokinetics and pharmacodynamics of intravenous levofloxacin in patients with early-onset ventilator-associated pneumonia. Clin Pharmacokinet **42:**589-598.
5. **Ambrose PG, Grasela DM, Grasela TH, Passarell J, Mayer HB, Pierce PF.** 2001. Pharmacodynamics of fluoroquinolones against Streptococcus pneumoniae in patients with community-acquired respiratory tract infections. Antimicrob Agents Chemother **45:**2793-2797.
6. **Cazzola M, Matera MG, Donnarumma G, Tufano MA, Sanduzzi A, Marchetti F, Blasi F.** 2005. Pharmacodynamics of levofloxacin in patients with acute exacerbation of chronic bronchitis. Chest **128:**2093-2098.
7. **Schentag JJ, Meagher AK, Forrest A.** 2003. Fluoroquinolone AUIC break points and the link to bacterial killing rates. Part 2: human trials. Ann Pharmacother **37:**1478-1488.
8. **Drusano GL, Preston SL, Fowler C, Corrado M, Weisinger B, Kahn J.** 2004. Relationship between fluoroquinolone area under the curve: minimum inhibitory concentration ratio and the probability of eradication of the infecting pathogen, in patients with nosocomial pneumonia. J Infect Dis **189:**1590-1597.
9. **Davies EA, O'Mahony MS.** 2015. Adverse drug reactions in special populations - the elderly. Br J Clin Pharmacol **80:**796-807.
10. **Arabyat RM, Raisch DW, McKoy JM, Bennett CL.** 2015. Fluoroquinolone-associated tendon-rupture: a summary of reports in the Food and Drug Administration's adverse event reporting system. Expert Opin Drug Saf **14:**1653-1660.
11. **Nicolle LE.** 1999. Quinolones in the aged. Drugs **58 Suppl 2:**49-51.
12. **Bidell MR, Lodise TP.** 2016. Fluoroquinolone-Associated Tendinopathy: Does Levofloxacin Pose the Greatest Risk? Pharmacotherapy **36:**679-693.
13. **Furlanut M, Brollo L, Lugatti E, Di Qual E, Dolcet F, Talmassons G, Pea F.** 2003. Pharmacokinetic aspects of levofloxacin 500 mg once daily during sequential intravenous/oral therapy in patients with lower respiratory tract infections. J Antimicrob Chemother **51:**101-106.
14. **Tachi T, Teramachi H, Asano S, Tanaka K, Fukuta M, Osawa T, Aoyama S, Yasuda M, Mizui T, Goto C, Tsuchiya T.** 2013. Impact of levofloxacin dose adjustments by dispensing pharmacists on adverse reactions and costs in the treatment of elderly patients. Pharmazie **68:**977-982.
15. **Child J, Mortiboy D, Andrews JM, Chow AT, Wise R.** 1995. Open-label crossover study to determine pharmacokinetics and penetration of two dose regimens of levofloxacin into inflammatory fluid. Antimicrob Agents Chemother **39:**2749-2751.
16. **Chow AT, Fowler C, Williams RR, Morgan N, Kaminski S, Natarajan J.** 2001. Safety and pharmacokinetics of multiple 750-milligram doses of intravenous levofloxacin in healthy volunteers. Antimicrob Agents Chemother **45:**2122-2125.
17. **Chien SC, Wong FA, Fowler CL, Callery-D'Amico SV, Williams RR, Nayak R, Chow AT.** 1998. Double-blind evaluation of the safety and pharmacokinetics of multiple oral once-daily 750-milligram and 1-gram doses of levofloxacin in healthy volunteers. Antimicrob Agents Chemother **42:**885-888.

18. **Flamant M, Haymann JP, Vidal-Petiot E, Letavernier E, Clerici C, Boffa JJ, Vrtovsnik F.** 2012. GFR estimation using the Cockcroft-Gault, MDRD study, and CKD-EPI equations in the elderly. Am J Kidney Dis **60:**847-849.
19. **Neely MN, van Guilder MG, Yamada WM, Schumitzky A, Jelliffe RW.** 2012. Accurate detection of outliers and subpopulations with Pmetrics, a nonparametric and parametric pharmacometric modeling and simulation package for R. Ther Drug Monit **34:**467-476.
20. **Brendel K, Comets E, Laffont C, Laveille C, Mentre F.** 2006. Metrics for external model evaluation with an application to the population pharmacokinetics of gliclazide. Pharm Res **23:**2036-2049.
21. **Mouton JW, Dudley MN, Cars O, Derendorf H, Drusano GL.** 2005. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. J Antimicrob Chemother **55:**601-607.
22. **Preston SL, Drusano GL, Berman AL, Fowler CL, Chow AT, Dornseif B, Reichl V, Natarajan J, Wong FA, Corrado M.** 1998. Levofloxacin population pharmacokinetics and creation of a demographic model for prediction of individual drug clearance in patients with serious community-acquired infection. Antimicrob Agents Chemother **42:**1098-1104.
23. **Kiem S, Ryu SM, Lee YM, Schentag JJ, Kim YW, Kim HK, Jang HJ, Joo YD, Jin K, Shin JG, Ghim JL.** 2016. Population pharmacokinetics of levofloxacin in Korean patients. J Chemother **28:**308-313.
24. **Noreddin AM, Marras TK, Sanders K, Chan CK, Hoban DJ, Zhanel GG.** 2004. Pharmacodynamic target attainment analysis against Streptococcus pneumoniae using levofloxacin 500 mg, 750 mg and 1000 mg once daily in plasma (P) and epithelial lining fluid (ELF) of hospitalized patients with community acquired pneumonia (CAP). Int J Antimicrob Agents **24:**479-484.
25. **Deguchi T, Nakane K, Yasuda M, Shimizu T, Monden K, Arakawa S, Matsumoto T.** 2010. Microbiological outcome of complicated urinary tract infections treated with levofloxacin: a pharmacokinetic/pharmacodynamic analysis. Int J Antimicrob Agents **35:**573-577.
26. **EUCAST.** 2016. Clinical breakpoints. European Committee on Antimicrobial Susceptibility Testing, Växjö, Sweden.
27. **Leroy B, Uhart M, Maire P, Bourguignon L.** 2012. Evaluation of fluoroquinolone reduced dosage regimens in elderly patients by using pharmacokinetic modelling and Monte Carlo simulations. J Antimicrob Chemother **67:**2207-2212.

Figure Legends:

FIG 1 Diagnostic plot for the final covariate model. Observed versus population predicted plasma concentrations (left panel) and individual predicted plasma concentrations (right panel) in plasma.

FIG 2 Visual predictive check of levofloxacin plasma concentrations versus time for the final covariate model.

FIG 3 Probability of achieving and AUC_{24h}/MIC value of ≥ 95.7 with the various permissible doses of levofloxacin in relation to different degrees of renal function and of susceptibility of the invading pathogen.

Table 1. Population characteristics

Patients' demographics	
Age (years), mean ± SD	81.2 ± 7.8
Gender (male/female), n (%)	103/65 (61.3/38.7)
Body weight (kg), median (IQR)	70 (65 - 80)
$CrCL_{CKD-EPI}$ (ml/min/1.73 m^2)[a], median (IQR)	30.2 (18.2 - 50.2)
Indication for levofloxacin use, n (%)	
Community acquired pneumonia	77 (45.8)
Urinary tract infections	22 (13.1)
Chronic obstructive pulmonary disease	19 (11.3)
Fever of unknown origin	12 (7.1)
Sepsis of unknown origin	13 (7.7)
Intra-abdominal infections	11 (6.6)
Skin and soft tissue infections	8 (4.8)
Bone and joint infections	6 (3.6)
Patients with identified microbiological isolates, n (%)	49 (29.2)
Levofloxacin treatment	
Duration of therapy (days), median (IQR)	10 (7-14)
Route of administration (oral/i.v.), n (%)	145/23 (86.3/13.7)
Clinical outcome, n (%)	
Cured	95 (56.5)
Improved	28 (16.7)
Failed	26 (15.5)
Dead/modified antibiotic therapy	19 (11.3)

[a] at first TDM

$CrCL_{CKD-EPI}$, creatinine clearance estimated by means of the CKD-EPI formula; i.v., intravenous route of administration; oral, oral route of administration; IQR, interquartile range; SD, standard deviation

Table 2. Parameter estimates for final population pharmacokinetic model of levofloxacin in older patients

Unit	*k*a (h^{-1})	*k*cp (h^{-1})	*k*pc (h^{-1})	CL (L/h)	V_c (L)	F_{os} (%)	Tlag (h)
Mean	16.15	0.63	1.77	2.53	52.95	0.83	1.47
Standard deviation	13.47	0.85	0.52	1.46	21.57	0.21	0.65
Coefficient of variation	83.41	133.52	29.47	57.84	40.73	24.83	43.95
Median	9.91	0.04	2.00	2.20	61.25	0.98	1.87

CL, total clearance of levofloxacin; *k*a, first-order transfer rate constant of absorption; *k*cp and *k*pc, first-order intercompartmental transfer arte constant connecting the central and peripheral compartments; F_{os}, oral bioavailability of levofloxacin; Tlag, time delay between drug administration and first observed concentration; V_c, volume of the central compartment.

Table 3. Probability of achieving underexposure (AUC_{24h} < 50 mg·h/L), normal target exposure (AUC_{24h} between 50-160 mg·h/L) and overexposure (AUC_{24h} > 160 mg·h/L) with different levofloxacin dosing regimens in older patients in relation to different classes of renal function.

Levofloxacin regimens	Classes of renal function (mL/min/1.73 m^2) and of levofloxacin AUC_{24h} (mg·h/L)														
	0-19			20-39			40-59			60-79			> 80		
	<50	50-160	>160	<50	50-160	>160	<50	50-160	>160	<50	50-160	>160	<50	50-160	>160
125 mg 48-hourly	91.8	8.2	0.0	99.8	0.2	0.0	99.8	0.2	0.0	99.9	0.1	0.0	100.0	0.0	0.0
250 mg 48-hourly	48.5	50.5	1.0	91.4	8.6	0.0	99.0	1.0	0.0	99.6	0.4	0.0	99.9	0.1	0.0
500 mg 48-hourly	6.4	77.2	16.4	32.2	67.0	0.8	81.6	18.4	0.0	95.7	4.3	0.0	97.2	2.8	0.0
750 mg 48-hourly	1.4	53.9	44.7	7.2	86.2	6.6	42.2	57.2	0.6	79.6	20.0	0.4	89.0	11.0	0.0
500 mg 24-hourly	2.3	50.3	47.4	5	81.3	13.7	22.2	76.0	1.8	59.2	40.1	0.7	78.7	21.0	0.3
750 mg 24-hourly	1.1	17.1	81.8	1.7	51.3	47.0	5.8	82.8	11.4	23.1	73.1	3.7	50.3	47.6	2.1
500 mg 12-hourly	3.3	3.6	99.7	0.2	12.3	87.5	0.1	39.0	60.9	1.5	70.1	28.4	2.8	82.8	14.4

Table 4. Bacterial pathogens (n = 43 yielded from 41 patients) included in the pharmacokinetic/pharmacodynamic analysis

Pathogen	No. of isolates	MIC range (mg/L)
Escherichia coli	12	0.03 - 4
Staphylococcus aureus	9	0.125 - 0.5
Pseudomonas aeruginosa	7	0.25 - 2
Klebsiella pneumoniae	4	0.06 - 1
Haemophilus influenzae	2	0.03
Klebsiella oxytoca	2	0.06 - 1
Staphylococcus epidermidis	2	0.25 - 4
Enterobacter aerogenes	1	0.125
Streptococcus pneumoniae	1	1
Staphylococcus saprofiticus	1	0.5
Staphylococcus schleiferi	1	0.25
Staphylococcus capitis	1	0.25

Table 5. Permissible dosing regimens of levofloxacin granting optimal PTA in older patients in relation to different degrees of renal function and of the susceptibility of the invading bacterial pathogen

MICs (mg/L)	Classes of renal function (mL/min/1.73 m^2)				
	0-19	20-39	40-59	60-79	> 80
0.125	125 mg every 48h	500 mg every 48h	500 mg every 48h	500 mg every 48h	750 mg every 48h
0.25	250 mg every 48h	500 mg every 48h	500 mg every 48h	750 mg every 48h	750 mg every 24h
0.5	500 mg every 48h	750 mg every 48h	500 mg every 24h	750 mg every 24h	500 mg every 12h

Table 6. Cumulative fraction of response of the permissible doses of levofloxacin against the invading pathogens more frequently yielded in the study population according to their EUCAST MIC distribution

Classes of renal function (mL/min/1.73 m^2)	Levofloxacin doses	SA	HI	EC	PA
0-19	125 mg every 48h	59.89	99.66	82.06	16.48
	250 mg every 48h	77.03	99.78	85.07	40.36
	500 mg every 48h	81.59	99.85	87.34	62.24
20-39	500 mg every 48h	79.22	99.79	85.80	47.07
	750 mg every 48h	81.26	99.84	87.12	59.63
	500 mg every 24h	81.49	99.85	87.43	63.08
40-59	500 mg every 48h	71.28	99.73	83.45	25.81
	750 mg every 48h	77.73	99.78	85.26	42.03
	500 mg every 24h	79.42	99.81	86.16	50.72
	750 mg every 24h	81.13	99.84	87.28	61.63
60-79	500 mg every 48h	57.19	99.65	81.57	14.41
	750 mg every 48h	70.61	99.73	83.52	26.68
	500 mg every 24h	74.86	99.76	84.55	36.08
	750 mg every 24h	79.16	99.81	86.20	51.22
>80	750 mg every 48h	60.72	99.67	82.12	18.21
	500 mg every 24h	67.91	99.71	83.27	25.50
	750 mg every 24h	75.51	99.77	84.90	39.43
	500 mg every 12h	81.67	99.85	87.52	63.81

SA, *S. aureus*; HI, *H. influenzae*; EC, *E. coli*; PA, *P. aeruginosa*