**Population pharmacokinetics and pharmacodynamics ~~of maximum permissible doses~~ 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 ~~maximum~~ permissible doses ~~(MPDs)~~ in older patients according to various degrees of renal function. CART analysis was used to detect the cut-off AUC24h/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∙CrCLCKD-EPI). Drug doses ~~The MPD~~ ranged between ~~250 and 750 mg~~ 500 mg every 48h and 500 mg every 12h in relation to different renal function. The identified cut-off AUC24h/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*. ~~The MPD of~~ 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 ~~spectrum~~ spectra of activity, encompassing both Gram-negative and Gram-positive organisms, atypical and anaerobic bacteria ([1](#_ENREF_1)). Accordingly, it has ~~being~~ 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](#_ENREF_2), [3](#_ENREF_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 ~~(CLCr)~~ (CrCL) has been demonstrated ([4](#_ENREF_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 (AUC24h)/minimum inhibitory concentration (MIC) ratio. Different AUC24h/MIC ratios have been proposed as optimal targets according to the invading pathogen. Although an AUC24h/MIC ratio of 25-30 may suffice for infections due to ~~Gram-positives~~ *S. pneumoniae* ([5](#_ENREF_5)), values of 100-125 have been recommended for efficacy against those due to Gram-negative pathogens ([6](#_ENREF_6), [7](#_ENREF_7)). Interestingly, an AUC24h/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](#_ENREF_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](#_ENREF_8)). Similarly, combination therapy was also present in the retrospective analysis by Schentag et al. ([7](#_ENREF_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](#_ENREF_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](#_ENREF_10), [11](#_ENREF_11)).

Accordingly, since levofloxacin toxicity is ~~exposure~~ dose dependent ([12](#_ENREF_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](#_ENREF_13), [14](#_ENREF_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 ~~maximum~~ permissible doses ~~(MPDs)~~ 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 Medicineof 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 AUC24h 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](#_ENREF_7), [15-17](#_ENREF_15)). This TDM-guided approach, by maintaining exposure within the expected normal range, is finalized to prevent theoretical overexposure (arbitrarily defined as AUC24h > 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 ~~(CrCL)~~ was estimated by means of the Chronic Kidney Disease Epidemiology (CKD-EPI) formula (CrCLCKD-EPI) ([18](#_ENREF_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](#_ENREF_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 ~~failure~~ 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 ~~the~~ 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](#_ENREF_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, Fos, Tlag) 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 χ2 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 ~~(OFV)~~ 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](#_ENREF_20)). NPDEs should be normally distributed when the model is appropriately fitted.

***Monte Carlo simulation for estimation of ~~the MPDs 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 AUC24h 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 m2).

In order to define the ~~MPDs of~~ permissible levofloxacin doses in the study population, we considered as desirable in this population the achievement of the exposure range that was observed in ~~subjects~~  healthy volunteers with normal renal function with the standard high dose of 500 mg every 12h (AUC24h of 50-160 mg∙h/L) (14-16). Consistently, AUC24h < 50 mg·h/L was defined as underexposure, AUC24h between 50 and 160 mg·h/L was defined as optimal target exposure, and AUC24h > 160 mg·h/L was defined as overexposure. Permissible doses ~~MPDs~~ were defined as those producing a less than 10% of probability of causing both drug underexposure and overexposure in each class of renal function. The identified levofloxacin doses ~~MPDs~~ were considered sufficiently safe for clinical use in this population, and were subsequently tested in the pharmacokinetic/pharmacodynamic analysis.

***Pharmacokinetic/pharmacodynamic (PK/PD) analysis***

AUC24h/MIC ratios were calculated for all of those patients who had bacterial clinical isolates yielded and tested for levofloxacin susceptibility. Considering that levofloxacin is approximately 30% plasma protein bound, all the pharmacodynamic targets were multiplied by factor 0.7 in order to obtain the free targets (*f*AUC24h/MIC), which were than included in the PK/PD analysis.

Logistic regression analysis was used to explore the relationship between drug exposure and other clinical factors on the probability of clinical outcome. For those patients who had antimicrobial combination therapy, we created a dichotomous categorical variable. Covariates resulting with a P < 0.20 at the univariate analysis were deemed of potential clinical relevance and then included in the multivariate model on the basis of a forwards stepwise approach.

Classification and regression tree (CART) analysis was used to develop a prediction model for detecting the cut-off value of AUC24h/MIC ratio that best correlates with favorable clinical outcome in the study population. Subsequently, the validity of the identified cut-off value was tested by means of receiver operating characteristic (ROC) analysis.

***Probability of target attainment (PTA) and cumulative fraction of response (CFR) at the cut-off AUC24h/MIC ratio associated with favorable clinical outcome***

We estimated the probability of target attainment (PTA) of the identified cut-off value of AUC24h/MIC ratio in relation to the various ~~MPDs of~~ levofloxacin doses. The cumulative fraction of response (CFR)([21](#_ENREF_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 AUC24h/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 χ2 test  ~~with Yates’s correction~~ or Fisher’s exact test ~~as necessary~~, 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 ~~zero~~ 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 CrCLCKD-EPI (OFV reduction from 2125 to 2086; p < 0.01). The final model for clearance was as follows:

Levofloxacin CL = 0.399 + 0.051∙CrCLCKD-EPI

where: CL is the value of levofloxacin clearance and CrCLCKD-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 ~~was~~ were close to zero and 1, respectively [Observed = 0.146 + 0.973∙Predicted (*r2* = 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 ~~the MPDs 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 MPDs predicted by Monte Carlo simulation as being safe for use in older patients with different degrees of renal function were as follows:~~ ~~250 mg every 24 h for CLCr~~~~CKD-EPI~~ ~~< 20 ml/min/1.73 m~~~~2~~~~; 750 mg every 48 h for CLCr~~~~CKD-EPI~~ ~~of 20-39 ml/min/1.73 m~~~~2~~~~; 500 mg every 24 h for CLCr~~~~CKD-EPI~~ ~~of 40-59 ml/min/1.73 m~~~~2~~~~; 750 mg every 24 h for CLCr~~~~CKD-EPI~~ ~~of 60-79 ml/min/1.73 m~~~~2~~ ~~and for CLCr~~~~CKD-EPI~~  ~~of > 80 ml/min/1.73 m~~~~2~~~~.~~  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 CrCLCKD-EPI < 20 ml/min/1.73 m2; 750 mg every 48 h for CrCLCKD-EPI of 20-39 ml/min/1.73 m2; 500 mg every 24 h for CrCLCKD-EPI of 40-59 ml/min/1.73 m2; 750 mg every 24 h for CrCLCKD-EPI of 60-79 ml/min/1.73 m2 and 500 mg every 12 h for CrCLCKD-EPI of > 80 ml/min/1.73 m2. Nevertheless, > 20% risk of underexposure could be expected when using 500 mg every 24 h or 750 mg every 24 h in patients with CrCLCKD-EPI of 40-59 and 60-79 ml/min/1.73 m2, 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 CrCLCKD-EPI of < 20 and > 80 ml/min/1.73 m2, 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 AUC24h/MIC ratio identified as valuable predictor of favorable clinical outcome at CART analysis was of ≥ 95.7. Among the 5 patients whose AUC24h/MIC ratios were below this breakpoint, in only one case (1/5, 20%) a positive clinical outcome was observed. Conversely, of the 36 patients having AUC24h/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, CrCLCKD-EPI, route of levofloxacin administration, AUC24h/MIC ratio ≥ 95.7, length of levofloxacin treatment, co-treatment with other antimicrobials), only weight (p = 0.117, log-likelihood = -21.399) and AUC24h/MIC ratio ≥ 95.7 (p < 0.05, log-likelihood = -19.328) were predictive of a favorable clinical outcome ~~(p < 0.05 and 0.117, respectively)~~. At the multivariate logistic regression analysis, only AUC24h/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 AUC24h/MIC ratio associated with favorable clinical outcome***

Fig. 3 shows the probability of achieving the AUC24h/MIC ratio cut-off value of ≥ 95.7 with the various permissible doses of levofloxacin. The analysis showed that the ~~MPDs of~~ permissible levofloxacin may achieve optimal PTAs only against those pathogens with an MIC for levofloxacin of ≤ 0.5 mg/L.

Table 5 summarizes the ~~MPDs of~~ levofloxacin doses that resulted effective AUC24h s in older patients in relation to different degrees of susceptibility of the pathogens to levofloxacin.

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

**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 ~~study~~ population was consistently lower (2.53 L/h) than that observed among healthy volunteers ([16](#_ENREF_16)), adult patients with normal renal function ([8](#_ENREF_8), [22](#_ENREF_22), [23](#_ENREF_23)), and elderly patients with CAP ([24](#_ENREF_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 CrCLCKD-EPI of 30.4 mL/min/1.73 m2).

The fact that CrCLCKD-EPI was the only covariate that improved ~~the~~ model fit is similar to previous findings in elderly patients ([25](#_ENREF_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 (CrCLCKD-EPI < 40 mL/min/1.73 m2), 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](#_ENREF_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).

~~Interestingly, a recent Monte Carlo simulation study showed that the lack of dosage adjustment of levofloxacin in presence of renal failure might cause drug accumulation in tendon tissue (~~[~~26~~](#_ENREF_26)~~). In that study, the administration of 500 mg once daily for seven consecutive days to patients with impaired renal function was estimated to provide in tendon tissue concentrations of the same magnitude of those that were previously associated with chondrotoxicity in experimental animal models (~~[~~27~~](#_ENREF_27)~~).~~

The opportunity of defining ~~MPDs~~ 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](#_ENREF_10), [12](#_ENREF_12)). This may furtherly strengthen the valuable role that ~~the~~ 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 AUC24h/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](#_ENREF_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* ([28](#_ENREF_28)). 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 AUC24h/MIC ratio > 100 only against pathogens with an MIC up to and including 0.5 mg/L ([23](#_ENREF_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 ([29](#_ENREF_29)). Besides, it is worth mentioning that our study is unique in that PTAs were estimated for various ~~MPDs~~ 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](#_ENREF_12)).

When looking at species-specific CFR, optimal CFR in older patients may be predicted in relation to the ~~MPDs~~ 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 AUC24h/MIC target is probably too high for *S. pneumoniae*, a pathogen for which an AUC24h/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 ~~MPDs~~ 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**

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**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 AUC24h/MIC value of ≥ 95.7 with the various ~~maximum~~ permissible doses of levofloxacin in relation to different degrees of renal function and of susceptibility of the invading pathogen.







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| 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) |
|  | CrCLCKD-EPI (ml/min/1.73 m2)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 TDMCrCLCKD-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 |

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| 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) | Vc (L) | Fos (%) | 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; Fos, oral bioavailability of levofloxacin; Tlag, time delay between drug administration and first observed concentration; Vc, volume of the central compartment. |

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| Table 3. Probability of achieving underexposure (~~UE =~~ AUC24h < 50 mg·h/L), normal target exposure (~~TE =~~ AUC24h between 50-160 mg·h/L) and overexposure (~~OE =~~ AUC24h > 160 mg·h/L) with different levofloxacin dosing regimens in older patients in relation to different classes of renal function. ~~Maximum permissible doses were considered those producing < 10% of probability of causing drug overexposure~~ |
| Levofloxacin regimens | Classes of renal function (mL/min/1.73 m2) and of levofloxacin AUC24h (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 |
| ~~250 mg 24-hourly~~ | ~~15.0~~ | ~~80.1~~ | ~~4.9~~ |  | ~~46.2~~ | ~~53.8~~ | ~~0.0~~ |  | ~~85.3~~ | ~~14.7~~ | ~~0.0~~ |  | ~~94.6~~ | ~~5.4~~ | ~~0.0~~ |  | ~~97.1~~ | ~~2.9~~ | ~~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 |

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| 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 |

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| 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 m2) |
|  | 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 |

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| 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 m2) | 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* |