

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

4

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25

26 **Abstract**

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

31 PopPK and Monte Carlo simulation were performed for defining the permissible doses in older
32 patients according to various degrees of renal function. CART analysis was used to detect the cut-
33 off AUC_{24h}/MIC ratio that best correlated with clinical outcome. Probability of target attainment
34 (PTA) of this value was calculated against different pathogens. 168 patients were included, and 330
35 trough and 239 peak concentrations were used for the popPK analysis. Creatinine clearance was the
36 only covariate that improved the model fit (Levofloxacin $CL=0.399+0.051 \cdot CrCL_{CKD-EPI}$). Drug
37 doses ranged between 500 mg every 48h and 500 mg every 12h in relation to different renal
38 function. The identified cut-off AUC_{24h}/MIC ratio (≥ 95.7) was the only covariate that correlated
39 with favorable clinical outcome at multivariate regression analysis (OR 20.85; 95% CI 1.56–
40 186.73). PTAs were optimal ($>80\%$) against *E. coli* and *H. influenzae*, borderline against *S. aureus*,
41 and suboptimal against *P. aeruginosa*. Levofloxacin doses defined in our study may be effective for
42 the treatment of infections due to bacterial pathogens with an $MIC \leq 0.5$ mg/L in older patients with
43 various degrees of renal function, while minimizing the toxicity risk. Conversely, the addition of
44 another active antimicrobial should be considered whenever treating infections caused by less
45 susceptible pathogens.

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

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51 **Introduction**

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

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

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

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

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

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

85 **Materials and Methods**

86 *Study design*

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

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

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

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

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

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

120

121 *Assessment of clinical outcome*

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

128

129 *Population pharmacokinetic modeling*

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

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

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

153

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

156 One thousand-subject Monte Carlo simulations were conducted using Pmetrics to estimate
157 the AUC_{24h} achievable with various candidate regimens of levofloxacin (125 mg every 48h, 250 mg
158 every 48h, 250 mg daily, 500 mg every 48h, 750 mg every 48h, 500 mg daily, 750 mg daily and
159 500 mg every 12h) for different levels of renal function (0-19, 20-39, 40-59, 60-79 and > 80
160 mL/min/1.73 m²).

161 In order to define the permissible levofloxacin doses in the study population, we considered
162 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 then 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*

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

194

195 *Statistical analysis*

196 The Kolmogorov–Smirnov test was used to assess whether data were normally or non-
197 normally distributed. Accordingly, the mean+SD or median with IQR were used in the descriptive
198 statistics. Categorical variables were compared by the χ^2 test or Fisher’s exact test, while
199 continuous variables were compared using the Student’s t-test or Mann–Whitney test. A P value <
200 0.05 was required to achieve statistical significance. All statistical analysis were performed using
201 Systat version 13 (Systat Software, Inc., USA).

202

203 **Results**

204 *Patients characteristics*

205 One-hundred and sixty eight acutely hospitalized older patients were included in this study.
206 Demographic and clinical data are summarized in Table 1. The majority of patients were males
207 (103/168, 61.3%), and the median (IQR) age of the study population was of 81 years (76 - 88).
208 Community acquired pneumonia, urinary tract infections and acute exacerbation of chronic
209 bronchitis accounted for most of the bacterial infections requiring levofloxacin treatments (118/168,
210 70.2%). Levofloxacin was administered mainly orally (145/168, 86.3%) for a median length of
211 treatment of 10 days. Favorable clinical outcome was reported in 73.2% of cases (123/168).

212
213 *Population pharmacokinetic analysis*

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

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

221
$$\text{Levofloxacin CL} = 0.399 + 0.051 \cdot CrCL_{CKD-EPI}$$

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

224 Fig. 1 shows the diagnostic plots for the final covariate model. After MAP-Bayesian
225 estimation, the observed versus predicted plot had an intercept and slope that were close to zero and
226 1, respectively [Observed = $0.146 + 0.973 \cdot \text{Predicted}$ ($r^2 = 0.905$; $p < 0.01$)]. Bias and precision were
227 acceptable (0.064 mg/L for bias and 1.64 mg/L for precision).

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

233

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

236 Table 3 shows the distributions of probabilities of simulated patients having underexposure,
237 optimal target exposure and overexposure with the various permissible doses of levofloxacin. The
238 regimens that were associated with the highest proportion of optimal target exposure and lowest risk
239 of under and/or overexposure were as follows: 500 mg every 48 h for $\text{CrCL}_{\text{CKD-EPI}} < 20$ ml/min/1.73
240 m^2 ; 750 mg every 48 h for $\text{CrCL}_{\text{CKD-EPI}}$ of 20-39 ml/min/1.73 m^2 ; 500 mg every 24 h for $\text{CrCL}_{\text{CKD-}}$
241 EPI of 40-59 ml/min/1.73 m^2 ; 750 mg every 24 h for $\text{CrCL}_{\text{CKD-EPI}}$ of 60-79 ml/min/1.73 m^2 and 500
242 mg every 12 h for $\text{CrCL}_{\text{CKD-EPI}}$ of > 80 ml/min/1.73 m^2 . Nevertheless, $> 20\%$ risk of underexposure
243 could be expected when using 500 mg every 24 h or 750 mg every 24 h in patients with $\text{CrCL}_{\text{CKD-}}$
244 EPI of 40-59 and 60-79 ml/min/1.73 m^2 , respectively. Similarly, $> 10\%$ risk of overexposure could
245 be observed when using 500 mg every 48 h or 500 mg every 12 h in patients with $\text{CrCL}_{\text{CKD-EPI}}$ of $<$
246 20 and > 80 ml/min/1.73 m^2 , respectively.

247

248 *PK/PD analysis*

249 Forty-nine patients had documented bacterial infections, but only 41 out of them (83.7%)
250 were eligible for the PK/PD analysis (4 had to be excluded because of infections caused by
251 levofloxacin-resistant pathogens, 3 because of death for other causes and 1 because of stopping
252 therapy for adverse events). Most of the eligible patients received levofloxacin as monotherapy
253 (56.1%) and had favorable clinical outcome (75.6%).

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

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

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

271

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

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

277 Table 5 summarizes the levofloxacin doses that resulted effective AUC_{24h} s in older patients
278 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

284 **Discussion**

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

288 Population pharmacokinetic modeling provided robust estimates of the pharmacokinetic
289 parameters in our population. The final model explained almost 91% of the variability of drug
290 concentrations over time, with acceptable bias and precision. The pharmacokinetic estimates of
291 levofloxacin in the study population are quite different from those previously described in other
292 cohorts. The mean CL of levofloxacin in our population was consistently lower (2.53 L/h) than that
293 observed among healthy volunteers (16), adult patients with normal renal function (8, 22, 23), and
294 elderly patients with CAP (24). Of note, this is in agreement with the fact that most of our patients,
295 differently from those of the other studies, were very old (mean age 81.2 years) and had impaired
296 renal function (median $\text{CrCL}_{\text{CKD-EPI}}$ of 30.4 mL/min/1.73 m²).

297 The fact that $\text{CrCL}_{\text{CKD-EPI}}$ was the only covariate that improved model fit is similar to
298 previous findings in elderly patients (25). This suggests that estimation of renal function by means
299 of this formula should be considered mandatory in older patients for calculating appropriate dose
300 adjustments of levofloxacin in order to avoid drug overexposure. Interestingly, our Monte Carlo
301 simulations provided a detailed stratification of dose adjustments of levofloxacin in relation to
302 different levels of renal function in older patients. It is worth noting that in patients with severe
303 renal impairment ($\text{CrCL}_{\text{CKD-EPI}} < 40$ mL/min/1.73 m²), levofloxacin dosage must be more than
304 halved in order to avoid overexposure.

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

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

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

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

324 Importantly, our pharmacodynamic analyses suggested that pathogens with an $MIC \leq 0.5$
325 mg/L are adequately treated. However, even if this value is lower than the EUCAST clinical
326 breakpoint of susceptibility of levofloxacin against Gram-negative and Gram-positive pathogens
327 which is set to 1 mg/L, it corresponds to that of USCAST for *S. aureus* and *E. coli* (26). In both
328 cases, this poses potential concerns about the efficacy of levofloxacin monotherapy in some
329 settings. Results similar to ours were reported in a population pharmacokinetic analysis of 38 adults
330 Korean patients. In that study a levofloxacin regimen of 250 and 500 mg once daily in patients with
331 CrCL of 20-50 and > 50 mL/min, respectively, resulted in AUC_{24h}/MIC ratio > 100 only against
332 pathogens with an MIC up to and including 0.5 mg/L (23). Conversely, in another study it was
333 shown that dosing regimens of 125, 250, and 500 mg once daily were predicted to ensure PTA $>$
334 90% against pathogens with an MIC up to 2 mg/L in patients with CrCL < 20 , 20-50 and > 50
335 mL/min respectively (27). Besides, it is worth mentioning that our study is unique in that PTAs

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

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

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

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

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

363

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

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452 **Figure Legends:**

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

456

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

459

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

Table 1. Population characteristics

Patients' demographics	
Age (years), mean \pm SD	81.2 \pm 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 rate 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)
<i>Escherichia coli</i>	12	0.03 - 4
<i>Staphylococcus aureus</i>	9	0.125 - 0.5
<i>Pseudomonas aeruginosa</i>	7	0.25 - 2
<i>Klebsiella pneumoniae</i>	4	0.06 - 1
<i>Haemophilus influenzae</i>	2	0.03
<i>Klebsiella oxytoca</i>	2	0.06 - 1
<i>Staphylococcus epidermidis</i>	2	0.25 - 4
<i>Enterobacter aerogenes</i>	1	0.125
<i>Streptococcus pneumoniae</i>	1	1
<i>Staphylococcus saprofiticus</i>	1	0.5
<i>Staphylococcus schleiferi</i>	1	0.25
<i>Staphylococcus capitis</i>	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*





