



A Prospective Population Pharmacokinetic Study on Morphine Metabolism in Cancer Patients

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Abstract

Background Oral and subcutaneous morphine is widely used for the treatment of cancer-related pain; however, solid pharmacokinetic data on this practice are lacking. Furthermore, it is largely unknown which factors contribute to the variability in clearances of morphine and its metabolites and whether morphine clearance is related to treatment outcome.

Methods Blood samples from 49 cancer patients treated with oral and/or subcutaneous morphine were prospectively collected and were used to develop a population pharmacokinetic model for morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). The

influence of age, gender, renal function and several polymorphisms possibly related to the pharmacokinetics of the three compounds was investigated. In addition, the relation between treatment failure and morphine and metabolite clearances was explored.

Results A one-compartment model including an extensive first-pass effect adequately described the data of morphine and its metabolites. Estimated mean area under the plasma concentration–time curve (AUC) ratios following oral versus subcutaneous administration were: M3G/morphine 29.7:1 vs. 11.1:1; M6G/morphine 5.26:1 vs. 1.95:1; and M3G/M6G 5.65:1 vs. 5.70:1. Renal function was significantly correlated with clearance of the metabolites, which increased 0.602 L/h per every 10 mL/min/1.73 m² increase of estimated glomerular filtration rate (eGFR), reaching a plateau for eGFR >90 mL/min/1.73 m². The clearance of morphine or its metabolites was not found to be correlated with treatment failure.

Conclusion The influence of age-, gender- and pharmacokinetic-related polymorphisms was not identified on the pharmacokinetics of morphine. Clearance of morphine or its metabolites was not found to explain treatment outcome; however, large variations in plasma concentrations of morphine, M3G and M6G support further studies on the relation between plasma concentrations and treatment outcome.

Dutch Trial Register ID: NTR4369.

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Electronic supplementary material The online version of this article (doi:[10.1007/s40262-016-0471-7](https://doi.org/10.1007/s40262-016-0471-7)) contains supplementary material, which is available to authorized users.

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Key Points

We describe the pharmacokinetics of morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) after subcutaneous and oral administration in cancer patients, including first-order systemic and additional first-pass formation from morphine for the metabolites.

Variations in area under the plasma concentration–time curve (AUC) ratios of M3G:morphine and M6G:morphine related to first-pass effect and renal function support further studies on the relation between plasma concentrations and treatment outcome.

We did not identify significant effects of age, gender and polymorphisms in *UGT2B7*, *SLC22A1* and *ABCC3* on total clearance of morphine and morphine metabolic clearances to M3G or M6G. Furthermore, failure of treatment could not be related to the clearance of morphine or its metabolites.

1 Introduction

Morphine is a widely used opioid analgesic and is one of the preferred treatment options for the treatment of cancer-related pain [1].

After intravenous administration, morphine is rapidly distributed from the central compartment to highly perfused tissues (distribution half-life [$t_{1/2}$] = 0.9–2.5 min), and thereafter the plasma concentrations versus time decay in a biphasic way, with a short mean terminal elimination $t_{1/2}$ of 1.4–3.4 h that is similar for intravenous, subcutaneous and oral administrations [2, 3]. After oral administration, morphine undergoes extensive hepatic first-pass metabolism [2, 3], and is predominantly metabolized through glucuronidation in the liver into the conjugates morphine-3-glucuronide (M3G; 45–55%) and morphine-6-glucuronide (M6G; 10–15%) [4–6]. While M6G is thought to contribute to the analgesic effects [7–9], the effects of M3G are unclear. It has been associated with (central) side effects and the development of tolerance to the analgesic effects in rats [10, 11], but direct administration to humans did not produce any clinical effects [12].

Morphine is available for different routes of administration. For fast titration in cases of severe pain, we mainly use continuous subcutaneous administration. This has been found to be safe and effective [13, 14], has advantages over

the intravenous route, and can also be applied safely in an out-of-hospital setting [13, 15].

Little is known about the pharmacokinetics of morphine after continuous subcutaneous administration in cancer patients, and solid pharmacokinetic data after oral administration are also lacking. Furthermore, while substantial inter- and intraindividual variability in plasma concentrations of morphine, M3G and M6G has been reported after oral as well as subcutaneous administration [16, 17], the causes for this variability and its effects on clinical outcomes of treatment are incompletely understood. Although treatment with morphine is unsuccessful in approximately 30% of patients [18], it is unknown what causes these treatment failures. A number of clinical factors such as age and gender, as well as genetic factors, have been associated with variability in pharmacokinetics and/or dynamics of morphine [19–22] but data are sparse and to date only a small part of variability can be explained at best.

The objectives of the current population pharmacokinetic analysis were to describe the pharmacokinetics and metabolic ratios of morphine, M3G and M6G following subcutaneous and oral administration of morphine. As a second objective, the influence of age, gender, renal function and polymorphisms in several pharmacokinetic-related genes on the pharmacokinetics of morphine, M3G and M6G was investigated. Finally, the relation between outcome of treatment and the clearance of morphine and its metabolites was explored.

2 Patients, Materials and Methods

Between February 2010 and March 2014, patients admitted to the Erasmus MC Cancer Institute (Rotterdam, The Netherlands) and treated with morphine for moderate to severe cancer-related nociceptive pain were asked to participate in the study. All patients treated with morphine were eligible, i.e. patients already treated with morphine before admission but also opioid-naïve patients or patients rotating to morphine after failure of treatment with another type of opioid. Morphine was available as hydrochloride-3-water (molecular weight 375.84 mg/mmol) 10 mg/mL for parenteral administration and as 5-sulphate-water (molecular weight 758.83 mg/mmol) extended-release (ER; tablet 10, 20, 60, 100 mg) and immediate-release (IR) formulation (liquid 20 mg/mL or dose unit 10, 30 mg) for oral administration. The starting dose in opioid-naïve patients is usually 10 mg twice daily or 1 mg/h parenterally, depending on the clinical circumstances. Doses in non-naïve patients are based on previous treatments. Patients treated with a fentanyl patch who were prescribed IR oral morphine for treatment of breakthrough pain could also be included in the study. In cases of severe pain, patients were

titrated by continuous subcutaneous infusion, with the possibility of an extra bolus every hour. Doses were titrated based on clinical effects. When pain control was reached and doses were stabilized, patients could be rotated to oral ER morphine, with IR morphine prescribed as needed, using a 1:3 dose conversion ratio [23]. Gender, age and weight (kg) at study entry were recorded, as well as baseline creatinine values ($\mu\text{mol/L}$). The Modification of Diet in Renal Disease (MDRD) formula was used to calculate the glomerular filtration rates for all patients, and values $>90 \text{ mL/min/1.73 m}^2$ were truncated:

$$\begin{aligned} \text{eGFR (mL/min/1.73 m}^2) &= 175 \times (0.0113 \\ &\times S_{\text{cr}[\mu\text{mol/L}]}^{-1.154} \\ &\times \text{age}_{[\text{years}]}^{-0.203} \\ &\times (0.742 \text{ if female}). \end{aligned}$$

For every patient treated with long-acting or continuous morphine, the outcome of treatment was classified as failure or non-failure. The response was classified as failure in cases of rotation to another type of opioid or treatment with intrathecal opioids because of insufficient pain control and/or side effects or the use of palliative sedation because of refractory symptoms associated with opioid treatment in the dying phase.

2.1 Pharmacokinetic Sample Collection

Patients were included in the study as soon as possible after hospital admission or after the start of morphine. Blood samples for pharmacokinetic analysis were taken during a maximum of 72 h after the start of morphine and after each change in the opioid regimen (dose, route of administration). The protocol prescribed sampling twice daily, just before the administration of oral ER morphine or around 8:00 am and 8:00 pm in cases of continuous administration, a baseline sample before every change in the regimen, and a series of samples maximally once daily around the administration of a subcutaneous bolus or oral IR formulation at baseline and 5, 15, 30 and 60 min after administration. Samples were collected using potassium EDTA tubes. After centrifugation of the tube, the supernatant was collected and stored at $-70 \text{ }^\circ\text{C}$ until analysis at the laboratory of Translational Pharmacology (Erasmus MC Cancer Institute).

2.2 Measurements of Plasma Concentrations of Morphine, Morphine-3-Glucuronide and Morphine-6-Glucuronide

Morphine and its metabolites in plasma were quantitated using a validated ultra performance liquid chromatography tandem mass spectrometer (UPLC-MS/MS) method consisting of a Waters Acquity UPLC sample manager

coupled with a triple quadruple mass spectrometer operating in the multiple reaction monitoring (MRM) mode with positive ion electrospray ionization (Waters, Etten-Leur, The Netherlands). The MRM transitions were set at $286 \rightarrow 201$ and $462 \rightarrow 286$ for morphine and M3G and M6G, respectively.

Chromatographic separations for morphine were achieved on an Acquity UPLC[®] BEH C18 $1.7 \mu\text{m}$ $2.1 \times 100 \text{ mm}$ column eluted at a flow rate of 0.350 mL/min on a gradient of methanol. The overall cycle time of the method was 6 min. The calibration curves were linear over the range of $1.00\text{--}100 \text{ ng/mL}$, with the lower limit of quantitation (LLQ) validated at 1.00 ng/mL for morphine. The within- and between-run precisions at five tested concentrations, including the LLQ, were ≤ 10.3 and $\leq 8.67\%$, respectively, while the average accuracy ranged from 91.9 to 96.9% . The interday coefficient of variation (CV) at five tested concentrations, including the LLQ, was $\leq 11.8\%$ in individual validation runs. The extraction of $200 \mu\text{L}$ of plasma involved a deproteinization step with acetone, followed by a simple liquid extraction with ethyl acetate. For M3G and M6G, chromatographic separations were achieved on a VisionHT C18-P $3 \mu\text{m}$ $2.1 \times 50 \text{ mm}$ column eluted at a flow rate of 0.250 mL/min on a gradient of acetonitrile. The overall cycle time of the method was 10 min. The calibration curves were linear over the range of $10.0\text{--}1000 \text{ ng/mL}$ for M3G and $2.00\text{--}200 \text{ ng/mL}$ for M6G, with the LLQ validated at 10.0 ng/mL for M3G and 2.00 ng/mL for M6G. In patients with metabolite concentrations above these values, samples were adequately diluted in blank human plasma prior to processing until the signal fell within the calibration range. The within- and between-run precisions at five tested concentrations in human potassium EDTA plasma for M3G, including the LLQ, were ≤ 5.16 and $\leq 2.18\%$, respectively, while the average accuracy ranged from 84.0 to 96.5% . For M6G, the within- and between-run precisions at five tested concentrations, including the LLQ, were ≤ 16.2 and $\leq 9.12\%$, respectively, while the average accuracy ranged from 87.0 to 105.5% . The interday CV at five tested concentrations, including the LLQ, was ≤ 8.1 and $\leq 8.2\%$ for M3G and M6G, respectively, in individual validation runs. The morphine glucuronides were extracted from $100 \mu\text{L}$ aliquots of plasma after the addition of $850 \mu\text{L}$ ammonium carbonate buffer pH 8.8 followed by a solid-phase extraction using Oasis[®] HLB 1 cc (30 mg) SPE columns.

2.3 Single Nucleotide Polymorphism Analysis

Single nucleotide polymorphisms (SNPs) that have been related to morphine pharmacokinetics were studied (Table 1). DNA was isolated from 1 mL EDTA blood on the MagNA Pure LC 2.0 instrument (Roche Diagnostics),

Table 1 Summary of selected genetic variants**UGT2B7**

UGT2B7 is a phase II (glucuronidation) metabolizing enzyme encoded by the *UGT2B7* gene

UGT2B7 is involved in the conversion of morphine into M3G and M6G

The G allele of polymorphism $-900G>A$ (rs7438135), which is in complete LD with polymorphism $802C>T$, has been associated with decreased glucuronidation [56, 57]

SLC22A1

OCT1 is encoded by the *SLC22A1* gene

OCT1, expressed at the sinusoidal membrane of the human liver, mediates the cellular uptake of morphine [51]

Healthy volunteers with *SLC22A1* polymorphisms have reduced morphine uptake in the hepatocytes [54]

Children with two loss-of-function *SLC22A1* alleles have lower morphine clearance than carriers of the active *SLC22A1* alleles [55]

ABCC3

ABCC3 is an organic anion transporter encoded by the *ABCC3* gene

ABCC3, expressed on the basolateral membranes of hepatocytes, mediates the efflux of M3G, and mostly likely also M6G, into the bloodstream [58]

ABCC3 polymorphism $-211C>T$ (rs4793665) was associated with a significantly altered mRNA expression [59, 60]

Children with the $-211CC$ genotype had significantly higher M3G and M6G levels (approximately 40%) than carriers of the $-211T$ allele [55]

UGT2B7 uridine 5'-diphospho-glucuronosyltransferase 2B7, *LD* linkage disequilibrium, *M3G* morphine-3-glucuronide, *M6G* morphine-6-glucuronide, *OCT1* organic cation transporter 1, *ABCC3* ATP-binding cassette C3, *mRNA* messenger RNA

with further analysis performed on the 7500 Real-Time PCR System (Life Technologies). Hardy–Weinberg equilibrium was calculated using the Chi-squared test. Additionally, the observed minor allele frequency (MAF) was compared using the European MAF from HapMap in dbSNP (National Center for Biotechnology Information). The *SLC22A1* haplotype (consisting of either two active alleles, a combination of one active and one inactive allele, or two inactive alleles) was estimated based on the expectation maximization (EM) logarithm with the R (version 3.1.1) *haplo.stats* package, using a posterior probability >0.98 .

2.4 Population Pharmacokinetic Modeling

The analysis of concentration–time data of morphine and its metabolites was conducted with the first-order conditional estimation method with eta-epsilon interaction through non-linear mixed-effects modeling in NONMEM (version 7.3; Icon Development Solutions, Hanover, MD, USA) [24]. Model building was supported by Perl-speaks-NONMEM version 4.2.0, Xpose version 4.4.1 [25], and R version 3.2.0.

Concentration data and doses of morphine were expressed as free base in molar units (nmol/L and nmol, respectively), the latter calculated taking into account the salt administered. All dosing history regarding administration of morphine before and during the period of sampling was included in the dataset. Concentrations below the LLQ comprised 7.6, 0.7 and 0.9% of the data of morphine, M3G and M6G, respectively, and were discarded from the analysis [26].

First, a pharmacokinetic model was developed for morphine following subcutaneous and oral administration, starting out from previously published models [27, 28]. Oral bioavailability was estimated under the assumption of complete subcutaneous bioavailability, as indicated in the current literature [29–31]. Thereafter, the model was extended to also describe the pharmacokinetics of the metabolites. The rate of appearance of the metabolites was parameterized as a fraction of the rate of elimination of morphine, with fractions fixed to literature values [4–6]. The inclusion of first-pass formation of metabolites following oral morphine was assessed in the model, and the sum of the estimated fractions of morphine reaching the systemic circulation unchanged or undergoing first-pass metabolism to metabolites was constrained to a maximum of 1. The influence of age and gender on the pharmacokinetic profiles was explored and the relationship between estimated glomerular filtration rate (eGFR) and clearance of the metabolites was assessed.

Interindividual variability (IIV) in pharmacokinetic parameters was modeled using log-normal models. Homoscedastic, heteroscedastic and combined residual error models were evaluated. The correlation between parent drug and metabolite concentrations from the same sample was taken into account utilizing the L2 data item in NONMEM.

Selection between alternative models was based on scientific plausibility, statistical significance, precision in parameter estimates and visual inspection of goodness-of-fit plots. Statistical significance was determined using the likelihood ratio test with the NONMEM objective function value (OFV). The OFV is given by minus twice the log

likelihood, and a difference in OFV (Δ OFV) between nested models is approximately Chi-square distributed. A Δ OFV of 6.64 and 10.8 corresponds to p values of 0.01 and 0.001, when one parameter is added to the model (1 df). The reliability of various diagnostic plots was judged based on the magnitude of η - and ε -shrinkage [32]. The precision of the model parameter estimates was obtained using the sampling importance resampling (SIR) method [33]. In addition to the general advantages of SIR (e.g. fast run times as it does not require estimation steps, flexibility in addressing asymmetric confidence intervals), SIR was deemed more appropriate than the bootstrap in this case because it is less sensitive to sample size and does not require stratification of the data, which is particularly useful with unbalanced study designs involving a few subjects. Further details on the SIR procedure are presented in electronic supplementary material. The predictive performance of the final model was evaluated using visual predictive checks (VPCs) or population prediction-corrected VPCs (pcVPCs) [34] for the observed concentrations, as well as for concentration ratios. The concentration ratios M3G:M, M6G:M and M3G:M6G, uniquely following the subcutaneous or oral route of administration, were calculated by dividing the respective observed or simulated concentrations.

2.5 Influence of Genetic Variants and Assessment of Treatment Failure

After finalization of the population pharmacokinetic model, the influence of *UGT2B7* (rs7438135), *SLC22A1* (rs72552763, rs12208357, rs34130495, rs34059508) and *ABCC3* (rs4793665) genetic variants were explored on total morphine clearance and morphine metabolic clearances to M3G and M6G; *ABCC3* (rs4793665) was also studied in relation to the clearance of the metabolites. In addition, the model was used to assess whether failure of treatment was related to a difference in clearance of morphine or metabolites. The influence of failure of treatment was tested in the model as a binominal variable on the clearance of morphine and its metabolites, not with the purpose of explaining parameter variability but to identify a possible association between failure of the treatment and clearance.

3 Results

3.1 Patients

The dataset contained 410 samples from 49 patients (Table 2). Treatment with oral and subcutaneous morphine in relation to the observations for all patients is shown in

Table 2 Baseline characteristics

Characteristics ($n = 49$)	No	% or range
Median age, years	60	38–80
Gender		
Male	27	55
Female	22	45
Median weight, kg	83	53–140
Body mass index, kg/m ²		
Underweight, <18.5	1	2
Normal range, 18.5–25	13	27
Overweight, 25–30	20	41
Obese, 30–40	13	27
Severely obese, >40	2	4
Race		
Caucasian	44	90
Latin American	1	2
Unknown/other	4	8
Median WHO performance status	2	0–3
Primary tumor localization		
Breast	11	22
Colorectal	7	14
Prostate	6	12
Sarcoma	4	8
Other	21	43
Distant metastasis present	44	89
Median creatinine, μ mol/L	72	25–190
Median estimated glomerular filtration rate, mL/min/1.73 m ²	81	33 >90
Median serum albumin, g/L	40	28–47
Routes of administration during sampling		
Subcutaneous	28	57
Oral extended and immediate release	12	24
Oral immediate release only	6	12
Both oral and subcutaneous consecutively	3	6
<i>UGT2B7</i> $G>A$		
GG, wild type	14	29
GA, heterozygous	26	53
AA, variant	9	20
<i>SLC22A1</i>		
2 active alleles	26	53
1 active allele/1 inactive allele	18	37
2 inactive alleles	5	10
<i>ABCC3</i> $C>T$		
CC, wild type	5	10
CT, heterozygous	28	57
TT, variant	16	33

UGT2B7 uridine 5'-diphospho-glucuronosyltransferase 2B7, *SLC22A1* solute carrier family 22A member 1, *ABCC3* ATP-binding cassette C3

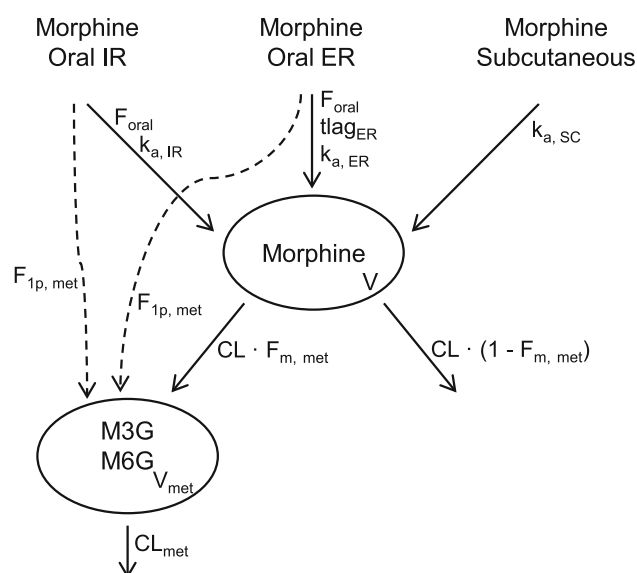


Fig. 1 Pharmacokinetic model developed to describe plasma concentrations of morphine and its metabolites (M3G and M6G) following oral IR, oral ER and subcutaneous administration. CL morphine clearance, CL_{met} metabolite clearance, ER extended release, $F_{1p,met}$ fraction of morphine converted to metabolites in first-pass effect, $F_{m,met}$ fraction of morphine clearance forming metabolites, F_{oral} oral bioavailability, IR immediate release, $k_{a,ER}$ absorption rate constant for oral ER morphine, $k_{a,IR}$ absorption rate constant for oral IR morphine, $k_{a,SC}$ absorption rate constant for subcutaneous morphine, $M3G$ morphine-3-glucuronide, $M6G$ morphine-6-glucuronide, $tlag_{ER}$ absorption lag-time for oral ER morphine, V morphine volume of distribution, V_{met} metabolites volume of distribution

electronic supplementary Fig. 1, and observations in relation to time after first dose are shown in electronic supplementary Fig. 2. Before the start of treatment with morphine, and/or inclusion in the study, 13 patients were opioid-naïve, 11 were treated with fentanyl, 19 were treated with oxycodone, and six patients were already treated with morphine. Of these, five patients were treated with oral ER morphine and one was treated with continuous subcutaneous administration. The median treatment doses were 2 mg/h for continuous and bolus subcutaneous morphine (ranges 0.6–14 mg/h and 0.6–10 mg, respectively), 40 mg twice daily for oral ER morphine (range 10–150 mg) and 10 mg for oral IR morphine (range 5–60 mg). Creatinine values were missing for four patients and were imputed based on linear regression of the available values of eGFR on age and gender. In seven patients, baseline eGFR was between 30 and 60 mL/min/1.73 m² (range 33–57 mL/min/1.73 m², median 43 mL/min/1.73 m²). All other patients ($n = 38$) had an eGFR ≥ 60 mL/min/1.73 m². In 12 of 43 patients treated with long-acting/continuous morphine, the outcome of treatment was classified as failure, in all due to the occurrence of dose-limiting side effects.

3.2 Morphine Pharmacokinetics

The pharmacokinetics of subcutaneous and oral morphine was adequately described by a one-compartment model with separate first-order absorption processes for each route. Parameters describing the absorption phases for subcutaneous and oral IR morphine were fixed to literature values [27, 28], and the value of the absorption rate constant for oral ER morphine was estimated ($p < 0.001$ when compared with a fixed value of 0.8 h^{-1}). The fractions of total morphine clearance forming M3G and M6G were fixed to 0.57 and 0.10, respectively [4–6].

The pharmacokinetics of M3G and M6G were appropriately described by first-order systemic and additional first-pass formation and first-order elimination according to a one-compartment model (Fig. 1). Note that given the parameterization of the model (fixed and estimated fractions of formation), the estimated disposition parameters reflect true, and not apparent, values. The inclusion of first-pass metabolism statistically significantly improved the fit of the parent-metabolite model ($p < 0.001$). The metabolite disposition parameters were estimated to common values, and the estimation of separate clearance and volume parameters for each metabolite was not found to be statistically significant ($p > 0.01$). The final population model parameters for morphine, M3G and M6G are presented in Table 3.

The model was found to fit the data well, as shown by the absence of major systematic trends in the goodness-of-fit plots and pcVPCs (Fig. 2), with only a small tendency of overprediction of variability at low concentrations.

Allometric body weight with theory-based exponents was included a priori on all disposition parameters of all entities [35]. Age did not statistically significantly improve the model fit ($p > 0.01$). The inclusion of an effect of gender on clearance of morphine resulted in a decrease of 17.0% for females ($p > 0.01$) but was not retained in the model. The clearance of the metabolites was found to change with eGFR ($p < 0.001$) and increased 0.602 L/h with every increase in eGFR of 10 mL/min/1.73 m² up to an eGFR of 90 mL/min/1.73 m², above which clearance was constant (Fig. 3).

The mean area under the plasma concentration–time curve (AUC) molar ratios for a typical patient (weight 70 kg and eGFR of 81 mL/min/1.73 m²) following oral morphine compared with the subcutaneous route of administration for the same dose were: M3G/morphine 29.7:1 vs. 11.1:1; M6G/morphine 5.26:1 vs. 1.95:1; and M3G/M6G 5.65:1 vs. 5.70:1. The concentration ratios over time and the respective model predictions by route of administration are shown in Fig. 4.

Table 3 Population pharmacokinetic parameter estimates for morphine, M3G and M6G following subcutaneous and oral administration of morphine

Parameter (units)	Description	Estimate	SIR RSE ^a (%)	SIR 95% CI
Morphine				
$t_{lag, ER}$ (h)	Absorption lag-time for oral extended-release	0.25 fixed	NA	NA
$k_{a, SC}$ (h^{-1})	Absorption rate constant for subcutaneous	3.96 fixed	NA	NA
$k_{a, IR}$ (h^{-1})	Absorption rate constant for oral immediate release	6.00 fixed	NA	NA
$k_{a, ER}$ (h^{-1})	Absorption rate constant for oral extended release	0.221	17.7	0.155–0.306
F_{oral} (%) ^b	Oral bioavailability	0.372	NA	NA
CL_{70kg} (L/h) ^c	Clearance	91.9	3.91	85.8–99.9
V_{70kg} (L) ^c	Volume of distribution	278	12.3	221–351
M3G and M6G				
$F_{m, M3G}$	Fraction of morphine clearance forming M3G	0.573 fixed	NA	NA
$F_{m, M6G}$	Fraction of morphine clearance forming M6G	0.104 fixed	NA	NA
θ_1^b	Parameter estimated to derive F_{oral} , $F_{1p, M3G}$ and $F_{1p, M6G}$	0.170	10.4	0.136–0.206
θ_2^b	Parameter estimated to derive F_{oral} , $F_{1p, M3G}$ and $F_{1p, M6G}$	0.953	8.95	0.796–1.14
θ_3^b	Parameter estimated to derive F_{oral} , $F_{1p, M3G}$ and $F_{1p, M6G}$	0.565	30.3	0.310–1.01
$F_{1p, M3G}^b$	Fraction of morphine converted to M3G in first-pass effect	0.355	NA	NA
$F_{1p, M6G}^b$	Fraction of morphine converted to M6G in first-pass effect	0.0631	NA	NA
$CL_{met, 70kg}$ (L/h) ^d	Clearance (common for metabolites)	4.71	5.24	4.24–5.20
$V_{met, 70kg}$ (L) ^c	Volume of distribution (common for metabolites)	25.8	6.12	22.8–29.0
eGFR on $CL_{met, 70kg}^d$	Fractional change in $CL_{met, 70kg}$ per mL/min/ $1.73 m^2$ eGFR relative to $CL_{met, 70kg}$ for subject with eGFR of 81 mL/min/ $1.73 m^2$	0.0128	12.9	0.00924–0.0156
Interindividual variability				
$k_{a, all}$ (%CV [η -shrinkage])		71.0 [25.3]	NA	NA
CL_{70kg} (%CV [η -shrinkage])		22.2 [17.9]	12.9	16.9–27.8
V_{70kg} (%CV [η -shrinkage])		74.7 [21.4]	9.71	60.4–88.6
CL_{M3G} (%CV [η -shrinkage])		36.2 [6.28]	10.6	29.5–44.4
CL_{M6G} (%CV [η -shrinkage])		36.8 [7.00]	11.8	29.6–46.2
Correlation CL_{M3G} – CL_{M6G}^c		0.912	11.8	0.864–0.952
V_{M3G} (%CV [η -shrinkage])		24.7 [30.7]	18.4	17.0–34.0
V_{M6G} (%CV [η -shrinkage])		24.3 [39.0]	20.5	16.3–34.6
θ_1 (%CV [η -shrinkage])		15.0 [62.7]	18.7	8.91–19.9
θ_3 (%CV [η -shrinkage])		98.2 [57.1]	25.1	50.1–146
Residual variability				
Morphine (%CV [ϵ -shrinkage])	Proportional residual error for morphine	28.6 [8.45]	4.31	26.5–31.2
M3G (%CV [ϵ -shrinkage])	Proportional residual error for M3G	20.0 [8.00]	4.14	18.5–21.7
M6G (%CV [ϵ -shrinkage])	Proportional residual error for M6G	23.9 [8.00]	4.04	22.2–26.0
Correlation morphine–M3G ^c		0.420	7.11	0.340–0.504
Correlation morphine–M6G ^c		0.386	7.57	0.302–0.477

Table 3 continued

Parameter (units)	Description	Estimate	SIR RSE ^a (%)	SIR 95% CI
Correlation M3G–M6G ^c		0.918	4.29	0.901–0.934

CI confidence interval, CV coefficient of variation, eGFR estimated glomerular filtration rate, M3G morphine-3-glucuronide, M6G morphine-6-glucuronide, NA not available, RSE relative standard error, SIR sampling/importance resampling

^a For interindividual and residual variability, %RSE is reported on the standard deviation scale

^b $F_{\text{oral}} = 1/(1 + \theta_1 + \theta_2 + \theta_3)$; $F_{1p, \text{M3G}} = \theta_2/(1 + \theta_1 + \theta_2 + \theta_3)$; $F_{1p, \text{M6G}} = \theta_1/(1 + \theta_1 + \theta_2 + \theta_3)$

^c Value for a 70-kg patient calculated as parameter = estimate_{70kg} × (weight/70)¹ for volumes or 0.75 for clearances

^d Value for a 70-kg patient with eGFR = 81 mL/min/1.73 m² calculated as CL_{met} = 4.71 × (weight/70)^{0.75} × (1 + 0.0128 × (eGFR - 81))

^e Correlation of the off-diagonal estimate calculated as cov(omega1, omega2)/sqrt(var(omega1) × var(omega2)) or cov(sigma1, sigma2)/sqrt(var(sigma1) × var(sigma2))

3.3 Influence of Genetic Variants

An effect of *UGT2B7*, *SLC22A1* and *ABCC3* SNPs on total clearance of morphine and morphine metabolic clearances to M3G or M6G could not be identified ($p > 0.01$). Similarly, an effect of *ABCC3* genotype on the clearance of the metabolites was not found ($p > 0.01$).

3.4 Assessment of Treatment Failure in Relation to Metabolism

Differences in clearance of morphine, M3G and M6G in patients in whom treatment with morphine failed ($n = 12$) compared with patients in whom treatment did not fail ($n = 31$) could not be identified ($p > 0.01$ in all cases).

4 Discussion

First, we developed a population pharmacokinetic model for morphine, M3G and M6G following subcutaneous and oral morphine administration from a high number of sparse samples. We found that a one-compartment model with separate first-order absorption processes for each route adequately describes the plasma concentrations of morphine, and a one-compartment model following first-order systemic and additional first-pass formation from morphine appropriately describes plasma concentrations of the metabolites. Our results are in line with literature data.

Pharmacokinetic data after subcutaneous administration are scarce. The model by Upton et al. [27], who reported a clearance of 79.8 L/h in a population of 22 postoperative patients aged 50 years or over, was the basis for the morphine model. In a study by Stuart-Harris et al. [29] in six healthy volunteers, clearances of 83.1 L/h (subcutaneous bolus), 95 L/h (intravenously) and 127.5 L/h (subcutaneous infusion) were reported. In a recent publication, a lower clearance of 47.5 L/h was reported in a slightly older and terminally ill population, with (compared with our cohort)

lower serum albumin values (median 26 g/L), shorter survival (median 33 days) and, most likely, lower body weight (not reported); these factors may suggest lower metabolic capacity and may explain the lower clearance in that study [36]. Thus, clearance in our study, estimated to be 92.9 L/h for a patient weighing 70 kg, is in reasonable agreement with previous data. In our analysis, we assumed complete bioavailability following subcutaneous administration. Although information available in the literature was limited, i.e. the studies comparing subcutaneous and intravenous administration usually involved a small number of patients and a crossover design was not used in all studies [29–31], we consider that the current literature supports the assumption made. The main consequence if this assumption is not true lies in the interpretation of the estimated pharmacokinetic parameters, i.e. they would correspond to apparent clearances and volumes of distribution, and oral bioavailability would be relative (to subcutaneous) instead of absolute.

Clearance for the metabolites was estimated to a common value of 4.71 L/h for a subject weighing 70 kg and with eGFR of 81 mL/min/1.73 m². The estimation of separate disposition parameters for M3G and M6G did not statistically significantly improve the model, proposing that these entities have the same clearance and volume of distribution, a finding that is true or may be due to modeling assumptions leading to difficulties in distinguishing different pharmacokinetic characteristics of the metabolites. Thus, the different pharmacokinetic profiles of the metabolites depend exclusively on the fraction of systemic (subcutaneous and oral) and first-pass formation (oral) of the metabolites, which is in line with the observed strong correlation ($R^2 = 0.963$) of the metabolites as reported in other studies [37, 38]. Furthermore, the suggestion of similar pharmacokinetic estimates for the metabolites is supported by results from independent pharmacokinetic studies following intravenous administration of M6G and M3G to healthy volunteers, which reported clearances for M6G and M3G of 10–11 and 10 L/h, respectively [39–41].

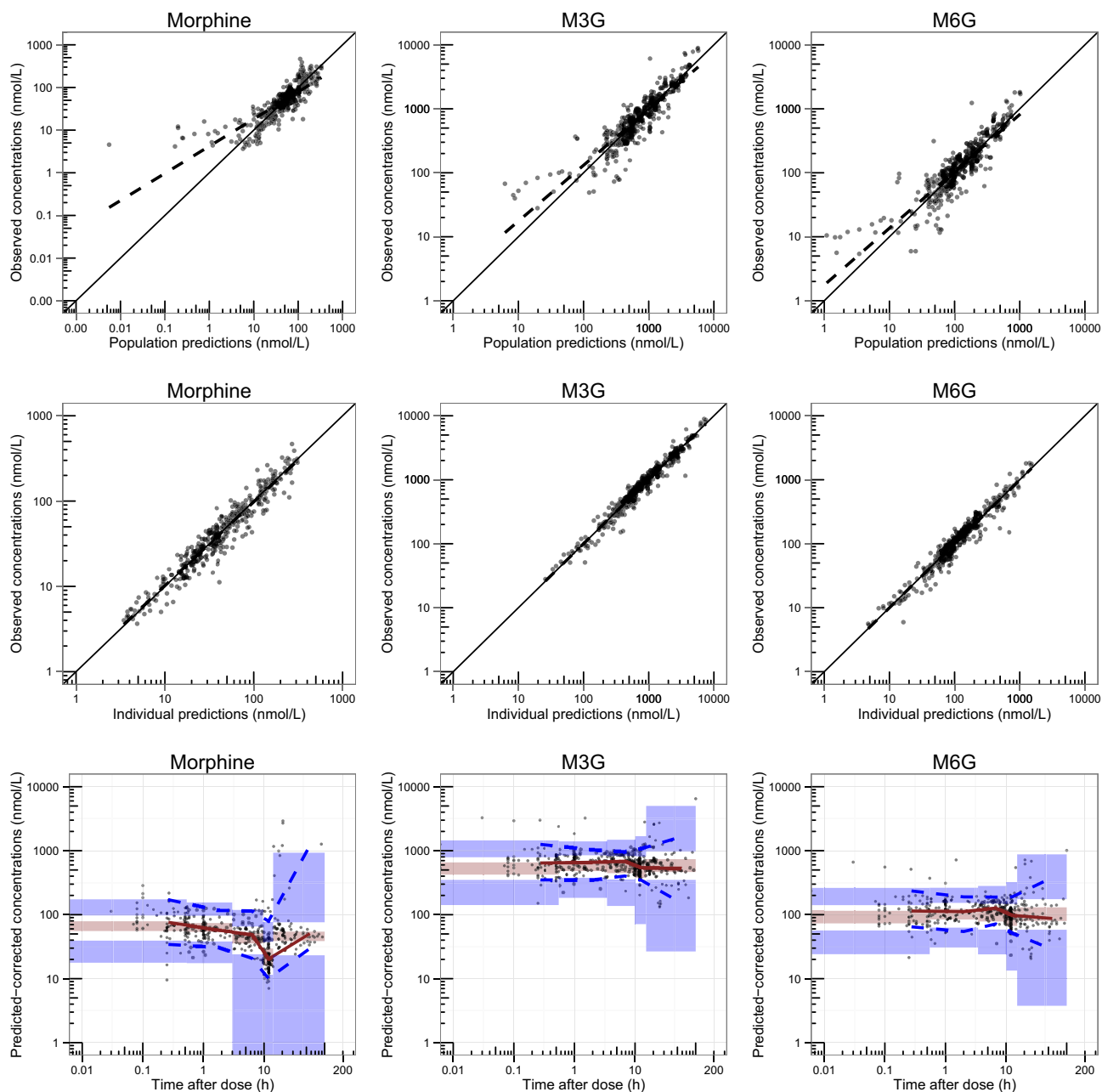


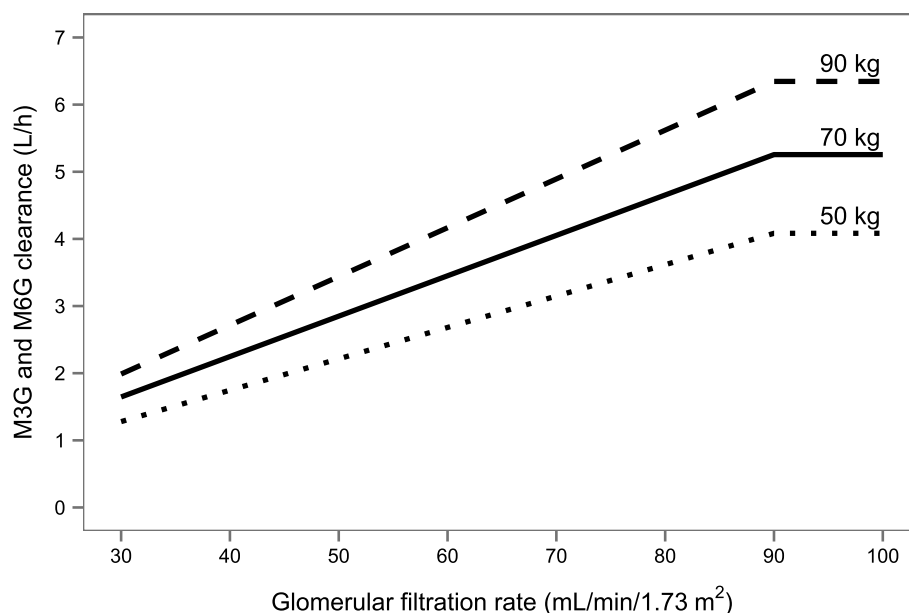
Fig. 2 Prediction- and simulation-based diagnostics for the final population pharmacokinetic model. Observed concentrations of morphine, M3G and M6G versus population predictions (*upper panel*), and observed concentrations versus individual population predictions (*middle panel*); the *solid line* represents a unity line and the *dashed line* represents a linear tendency line. Prediction-corrected

visual predictive checks (*lower panel*) through 1000 replications; *dots* represent the predicted-corrected concentrations of each entity, the *solid red line* and *dashed blue lines* represent the observed median and 5th and 95th observed percentiles, and the *shaded areas* represent the 95% confidence interval for the respective percentiles. *M3G* morphine-3-glucuronide, *M6G* morphine-6-glucuronide

Moreover, the lower clearance estimated in our study (4.7 L/h) is reasonable given the lower renal function in cancer patients compared with healthy volunteers, and is in agreement with clearance for M6G of 5.7 L/h following intravenous administration in cancer patients [42]. In the four cited studies, volume of distribution was estimated in the range of 20–30 L, which is consistent with our estimate.

Concentration–time data following the administration of subcutaneous and oral morphine allowed estimation of the oral morphine bioavailability (37.2%) and, in addition, the fractions of the oral morphine dose that undergo first-pass metabolism to M3G (35.5%) and M6G (6.31%). It is not expected that the fractions are in agreement with the F_m , i.e. the fractions of morphine clearance forming the two metabolites. The fraction of the dose formed into a

Fig. 3 M3G and M6G clearance in relation to eGFR and body weight. *eGFR* glomerular filtration rate, *M3G* morphine-3-glucuronide, *M6G* morphine-6-glucuronide



metabolite in the first pass is dependent on several factors: whether metabolism occurs in the intestinal wall in addition to the liver; whether all metabolites are formed in the first passage (i.e. whether total clearance is equal to hepatic clearance); and the blood-to-plasma ratio of morphine. The values estimated are in line with hepatic first-pass metabolism only (for all pathways), but this is not solid evidence of lack of intestinal wall formation.

As expected, the subcutaneous route of administration, which avoids first-pass metabolism, resulted in lower metabolite:morphine concentration ratios compared with the oral route. According to Hasselstrom and Sawe [5], and supported by our data, this difference is due to higher morphine plasma concentrations, and therefore the AUCs of the metabolites formed following both routes of morphine administration are similar. The observed and model-predicted ratio M3G:M6G remained constant regardless of the level of renal impairment or route of administration. The clinical consequences of the differences in metabolite:morphine ratios are uncertain. We could only find one study comparing oral and subcutaneous administration using a crossover design [23]. This study reported less nausea and somnolence during treatment with subcutaneous morphine, a finding that we recognize from our daily clinical practice. However, the relationship between plasma concentrations of morphine and its metabolites and clinical effects is not clear because some studies have failed to show a correlation [43–45], while others reported an association [9, 37, 46]. Although we did not perform a full pharmacokinetic-pharmacodynamic analysis, we tried to identify an association between outcome of treatment and clearance of morphine. The outcome of treatment may not be associated with a different clearance of morphine,

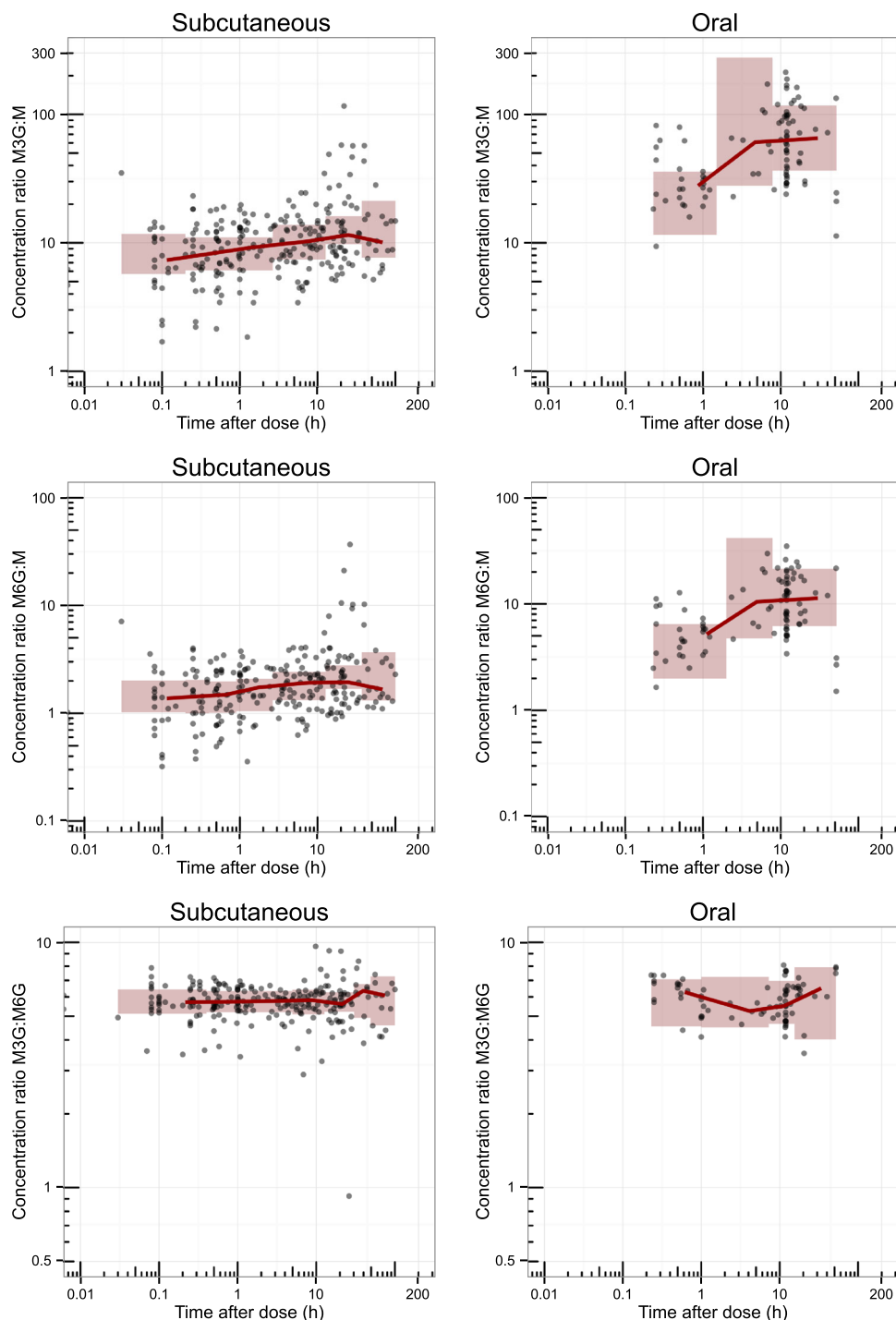
and other factors may be more important in this regard. The relation between clearances and plasma concentrations of morphine and the metabolites and outcomes of treatment deserves further study.

Second, in an attempt to explain variability in pharmacokinetic parameters, we studied the role of several clinical and genetic covariates on the clearances of morphine and the metabolites. In our study, inclusion of both age and gender did not statistically significantly improve the model, although we estimated a 17% lower clearance in females. Reported data on the effects of age are conflicting. Age was reported to predict postoperative morphine requirements [47], and pharmacokinetic studies have reported either lower clearances and volume of distribution in elderly patients [20] or higher plasma concentrations of M6G and/or M3G [48, 49], while others found no significant impact of age [50]. A possible explanation for these findings is the fact that in most studies renal function, which declines with age, was not taken into account. However, in the study by Klepstad et al. [49], serum creatinine and age were found to be independent contributors to outcome in a multivariable analysis.

With regard to gender, in a systematic review and meta-analysis, Niesters et al. [21] found that women display greater opioid analgesia than men and this effect was largest when the analysis was restricted to patient-controlled analgesia studies with morphine. However, it is unclear if this gender difference can be attributed to pharmacokinetic differences. While McQuay et al. [48] found lower plasma concentrations of morphine and M6G in men compared with women, the effect of gender was also non-significant in other modeling studies [27, 50].

Furthermore, clearance of the metabolites was found to be a function of body weight and renal function (Fig. 3),

Fig. 4 Observed and simulated (1000 replications) metabolic concentration ratios (M3G:morphine, M6G:morphine and M3G:M6G) over time stratified by route of administration; *dots* represent the observed metabolic concentrations ratios, the *solid red line* represents the observed median, and the *shaded area* represents the 95% confidence interval for the simulated median. *M3G* morphine-3-glucuronide, *M6G* morphine-6-glucuronide



while no correlation was found between these two (data not shown). The consequence of this combined finding for dose recommendation is not clear, and would demand to simultaneously take into account the systemic exposure of morphine and its metabolites. Although accumulation of M3G and M6G in patients with impaired renal function is widely reported [51], data on the clinical effects of morphine treatment in these patients are scarce and conflicting.

Reducing the frequency of administration or the dose are carefully suggested in guidelines [1, 52], but opioid rotation to an opioid without renally excreted active metabolites, such as fentanyl, should also be considered.

In addition, we did not identify the significant effects of genetic variants in transporters (*OCT1*, *ABCC3*) and the phase II metabolizing enzyme (*UGT2B7*) on morphine pharmacokinetics. Remarkably, almost all of the

previously identified effects of these genetic variants were found in children and mainly in small patient populations (Table 1). In our adult population, we were unable to confirm previously identified effects, which may be caused by lower rates of glucuronidation in children and possibly overcapacity in adult livers. Additionally, the absence of a genotypic OCT1 effect could be due to construction of the OCT1 haplotype in the current study. Recently, a study addressing worldwide genetic variability within this gene, and assessing the effect of several genetic variants (among others *SLC22A1**2-*6 alleles) on 10 probe compounds, found that the effect of the *2 allele on the transport function is substrate-dependent [53]. This makes the *2 allele a rather reduced function allele against morphine than total loss-of-function, as previously suggested [54, 55].

5 Conclusions

We found that a one-compartment model adequately described the pharmacokinetics of morphine after subcutaneous and oral administration, and a one-compartment model following first-order systemic and additional first-pass formation from morphine appropriately described the plasma concentrations of the metabolites. The estimated relative bioavailability of 37.2% for oral morphine confirms the dose conversion ratio of 1:3 when converting subcutaneous to oral morphine. Age and gender did not significantly influence the clearance of morphine, while clearance of the metabolites was found to be a function of body weight and glomerular filtration rate. We identified no significant effects of polymorphisms in *UGT2B7*, *SLC22A1* and *ABCC3*, and no difference in morphine and metabolite clearance between patients in whom treatment failed versus patients in whom treatment did not fail. Further research is therefore needed to explain the variability in treatment doses as well as clinical outcomes.

Acknowledgements The authors thank Tilly Baan for her help with the conduct of this study.

Compliance with Ethical Standards

This study was approved by the Medical Ethics Review Board (MEC 09.332) and was conducted in accordance with the Declaration of Helsinki. The trial was registered in the Dutch Trial Register (Trial Registration ID: NTR4369), and written informed consent was obtained from all patients.

Funding No sources of funding were used in the preparation of this manuscript. This study was financially supported by the Netherlands Organisation for Health Research and Development (ZonMw Project Number 1151.0014), Cornelis Vrolijk Fund and Stichting Voorzieningsfonds Palliatieve Zorg Dirksland.

Conflict of interest Astrid W. Oosten, João A. Abrantes, Siv Jönsson, Maja Matic, Ron H.N. van Schaik, Peter de Bruijn, Carin C.D. van der Rijt and Ron H.J. Mathijssen have no conflicts of interest relevant to the content of this paper.

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