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ORIGINAL RESEARCH ARTICLE

Predictive Potential of BCS and Pharmacokinetic Parameters on Study Outcome: Analysis of 198 In Vivo Bioequivalence Studies

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Abstract

Background and Objectives Understanding predictive potential of parameters to perform early bioequivalence (BE) risk assessment is crucial for good planning and risk mitigation during product development. The objective of the present study was to evaluate predictive potential of various biopharmaceutical and pharmacokinetic parameters on the outcome of BE study.

Methods Retrospective analysis was performed on 198 Sandoz (Lek Pharmaceuticals d.d., A Sandoz Company, Verovskova 57, 1526 Ljubljana, Slovenia) sponsored BE studies [52 active pharmaceutical ingredients (API)] where characteristics of BE study and APIs were collected for immediate-release products and their predictive potential on the study outcome was assessed using univariate statistical analysis.

Results Biopharmaceutics Classification System (BCS) was confirmed to be highly predictive of BE success. BE studies with poorly soluble APIs were riskier (23% non-BE) than with highly soluble APIs (0.1% non-BE). APIs with either lower bioavailability (BA), presence of first-pass metabolism, and/or being substrate for P-glycoprotein substrate (P-gP) were associated with higher non-BE occurrence. In silico permeability and time at peak plasma concentrations (T_{max}) were shown as potentially relevant features for predicting BE outcome. In addition, our analysis showed significantly higher occurrence of non-BE results for poorly soluble APIs with disposition described by multicompartment model. The conclusions for poorly soluble APIs were the same on a subset of fasting BE studies; for a subset of fed studies there were no significant differences between factors in BE and non-BE groups.

Conclusion Understanding the association of parameters and BE outcome is important for further development of early BE risk assessment tools where focus should be first in finding additional parameters to differentiate BE risk within a group of poorly soluble APIs.

1 Introduction

Conclusion of BE between a test product and a comparator product (from hereon reference product) is a critical step in the development of generic or innovative medicine, e.g., fixed-dose combination (FDC), new modified-release product, change of manufacturing site, etc. In vivo BE study is a simpler and more discriminatory surrogate for therapeutic equivalence clinical study and can evaluate equivalency of safety and efficacy profile between the generic and the reference product. BE study needs to be performed on a representative batch of the generic product, and hence is performed in late stages of the development. BE study represents a significant part of the development budget. On the other hand, affordability is inversely proportional to the product development budget and is one of the key benefits of generic medicines. The introduction of generic drugs saved the US health system nearly $1.5 trillion between 2004 and 2013 [1].

Quality by design and related paradigms call for structured learning about product characteristics and assurance of continuous built-in quality of product throughout its lifecycle [2]. Guidelines provided by the health authorities are an appreciated step towards standardized and optimal BE study design. Namely, protocol predefined parameters and criteria for conclusions of BE are important for making science-based decision about equivalency of generic and reference products and protecting patient's interest and well-being.

Extended author information available on the last page of the article

Key Points

Biopharmaceutics Classification System (BCS), bioavailability, first-pass metabolism, active pharmaceutical ingredient (not) being P-glycoprotein substrate, time at peak plasma concentration, permeability, and number of compartments in drug disposition model were found to be associated with outcome of bioequivalence (BE) studies in fasting conditions (but not in fed condition) and are parameters to be considered for early-stage BE risk assessment.

Other tested parameters: intra-individual variability of peak plasma concentration (C_{max}) and area under the plasma concentration time curve (AUC), volume of distribution (Vd), elimination half-life ($t_{1/2}$), C_{max}/AUC, AUC-to-dose ratio (AUC/*D*), and plasma protein binding (PPB) did not show association with outcome of BE studies in fasting and/or fed conditions.

Pharmaceutical development is on the other hand interested also in the probability of BE study success. A good way to predict BE study outcome is to build in vitro–in vivo relationship [3]. This can be done in later stages of the development, but extensive in vitro and in vivo data (BE studies) are needed and this requires a large amount of development time and budget. Probability of the success of a BE study needs to be evaluated as well in the early stages of product development where extensive data are not available. An early initial assessment of the risk related with the BE study outcome is needed for good planning and risk mitigation during product development. For such assessment, the impact of different factors on BE study outcome needs to be understood.

Numerous interrelated factors impact the outcome of the BE study. Thus, it is very challenging to predict it. The BCS [4] biowaiver approach is an example where one can, on the basis of certain API and product characteristics profiles, anticipate positive outcome of a BE study and can even completely waive a BE study in some situations. Impact of BCS on BE study outcome has been explored in real sets of data, published in conference abstracts and research articles. Two conference abstracts [5, 6] report work on large databases of BE studies (918 and 1200, respectively), but information available from these reports is very limited. On the other hand, two full-length research articles [7, 8] report the analyses of 124 and 500 BE studies, respectively, and focus mainly on the impact of BCS or Biopharmaceutical Drug Disposition Classification System (BDDCS), on acceptability of the BCS-based biowaiver approach and discriminative power of the in vitro methods. Comprehensive research for finding additional discriminatory features within each BCS Class that could help us additionally improve the risk assessment is limited. It includes research articles where the impact of area under the plasma concentration time curve to dose ratio (AUC/*D*) on BE outcome is explored on real-world datasets of BE studies with all BCS types [9] and research articles where in silico (i.e., simulation) methodology is used to predict the impact of first-pass metabolism and intrinsic clearance variability on BE outcome [10] and impact of T_{max} and affinity for P-glycoprotein on BE study outcome for APIs belonging to BCS I and III [11].

Comprehensive research on the real set of BE study data that would assess the impact of additional biopharmaceutical and pharmacokinetic parameters, such as absolute BA, affinity for P-glycoprotein, time at which maximum plasma concentration is achieved, elimination half-life ($t_{1/2}$), volume of distribution, number of compartments in a compartmental model by which pharmacokinetics can be best described mathematically, in-silico permeability, plasma protein binding (PPB), etc., that would help identifying clusters of high-risk APIs within specific BCS class and that would set grounds for extended risk classification is missing.

For this reason, a retrospective study was performed where a wide range of characteristics of BE study, API and product were collected, and their predictive potential of the study outcome was assessed.

2 Methods

2.1 Database Preparation

All data from in-house BE studies sponsored by Sandoz that satisfied the inclusion criteria were included in the analysis. Criteria for inclusion of the study into the database were:

- BE study date of completion was within the prespecified time period;
- Study was a pivotal BE study (i.e., pilot studies were not in the scope of this analysis, since the power of such studies is very likely insufficient);
- Study was completed (i.e., study phases were completed as per protocol and a report was issued);
- Results were not inconclusive (i.e., there was no clinical or bioanalytical deviation that would impact study results).
- Test product and reference product were both immediate-release products.
- Test product and reference product contained one API or were FDC products containing two or more APIs. In the

latter case the study was treated as two or more independent BE studies for each API.
– BA of test and reference products were compared under the same condition (either fasting or fed).

Information collected or calculated for each study is presented in Table 1.

Database included BE studies under fasted or fed conditions. BE study was considered successful when the BE criteria defined in the study protocol were satisfied and the compared products were concluded to be BE. In case of FDC, products for each API success of BE study were evaluated separately. For example, it could happen that for API 1 of a fixed dose combination product BE was concluded and for API 2 BE was not concluded. Such product cannot be concluded as BE, but for the purpose of this analysis the outcome for the API 1 was still considered as BE. The term non-BE is used intentionally since failure to conclude BE does not lead to conclusion of bioinequivalence.

In addition to the data on BE studies, we have collected biopharmaceutical and pharmacokinetic parameters of each API as summarized in Table 2. Data on API pharmacokinetics (T_{max}, peak plasma concentration (C_{max}), area under the concentration time curve from zero extrapolated to infinity (AUCi), and $t_{1/2}$ were collected from the in-house studies. Parameters AUCi/*D* and C_{max}/AUCi were calculated from the dose administered, while C_{max} and AUCi were used as observed in the study. Some parameters were used to create classes, e.g., variable T_{max} class (short T_{max} and long T_{max}) was created by splitting database through T_{max} cut down at 1.5 h (90 min)—the upper estimated range of gastric emptying time for a capsule in fasted state [12]. Other biopharmaceutical and pharmacokinetic characteristics, including absolute BA, presence of first-pass metabolism, apparent volume of distribution (Vd), plasma protein binding (PPB), number of pharmacokinetic compartments, substrate for P-gP transporter were collected from the literature. There were two conditions to classify drugs to have first-pass metabolism: the first was BA $\leq$ 80%, and the second was nonclinical or clinical literature evidence indicating first-pass metabolism. The 80% limit is arbitrary and comes from the fact that, in BE testing, $\pm$ 20% (on a normal scale) is considered a relevant BA difference. Literature claiming that API is a substrate for P-gP was the criterion for assigning API as a P-gP substrate. In the literature this claim was made on the basis of in vitro assessment, and in some cases

Table 1 Type of information collected or calculated for each BE study

Parameter	Variable type	Unit of measurement	Description
Dose	Numerical	mg	Dose of the API in the investigational product administered in the study
Food	Categorical (fast, fed)	No unit	BE study is performed in fasted or fed conditions
FDC	Categorical (yes, no)	No unit	Is investigational product a fixed-dose combination product?
T_{max}	Numerical	h	Time to peak concentration. Median[a]
T_{max} class	Categorical (short T_{max}/long T_{max})	No unit	Two categories: short T_{max} ($T_{max} \leq 1.5$ h), long T_{max} ($T_{max} > 1.5$ h)
$t_{1/2}$	Numerical	h	Terminal-phase elimination half-life. Arithmetic mean[a]
C_{max}	Numerical	ng/mL	Peak plasma concentration. Arithmetic mean[a]
AUCi	Numerical	ng h/mL	Area under the plasma concentration time curve from time zero extrapolated to infinity. Arithmetic mean[a]
AUCi/D	Numerical	(h/L)	Ratio of AUCi and dose of API administered in the study. Arithmetic mean[a]
C_{max}/AUCi	Numerical	1/h	Ratio of C_{max} and AUCi. Arithmetic mean[a]
Intra-CV C_{max}	Numerical	%	Estimate of the intra-individual coefficient of variation for C_{max}
Intra-CV AUC	Numerical	%	Estimate of the intra-individual coefficient of variation for AUC
BE criteria for C_{max}	Numerical	No unit	BE criteria for C_{max}[b] as defined in protocol (80–125% or wider for highly variable drugs)
N completed	Numerical	No unit	Number of subjects that completed the study and were included in statistical analysis
Study design	Categorical (crossover/semi-replicate/full-replicate)	No unit	Statistical study design
BE outcome	Categorical (BE/non-BE)	No unit	Outcome of the study based on passing BE criteria for C_{max}[b]

AUC area under the plasma concentration time curve, *AUCi* area under the plasma concentration time curve from time zero extrapolated to infinity, *BE* bioequivalence, C_{max} peak plasma concentrations, *D* dose, *FDC* fixed dose combination, T_{max} time at peak plasma concentration

[a]Estimate for the reference product

[b]In all cases C_{max} was more discriminating for conclusion of BE than AUC

the claim was supported by clinical data. GastroPlus v. 9.6 (Simulations Plus Inc., 42505 10th St W, Lancaster, CA 93534, USA) was used to calculate effective permeability (P_{eff}) for each API using absorption, distribution, metabolism, excretion, and toxicity (ADMET) Predictor and .mol file of the API.

2.2 Statistical Analysis

For non-BE studies, post-hoc power was calculated considering number of subjects that completed the study, observed intrasubject variability for C_{max}, BE criteria for C_{max} parameter defined in the study protocol, study design, expected geometric mean ratio of C_{max} of 95%, and type I error rate of 5%. Post-hoc power was calculated to determine whether the study failed due to inappropriate design (post-hoc power under 80%) or due to more than 5% difference in the BA of the products (post-hoc power above or equal to 80%). R version 3.5.3, RStudio version Version 1.1.456, and package PowerTOST version 1.4-9 were used to calculate post-hoc study power.

Descriptive statistical analysis was performed to summarize various API parameters and BE study data within the highly and poorly soluble groups of APIs. For comparison purposes, descriptive statistics were calculated on a subset of poorly soluble APIs for the BE and non-BE group. Descriptive statistics were reported as mean and coefficient of variance (CV) for BA and permeability. For variables Vd, T_{max}, PPB, $t_{1/2}$, AUC/D, C_{max}/AUC, intra-individual coefficient of variation (intra-CV) for C_{max}, and intra-CV for AUC, where the distribution departures from normal, descriptive statistics were reported as median and interquantile range (IQR) (Table 3).

To assess the association between various numerical features of poorly soluble APIs, nonparametric Spearman rank correlations tests were performed and Spearman coefficients (and associated P values) were used to assess the correlation. Correlation was assessed as weak, moderate, and strong for absolute values of Spearman coefficients below 0.4, between 0.4 and 0.7, and above 0.7, respectively.

A set of univariate tests was performed to test how different variables are associated with the BE outcome. To test the association between categorical variables (Table 4) and BE outcome, a chi-square test was applied. In case any cells in contingency table had five or fewer observations Fisher's exact test was applied instead of chi-square test. Association between the numeric variables and BE outcome was assessed by analysis of variance (ANOVA) or nonparametric Kruskal–Wallis test in case of deviation from the normal distribution or outlying values. P values of < 0.1 and < 0.05 were set for conclusion of association and strong association,

Table 2 Type of information collected about each API

Parameter	Variable type	Unit of measurement	Description
BCS[a,b]	Categorical (I/II/III/IV)	No unit	BCS class was determined in-house or on the basis of literature solubility, literature permeability data, and/or literature BCS evaluations
First-pass metabolism[a]	Categorical (yes, no)	No unit	"Yes" indicates presence of first-pass metabolism assessed on the basis of published literature. Refer to Sect. 2.1
P-gP substrate[a]	Categorical (P-gP substrate/not P-gP substrate)	No unit	Indicates whether API is a substrate for P-gP efflux transporter. Refer to Sect. 2.1
BA[a]	Numerical	%	With the abbreviation BA we are referring to absolute bioavailability, if not specified otherwise
BA class[c]	Categorical (low BA, high BA)	No unit	Classification of API in one of the two categories: low BA (BA ≤40%), high BA (BA>40%). Cutoff was the median of all the BA values, i.e., 40%
Permeability[c]	Numerical	cm/s $\times 10^{-4}$	Calculated human effective jejunal permeability determined by ADMET Predictor of GastroPlus software
Vd[a]	Numerical	L/kg	Volume of distribution based on IV data
PPB[a]	Numerical	%	Plasma protein binding
Number of pharmacokinetic compartments	Categorical (1/2/3/4)	No unit	Number of compartments in a pharmacokinetic model that best describes disposition of API

ADMET absorption, distribution, metabolism, excretion, and toxicity, *API* active pharmaceutical ingredient, *BA* bioavailability, *BCS* Biopharmaceutics Classification System, *IV* intravenous, *PPB* plasma protein binding, *Vd* volume of distribution

Obtained from: [a]literature, [b]in-house data, [c]in silico

respectively. As this was an exploratory analysis, there was no correction for multiple comparisons (i.e., type I error rate was not controlled).

To assess how well each significant parameter alone distinguishes between BE and non-BE outcome we have created receiver operating characteristic (ROC) curves and calculated area under the ROC curve (ROC AUC).

BCS was the only variable tested for the association with the BE outcome on the complete set of data. Based on the information from the BCS analysis, all subsequent analyses (on categorical and numerical variables) were performed on subsets of poorly soluble APIs, which included all of the BCS II and IV APIs. Additional tests for the impact of first-pass metabolism and P-gP substrate were restricted to APIs with the absolute BA < 40%. All these analyses were repeated on subsets of studies under fasting and fed conditions to explore if the conclusions are similar when taking into account the impact of food.

For the purpose of comparison with the results reported in the literature, some analyses were performed also on a subset of highly soluble APIs. These analyses included: descriptive statistics for parameters BA, first-pass metabolism, P-gP substrate, intrasubject CV, and T_{max}.

Tests used for specific parameters are presented in Table 4. Data were analyzed using Minitab 19.2020.3 (Minitab, Inc., 1829 Pine Hall Rd, State College, PA 16801, USA).

Table 3 Ranges of numeric parameters related to API and BE studies for 26 highly and 26 poorly soluble API

Parameter	Solubility	
	Highly soluble APIs (N = 128)	Poorly soluble APIs (N = 145)
BA (%)	18–100	4–100
Vd (L/kg)	0.16–64	0.11–142
T_{max} (h)	0.3–6	0.3–7
PPB (%)	0.1–99.8	0.1–99.9
Permeability (cm/s × 10^{-4})	0.3–7.1	0.3–9.2
$t_{1/2}$ (h)	0.68–65	2–64
C_{max}/AUCi (1/h)	0.03–1.3	0.02–1.6
Intra-CV C_{max} (%)	7–32	8–62
Intra-CV AUC (%)	3–37	5–41

API active pharmaceutical ingredient, *AUC* area under the concentration time curve, *BA* bioavailability, C_{max} peak plasma concentration, *intra-CV* intra-individual coefficient of variation, *Vd* volume of distribution, *PPB* plasma protein binding, $t_{1/2}$ elimination half-life, T_{max} time at peak plasma concentrations

3 Results

3.1 Full Dataset (N = 273)

The database consisted of 198 pivotal BE studies with immediate-release products containing 52 different APIs. There were no missing data for any of the observations.

Among these 198 BE studies, 63 were conducted with FDC products containing two or three APIs. Each API was considered as a separate observation; thus, the database consisted of 273 observations. Before treating each API as a separate observation, FDCs were checked for pharmacokinetic drug–drug interactions. Among 17 unique API FDCs, 7 cases were found to have absence of pharmacokinetic interaction. In six cases pharmacokinetics of highly soluble API(s) in FDCs were slightly impacted by poorly soluble API (although the pharmacokinetic interaction was never clinically significant), whereas pharmacokinetics of poorly soluble API in FDC was not impacted by highly soluble API; thus, this did not impact our analysis. In four cases pharmacokinetic interactions were found also for poorly soluble APIs in FDC; however, all were reported as clinically insignificant. In two out of these four cases we confirmed these interactions do not impact our analysis by comparing pharmacokinetics when APIs were administered alone or in FDCs. Pharmacokinetic parameters in our BE studies could not be considered different. In two remaining cases only FDCs BE studies were available, so there was no confounding of single API or FDC influences. Thus, we concluded that our analysis is not impacted by pharmacokinetic drug–drug interactions.

Of all 273 observations in the database, 34 were concluded as non-bioequivalent (non-BE) and 239 as BE. In all non-BE cases the results were out of limits for C_{max} parameter (only in few of the cases also for AUC). Among non-BE studies, all except one had post-hoc study power above 80%, indicating non-BE results were not caused by insufficient study design.

In 229 cases APIs were in the dosage form of tablets, in 18 cases oral suspensions, in 16 cases dispersible tablets, and in 10 cases hard-gelatin capsules. Apart from two non-BE cases with oral suspensions, other non-BE cases occurred when APIs were in the tablet formulation.

Out of 52 different APIs, 26 were considered as highly soluble and 26 were considered as poorly soluble according to BCS classification. Hypothesis of existing association between a variable and BE outcome were accepted for BCS (at 5% significance) on the complete set of observations (N = 273). ROC AUC for BCS was 0.78 (refer to supplemental data). There were no non-BE studies with BCS class I API (N = 37), and there was only one for BCS class III API (N = 91). BCS class II had the highest occurrence of non-BE

Table 4 Number of studies shown as variable (parameters) levels by BE outcome and descriptive statistic of parameters by BE outcome

Parameter	BE study outcome		*P* value
	BE	Non-BE	
All studies ($n = 273$)	**238 (87)**	**35 (13)**	
BCS			**< 0.001**[b]
Class I	37 (100)	0 (0)	
Class II	84 (74)	30 (26)	
Class III	90 (99)	1 (1)	
Class IV	27 (87)	4 (13)	
Low-solubility APIs (BCS II and IV) ($n = 145$)	**111 (77)**	**34 (23)**	
BA class			**0.006**[b]
Below 40%	70 (70)	30 (30)	
Above 40%	41 (91)	4 (9)	
P-gP substrate			**0.051**[b]
Yes	74 (72)	29 (28)	
No	37 (88)	5 (12)	
First-pass metabolism			**0.021**[b]
Yes	79 (72)	31 (28)	
No	32 (91)	3 (9)	
T_{max} *class*			**0.050**[a]
Short T_{max} ($\leq$ 1.5 h)	41 (68)	19 (32)	
Long T_{max} (> 1.5 h)	70 (82)	15 (18)	
Number of compartments in pharmacokinetic model			**0.070**[b]
1	16 (89)	2 (11)	
2	80 (78)	22 (22)	
> 2	15 (60)	10 (40)	
Intra-CV for Cmax class			0.290[a]
Low CV (< 30%)	70 (80)	18 (20)	
High CV ($\geq$ 30%)	41 (72)	16 (28)	
Intra-CV for AUC class			0.190[a]
Low CV (< 30%)	50 (82)	11 (18)	
High CV ($\geq$ 30%)	61 (73)	23 (27)	
FDC			0.295[a]
Yes	38 (72)	15 (28)	
No	73 (80)	19 (20)	
Low-solubility APIs with BA below 40% (BCS II and IV) ($n = 100$)	70 (70)	30 (30)	
First-pass metabolism			0.854[a]
Yes	66 (70)	28 (30)	
No	4 (67)	2 (33)	
P-gP substrate			**0.075**[a]
Yes	46 (65)	25 (35)	
No	24 (83)	5 (17)	
Low-solubility APIs (BCS II and IV) (n=145)			
BA[#] (%)	48 (52)	33 (59)	**0.003**[c]
Vd[$] (L/kg)	1.5 (6)	1.5 (4)	0.670[d]
T_{max}[$] (h)	2.3 (3)	1.4(2.5)	**0.078**[e]
PPB[$] (%)	98 (9)	98 (9)	0.806[e]
Permeability[#] (cm/s $\times 10^{-4}$)	2.3 (81)	3.3 (68)	**0.004**[c]
$t_{1/2}$ (h)	13.4 (11)	15 (6.6)	0.338[d]
[$]$C_{max}$/AUCi[$] (1/h)	0.11 (0.1)	0.13 (0.12)	0.417[d]
AUC/D[$] (cm/s $\times 10^{-4}$)	5.7 (11.6)	5.2 (9.2)	0.192[d]
Intra-CV[$] C_{max} (%)	26 (14)	29 (12)	0.135[e]

Table 4 (continued)

Parameter	BE study outcome		*P* value
	BE	Non-BE	
Intra-CV[$] AUC (%)	15 (5.5)	16.4 (5)	0.110[e]

Bold values indicate $p < 0.1$

API active pharmaceutical ingredient, *AUC* area under the concentration time curve, *BA* bioavailability, *BA_class* high or low BA class based on cutdown 40%, *BCS* Biopharmaceutics Classification System, *BE* bioequivalence, C_{max} peak plasma concentration, *D* dose, *FDC* fixed-dose combination, *intra-CV* intra-individual coefficient of variation, *P-gP* P-glycoprotein, *PPB* plasma protein binding, $t_{1/2}$ elimination half-life, T_{max} time at peak plasma concentrations, T_{max_class} high or low T_{max} class based on cutdown 1.5 h, *Vd* volume of distribution

Data reported as number (%) of studies. [#]mean (%CV) or [$]median (IQR), [a]Chi-square test, [b]Fisher exact test, [c]ANOVA, [d]logarithmic transformation and ANOVA, [e]Kruskal–Wallis test

results (25.7%), and BCS class IV was the second-riskiest class with 12.9% non-BE cases (Table 4 and Fig. 1).

3.2 Highly Soluble APIs (*N* = 128)

Highly soluble APIs had a wide range of BA, between 18% and 100%, wide range of permeability, and versatile absorption, distribution, and elimination characteristics (Table 3). Regardless, only one BE study had non-BE results. When BA was above 85% all BE studies were successful. In our database, 41% of highly soluble APIs were subject to first-pass metabolism, including the API with the one non-BE result. Only 6% of cases were APIs with first-pass metabolism and high (> 30%) intrasubject variability of C_{max}. However, non-BE results occurred for API with low intra-CV (< 30%). Of all highly soluble APIs, 17% were substrate of P-gP; however, the API with non-BE outcome was not a P-gP substrate. The study with the only non-BE result was conducted under fasted conditions.

3.3 Poorly Soluble APIs (*N* = 145)

Poorly soluble APIs had similarly versatile absorption, distribution, and elimination characteristics as highly soluble APIs, but as expected, even wider range of BA (4–100%) and volume of distribution (Table 3). There were no strong associations between the numerical variables among poorly soluble APIs (refer to supplemental data).

No association was found between BE outcome and APIs being part of or not part of an FDC (Table 4).

Strong association was shown between BE outcome and BA class, first-pass metabolism, and presence of P-gP efflux. For APIs with BA above 40%, absolute risk reduction for non-BE outcome was 21% compared with APIs with BA below 40%. For APIs without first-pass metabolism absolute risk reduction was 19% compared with APIs with first-pass metabolism. When API was not a P-gP efflux transporter substrate, absolute risk reduction for non-BE was 16% compared with studies with APIs that were substrates of P-gP (Table 4 and Fig. 1).

Association with BE outcome was observed also for number of compartments in a pharmacokinetic model of API and T_{max} class (Table 4 and Fig. 1). For APIs with short T_{max} ($\leq$ 1.5 h) absolute risk for non-BE outcome increased by 14% compared with APIs with longer T_{max} (> 1.5 h) (Table 4).

A significant difference (at $\alpha = 5\%$) was observed for either mean or median difference between group of BE and non-BE study outcome for BA, T_{max}, and permeability (Table 4). When BA was above 85% all BE studies were successful. Average BA of APIs with non-BE study outcome was 15% lower (Table 4 and Fig. 2A). Median T_{max} of non-BE group was significantly lower than median of BE group (Table 4). Similar trend, where T_{max} was lower for non-BE group, was observed within fasting and fed BE studies (Fig. 2C). Average permeability of APIs with non-BE study outcome was 1 cm/s $\times 10^{-4}$ higher (Table 4). A similar trend, where permeability was higher for the non-BE group, was observed within low BA (<40%) and high BA (>40%) poorly soluble API classes (Fig. 2B).

ROC AUC values for significant parameters mentioned in Sect. 3.3, were between 0.6 and 0.7 (please refer to supplemental data).

On the other hand, no significant differences ($P > 0.05$) between the groups with BE and non-BE study outcome was observed for parameters Vd, AUC/*D*, C_{max}/AUC, PPB, $t_{1/2}$, intra-CV C_{max}, and intra-CV AUC (Table 4). None of the non-BE studies had PPB below 90% (Fig. 2D).

Of all studies with poorly soluble APIs, 113 observations were attributable to BE studies under fasting conditions (with 24% non-BE occurrence) and 32 to BE studies under fed conditions (with 22% non-BE occurrence). The analysis on the subset of data in fasting conditions yielded the same conclusions as the analysis on the combined set of

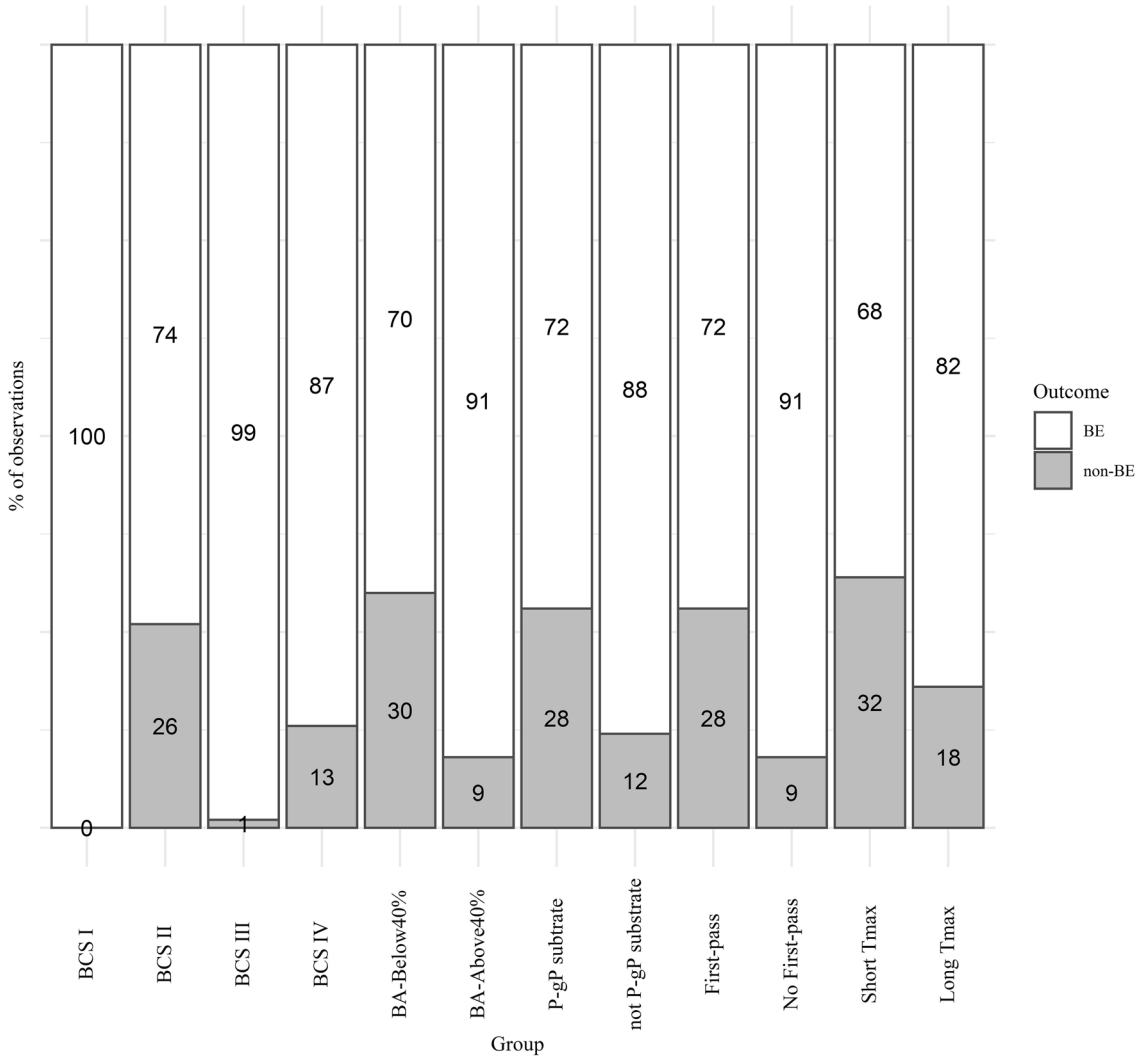

Fig. 1 Proportion (%) of bioequivalence (BE) outcome (BE or non-BE studies) by parameters with highest association to BE outcome: Biopharmaceutics Classification System (BCS) analysis in full dataset. BA class, P-gP subtrate, first-pass metabolism, and T_{max} class analysis on dataset of poorly soluble APIs. *API* active pharmaceutical ingredient, *BA* bioavailability, *BCS* Biopharmaceutics Classification System, *P-gP* P-glycoprotein, T_{max} time at peak plasma concentrations

poorly soluble APIs (fasting and fed conditions) presented in Table 4. However, analysis on the subset of data with food revealed no significant difference between the BE and non-BE groups (Table 4). This could be attributable to the lower discriminatory power of the parameters under fed conditions, or to the smaller sample size (power) of the fed subset.

3.4 Poorly Soluble APIs with BA below 40% (*N* = 100)

Association between P-gP transport involvement and BE outcome was found for the subset of poorly soluble APIs with low BA (< 40%) (Table 4 and Fig. 2). No association between first-pass metabolism and BE outcome could be concluded on the subset of poorly soluble APIs with low BA (<40%).

4 Discussion

4.1 Biopharmaceutics Classification System

Significantly different percentages of non-BE studies were found across different BCS classes in our database (Table 4 and Fig. 1), indicating high association between BCS and BE study outcome. This is in line with a number of publications that supported in vivo predictive nature of BCS [6–8].

Failure rate within the group of highly soluble APIs was negligible (~ 1%) and even lower than that found in the literature (10–16%), regardless of the wide range of BA

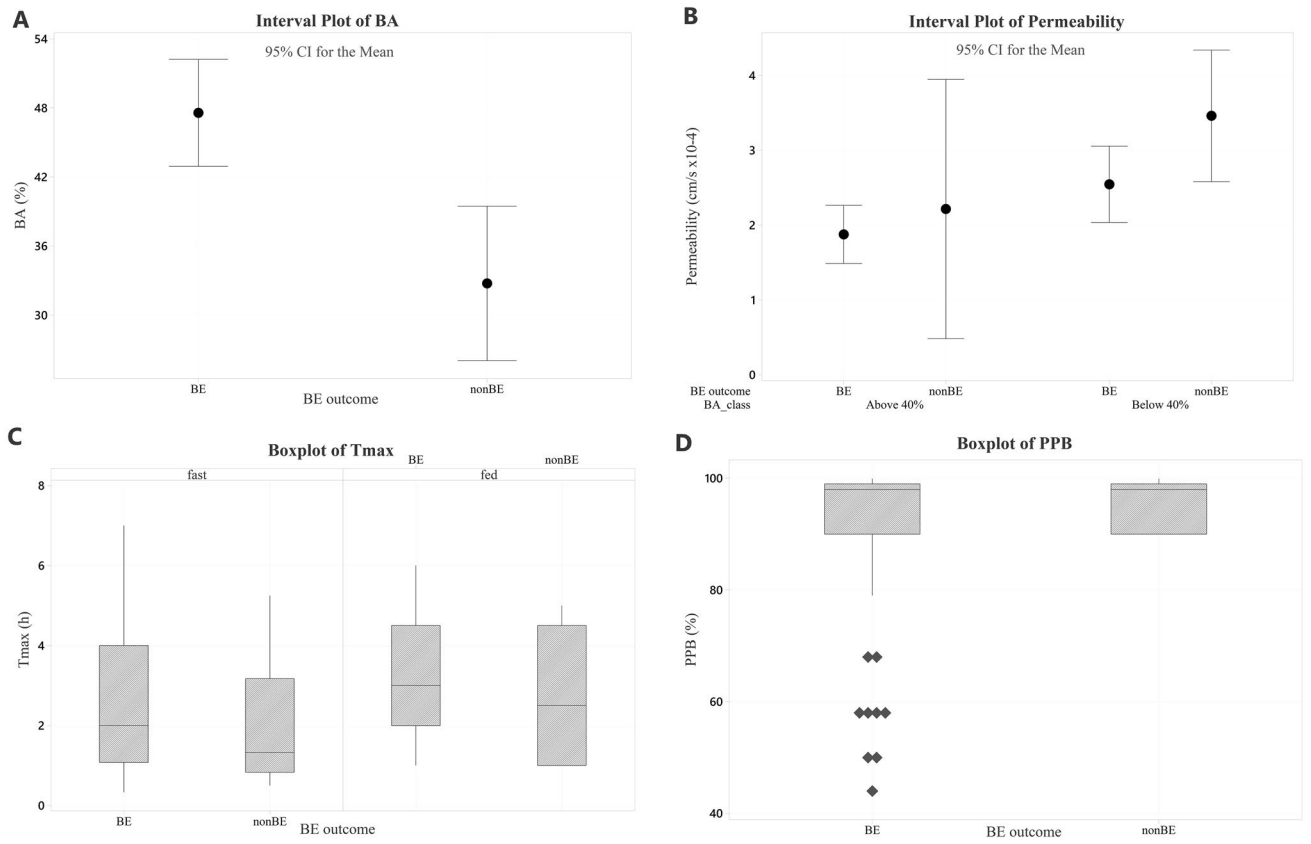

Fig. 2 Interval plot for BA by BE outcome (**A**), interval plot for permeability within BA class by BE outcome (**B**), boxplot T_{max} within fasting and fed by BE outcome (**C**), boxplot plot for PPB by BE outcome (**D**) for dataset of poorly soluble APIs. *API* active pharmaceutical ingredient, *BA* bioavailability (%), *PPB* plasma protein binding (%), T_{max} time at peak plasma concentrations (h)

(18–100%), presence of first-pass metabolism, and/or P-gP mediated efflux attributable to some highly soluble APIs. Low occurrence rate of non-BE results in our database supports BCS biowaiver approach for class I and III APIs implemented by numerous health authorities. In theory, BCS class I and III drugs were presented as less risky for non-BE outcome compared with classes with poorly soluble APIs, while the publications often reported similarly low failure rate for classes I, III, and IV, ranging from 10% to 16%. Similarity of BCS class IV APIs to BCS class I and III was usually attributed to smaller sample size of BCS IV group (i.e., insufficient power to detect differences) [6–8]. Our estimated failure rate of 12.5% within BCS class IV seemed comparable to that reported in the literature; however, in our case the difference between highly soluble group of BCS and BCS class IV was obvious due to negligible failure rate in the highly soluble BCS classes.

BCS class II was previously reported as the most critical for conclusion of BE, where failure rate ranged from 28% to 39% [6–8]. When considering only highly variable BCS class II APIs, Lamouche reported high, flip-of-a-coin-like 54% failure rate of BE studies [6]. In accordance with the literature data, our estimates showed that BCS class II has the highest occurrence of non-BE results (Table 4 and Fig. 1).

Since the BCS classification can be done after assessment of solubility and permeability, and the assessment of permeability is sometimes challenging in the early stages of development, we decided to combine BCS class II and IV APIs into one poorly soluble group for further subanalysis. In addition, the classification available in the literature may sometimes be misleading due to the fact that the permeability assessment for BCS based biowaiver approach is many times surrogated by in vivo BA assessments. Thus, there are sometimes inconsistencies in BCS classification between BCS II and IV classes; for example, BCS II APIs with low BA but high permeability may be misclassified as BCS IV APIs. Combining both poorly soluble BCS classes we avoided any assumptions regarding permeability while assessing factors influencing BE study outcome.

4.2 Bioavailability

When BA was above 85%, either for BCS class I or II, all BE studies ($N = 33$) were successful. Studies are usually powered at 80% or 90%, so the probability of study success, if product is indeed BE, is 80% or 90%, respectively. That further means that the scenario of observing zero non-BE studies among 33 is 0.1% (0.8^33) or 3.1% (0.9^33), which labels observation of zero non-BE studies in 33 cases, as unlikely. BCS biowaiver guidelines define BA of 85% as the limit for fraction of absorption which defines highly permeable APIs. Above this limit, less strict dissolution criteria and formulation differences requirements are set for the product being eligible for BCS biowaiver [13, 14]. This suggests that APIs with high extent of absorption are less risky for BE testing. This was also observed in our analysis. It seems that high permeability along with absence of presystemic API degradation/extraction processes decreases risks for BE study failure. On the other hand, regardless of wide BA range (18–100%) within highly soluble APIs, there was only one case of non-BE result. This supports acceptability of the BCS biowaiver approach for APIs with high (BCS class I) or low permeability (BCS class III).

4.3 Bioavailability within the Group of Poorly Soluble APIs

APIs with a wide range of BA were included in analyses. BA was a significant feature in all of the relevant analyses, i.e., lower BA (especially below 40%) was one of the key indicators for problems with BE. Low solubility might be the reason for low BA; however, it can also be caused by the low permeability, gastrointestinal instability, first-pass metabolism, and/or P-gP-mediated efflux. Some of these factors and their association with non-BE results are discussed in the Sects. 4.4, 4.5, and 4.6.

4.4 Permeability within the Group of Poorly Soluble APIs

As a general rule, higher permeability of poorly soluble APIs was more risky for conclusion of BE (Table 4 and Fig. 2B). Namely, for APIs with high permeability, dissolution rate or solubility becomes a limiting factor for absorption, and these conditions are more challenging in terms of BE outcome where formulation performance is compared. These results correspond well with the BCS class II APIs being the most challenging group of APIs. The fact that this permeability is determined in silico by GastroPlus is at the same time an advantage, as it is easily determined early in the development, and also disadvantage, as the in silico estimate may be associated with less precision.

4.5 First-Pass Metabolism

First-pass metabolism can significantly affect BA and is as such of particular interest when assessing risk for concluding BE. Association between first-pass metabolism, present at 40% of highly soluble APIs, and BE outcome could not be explored within highly soluble APIs, since there was only one non-BE study. Most, i.e., 94%, of these APIs with first-pass metabolism had low variability of pharmacokinetics. The low occurrence of non-BE results is in agreement with prediction of Fernández and coworkers that highly soluble APIs with first-pass metabolism and low variability held less risk for concluding BE [10].

On the other hand, our analyses infer that first-pass metabolism is associated with higher incidence of non-BE results in studies with poorly soluble APIs. These results are in line with the work of Cristofoletti and coworkers, where drug disposition and metabolism based classification (BDDCS) held similar predictive value for BE outcome as BCS [8]. For the subset of APIs where BA was below 40%, presence of first-pass metabolism did not increase the absolute risk for non-BE. However, we have to take the latter conclusion with caution since there were only six observations in the group of APIs without first-pass metabolism.

First-pass metabolism may be influenced by excipients [15–17], which can differ between the generic and reference product and thus impact BE outcome, and by different physiological or pathophysiological conditions, which may increase the variability of exposure. However, the impact of the latter can be excluded in our analysis since all BE studies in our database were performed with healthy volunteers.

4.6 Impact of P-gP Efflux

Another process that may impact BA is efflux of API from enterocytes. There are numerous transporters that perform this task. However, most often this process is governed by P-gP (MDR-1) transporter. H. Kortejärvi and coworkers have shown with simulations that, if a highly soluble API is a substrate for P-gP, this may significantly influence the outcome of BE studies [11]. This risk cannot be observed in our database, since all studies with highly soluble APIs that were substrates of P-gP concluded BE.

On the other hand, our analysis revealed that for poorly soluble APIs involvement of P-gP efflux transporter in pharmacokinetics of API seemed to significantly increase occurrence of non-BE results (Table 4). Our analysis also suggests that the risk for non-BE outcome may be higher if poorly soluble API has low BA (<40%) and is a substrate for P-gP efflux. These results were not surprising, since P-gP efflux may also be influenced by excipients, which can differ between the generic and reference product and thus impact

BE outcome. In addition, P-gP may impact variability of drug exposure [18].

4.7 Time to Peak Concentration

If rapid release is claimed to be clinically relevant and important for onset of action or is related to adverse events, then there should be no apparent differences in median T_{max} and its variability between the test and reference product [14]. In such case, T_{max} would be a significant parameter determining the BE outcome; however, this was not the case in any of our BE trials, where primary parameters were always C_{max} and AUC. Since T_{max} is a composite parameter of elimination and absorption rate, the latter can be impacted by formulation (differences); thus, T_{max} parameter could indeed hold relevant information regarding the study outcome. Furthermore, our hypothesis was that if T_{max} is very short, gastric emptying is not playing a role in limiting the absorption rate and the absorption is rather limited by the release of API from the formulation.

This was confirmed by our analysis of poorly soluble APIs where median T_{max} of the non-BE group was 0.9 h lower than the median of the BE group (Table 4). The trend was similar when the analysis was split for fasting and fed conditions (Fig. 2C). Similarly, the association between T_{max} class (below or above 1.5 h) and BE outcome was observed (Table 4). Splitting the dataset to fasting and fed conditions resulted in a similar conclusion under fasting conditions, but loss of association under fed conditions (even when a cutoff value higher than 1.5 h was considered). This is not surprising since under fed conditions gastric emptying limits the absorption rate. The T_{max} could lose its predictive value for this reason.

The authors acknowledge that the cutoff at 1.5 h might also be too strict to capture all cases with the fast absorption (not limited by gastric emptying), since the slow distribution/elimination can manifest in prolonged T_{max} values. On the other hand, 1.5 h cutoff might be just what we are looking for in BE risk assessment, since the risk for non-BE is particularly high for APIs with fast absorption and fast distribution/elimination, resulting in narrow peaks in plasma concentration profile. See also Sect. 4.14 where we discuss the association between number of compartments to describe distribution/elimination and BE outcome [19].

Finally, Kortejärvi and coworkers have used simulations to show that the risk for not concluding BE is increased for highly soluble and highly permeable APIs with very short T_{max} [11]. However, the higher risk for non-BE results predicted by simulations has not been confirmed in our analysis of highly soluble APIs.

4.8 C_{max}/AUC within the Group of Poorly Soluble APIs

Ln(C_{max}/AUCi) of the non-BE group was lower than that of the BE group, but statistical significance could not be shown (Table 4). C_{max}/AUC was previously recommended as a less polluted measure of absorption rate as C_{max} [20], but was later shown to have similar flaws as C_{max} in lacking sensitivity in indicating changes in absorption rate constant [19]. This might be why C_{max}/AUC was not discriminatory in detecting non-BE results.

4.9 Variability of Drug Exposure

Variability of drug exposure after oral administration poses a significant challenge in modern drug development. Factors impacting variability are diverse and include human physiology variation, bioanalytical variation, and formulation technology variation [18]. It is generally recognized that, with oral administration, distribution and elimination of API cannot be influenced by differences in formulations. For this reason, a crossover design is usually implemented for testing of BE, i.e., testing whether there are any differences in formulation performance. In such setting, "quazi" intra-individual variability (intra-CV of C_{max} and AUC) is the variability of interest. It eliminates variability that may arise from differences related to distribution and excretion of drug between different subjects. Regardless, high intra-CV could still be one of the reasons for decreased power and was more problematic in the past when tools for handling of high intra-CV were not available or accepted by regulatory agencies. These problems were manifested in high occurrence of non-BE results, e.g., 54% of non-BE studies with BCS class II APIs with variability higher than 30% (i.e., high variability) reported by Lamouche [6]. Examples of tools that tackle high variability are higher-order crossover design accompanied by scaling [21] or widening of BE limits [22].

All studies in our database of poorly soluble APIs were crossover studies (2×2 or higher order). Intra-CV of C_{max} and AUC was not significantly different between non-BE and BE group in our database (Table 4). This is not surprising considering the majority of non-BE studies had post-hoc power above 80%, meaning intra-CV was adequately considered in study design. It is noteworthy that higher average intra-CV of C_{max} and AUC was observed for APIs with BA below 40% compared with APIs with BA above 40%, which suggests that processes decreasing BA increase variability of exposure.

Regardless of the crossover nature of BE study, distribution and excretion properties and inter-occasion differences within one subject can still impact intra-CV and/or create more or less discriminatory environment for testing of BE. For this reason, differences between non-BE and BE group

were explored for parameters that describe distribution and elimination of drug: Vd, AUC/*D* (inverse of apparent clearance), PPB, $t_{1/2}$, and number of compartments in pharmacokinetic models that best describe plasma profiles.

4.10 Volume of Distribution within the Group of Poorly Soluble APIs

The ln(Vd) between the BE and non-BE groups was not significantly different (Table 4). Vd did not seem to correlate with intra-CV of AUC, but a moderate correlation between Vd and intra-CV of C_{max} was found (refer to supplemental data). Apart from the impact on variability we could not conclude anything about predictable value of Vd for non-BE outcome.

4.11 Inverse of Apparent Clearance (AUC/*D*) within the Group of Poorly Soluble APIs

Sakuma and coworkers suggested a correlation between AUC/*D* (fraction of absorption/clearance) and number of subjects in a BE study with highly soluble APIs [23]. Yamashita and colleagues explored the correlation of AUC/*D* ratio and parameters that impact BE study success. AUC/D correlated with the width of the 90% confidence interval (i.e., with variability) in BCS classes I and III [9]. Our analysis showed that the mean ln(AUCi/*D*) was not significantly different between the BE and non-BE groups (Table 4), although the mean ln(AUCi/D) of non-BE group was 0.5 h/L lower. This trend agrees with observations of Yamashita and coworkers where lower AUC/D implied higher chance of non-BE results, i.e., APIs with fast clearance, low permeability, high first-pass metabolism, and low GIT stability, were those that held the highest risk [9]. In addition, we have also found a correlation between ln(AUCi/*D*) and intra-CV for C_{max}. In contrast to the work by Yamashita and coworkers, the correlation was found not only for the highly soluble but also for the poorly soluble APIs (please refer to supplemental data). We could not conclude anything on the association of non-BE outcome and AUCi/D, but if the association exists, it may be confounded with the intra-CV.

4.12 Plasma Protein Binding within the Group of Poorly Soluble APIs

Protein binding may serve as a reservoir from which the API is slowly released as the unbound form and can prolong $t_{1/2}$ of the API. When an API is highly bound to plasma proteins, it typically has lower volume of distribution [24]. As such, PPB may impact BA and could hypothetically create more or less discriminatory environment for testing of BE.

Our analysis showed that PPB medians of BE and non-BE groups were not significantly different (Table 4). Further visual analysis showed a group of poorly soluble APIs ($N=11$) with PPB below 90% where there are no non-BE results (Fig. 2D). Considering the study power of 90%, there was still a high 31% chance of not observing any non-BE among 11 studies (0.9^11). These APIs belonging to BCS classes II and IV, with BA in the range from 37% to 100%, had representatives within all groups in terms P-gP efflux, first-pass metabolism, and number of compartments in a pharmacokinetic model (1 and 2). Lipophilicity and acid–base properties of an API correlate significantly with PPB [25], and they also both essentially impact effective permeability. Our analysis shows the higher success rate when PPB was below 90% can be in all but one cases correlated with permeability being below 2 cm/s $\times 10^{-4}$. It seems that for a specific group of poorly soluble APIs lower PPB might be associated with lower risk for non-BE result, but the impact might be to certain extent confounded by other parameters that correlate with lipophilicity and acid–base characteristic of API.

4.13 Elimination Half-Life within the Group of Poorly Soluble APIs

For an immediate-release product, terminal half-life of an API is a hybrid measure of clearance and volume of distribution. Based on the analysis of Vd and AUC/*D*, it was not expected that $t_{1/2}$ would have a direct influence on the BE study outcome. This expectation was confirmed within the group of poorly soluble APIs where no significant difference in ln($t_{1/2}$) was observed between the BE and non-BE groups (Table 4).

4.14 Number of Compartments in a Pharmacokinetic Model within the Group of Poorly Soluble APIs

Distribution of API into a peripheral compartment impacts concentration in a central compartment and can, as such, hypothetically create more or less discriminatory conditions for testing BE, i.e., the more compartments we need to describe the pharmacokinetics of API, the more complex are its distribution and elimination processes, and hypothetically, the risk for a non-BE study result is higher. We have confirmed this with our analysis, where non-BE results occurred in significantly different 11%, 22%, and 40% cases (Table 4) when APIs pharmacokinetics was described by one, two, and more than two compartments, respectively. However, it should be considered that in our database we had only one API with more than two compartments.

4.15 Risk Mitigation Strategy

Identification of parameters that are associated with non-BE outcome calls for mitigation strategy. Noncomprehensive set of examples that may guide reader towards creation of such strategy are: (1) If bioequivalence risk assessment is early, then we can guide development to select or control excipients to minimize impact on pharmacokinetics of APIs that are subject to first-pass metabolism or P-gP transport. (2) Identification of risk parameters also directly guides selection of appropriate methodology (in vitro, ex vivo, animal in vivo, in silico) that is used in predicting human in vivo behavior of API. (3) Lastly, also appropriate BE study design is important: inclusion and exclusion criteria need to be comprehensive when API is subject to first-pass metabolism and/or P-gP transport and blood sampling schedules plan needs to be adjusted when T_{max} is very short or when disposition is described by multicompartment models.

5 Conclusion

BCS was confirmed to be highly predictive for BE success. Only one non-BE study was determined within the group of products with a highly soluble API with wide range of BA (18–100%). This supports the BCS biowaiver approach for class I and III APIs implemented by numerous health authorities. Immediate-release products with BCS class II APIs are confirmed again to have the highest risk for non-BE results. Within groups of poorly soluble APIs (where the majority of non-BE results were observed), absolute BA was shown to be significantly lower for the group of non-BE results. This is in line with the significantly higher occurrence of non-BE results for poorly soluble API with presence of first-pass metabolism and affinity for P-gP transport (efflux). In silico estimated permeability and T_{max} were shown as potentially relevant features for predicting BE outcome. As expected, Vd, total clearance, and $t_{1/2}$ were not associated with BE outcome. PPB between BE and non-BE group was not different; however, we have not observed any non-BE results for poorly soluble API with PPB below 90%. Our analysis also showed significantly higher occurrence of non-BE results for poorly soluble APIs with pharmacokinetics described by multicompartment model (two or more than two compartments). The conclusions for poorly soluble APIs were the same on a subset of fasting BE studies; for a subset of fed studies there were no significant differences between factors in BE and non-BE groups.

One possible extension of our work could be to include additional acido-basic and specific solubility characteristics of APIs and see how these differentiate studies with regards to the BE outcome. On the other hand, it is easy to see how additional parameters showing differences in dosage form characteristics, e.g., process, composition, in vitro dissolutions, etc., could improve BE risk assessment. However, the aim of this research was to evaluate to what extent the BE risk could be predicted at the early beginning of the product development when the parameters related to the dosage form are limited or unknown for generic as well as for the innovator product.

Univariate analysis or plots are simple but essential approaches to exploring the basic relationships of parameters in the dataset. There are some limitations to such approach. Firstly, the type I error is not controlled so the conclusions are to be taken with caution. Secondly, many interactions between parameters may not be found or dealt with, especially, when dealing with such interrelated parameters as presented and discussed in this paper. There are tools available to tackle these problems ranging from simple linear or logistical regression analysis to machine learning/artificial intelligence techniques with different levels of complexity. Considering the limitations, one should use findings presented in this paper as a groundwork for the further research and development of tools for early BE risk assessment.

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Declarations

Conflict of Interest Dejan Krajcar, Rebeka Jereb, Igor Legen, and Jerneja Opara declare employment at Sandoz d.d. Iztok Grabnar declares employment at University of Ljubljana. The authors declare that they have no conflicts of interest.

Ethical Considerations All clinical trials included in this research were conducted in accordance with 1964 Declaration of Helsinki and its latest amendments, good clinical practice guidelines, and other local regulatory laws and guidelines and were approved by applicable ethics committee.

Consent to Participate Written informed consent was obtained from all participants in all clinical trials included in this research.

Consent for Publication Not applicable.

Code Availability Not applicable.

Availability of Data and Materials Not applicable.

Author contribution (CrediT) Dejan Krajcar: conceptualization, data curation, writing—original draft, methodology, formal analysis, visualization. Iztok Grabnar: conceptualization, writing—review and edit-

ing. Rebeka Jereb: data curation, writing—review and editing. Igor Legen: conceptualization, supervision, writing—review and editing. Jerneja Opara: data curation, writing—review and editing.

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