

Consideration of Endogenous Backgrounds in Pharmacokinetic Analyses

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ABSTRACT

Objective: The pharmacokinetic analysis of biologic compounds is frequently disturbed by the presence of endogenous levels, which cannot be discerned from exogenous levels. The frequently used method of subtracting baseline levels from subsequent measurements was compared to a fully adjusted regression model in a simulation study.

Methods: Simulations (5,000 each) were carried out for a standard one-compartment model with rich (n=10) and poor (n=6) post dose sampling, using unweighted as well as two weighted types of non-linear regression.

Results: Whereas the fully adjusted model performed properly across various scenarios, the subtraction method showed a noteworthy bias (up to 14%) for AUC and elimination half-life with weighted non-linear regression. For estimation of the parameter $C_{\max}$ using any weighting scheme, and of any parameter using unweighted non-linear regression, the two methods performed equally well. As expected, poor in contrast to rich sampling resulted in larger coefficients of variation, but also in increasing failures (4.4%) of the regression algorithm (failure to converge, negative $C_{\max}$ or half-life) for the subtraction method, when it was combined with the weighting scheme giving highest weight to small concentrations.

Conclusion: The risk of biased results may result from the subtraction method, which may also affect the analysis of dose linearity, bioequivalence and population kinetic studies with biologic compounds. When background endogenous levels are not negligible, a fully adjusted model is recommended.

Key Words: pharmacokinetics; biologic compounds; endogenous background; PK model; simulation

INTRODUCTION

The pharmacokinetic (PK) analysis of biologic compounds, which are defined here as blood derived products and their counterparts obtained via biotechnology (e.g. recombinant proteins), is often disturbed by the presence of endogenous levels which cannot be discerned from exogenous levels following administration of the compound. In addition, disposition of the exogenous compound can be subject to various endogenous processes, such as saturable enzyme processes, active or diffusional transport, feedback processes and renal threshold [1].

If endogenous synthesis of the compound can be regarded as stable, a frequently recommended method of data analysis consists in subtracting baseline (SB) levels (e.g. obtained from a pre-dose sample) from all subsequent measurements [2]. The use of radio-labelling to discern between exogenous and endogenous compound is less recommended, since early dissociation of the external radiolabel (e.g. I-125) cannot be ruled out, and radio-labelled amino-acids following the catabolic process may be incorporated into other proteins, thus creating pharmacokinetic artefacts [2]. In the absence of isotope effects, provided that a specific assay is used, labelled compounds would be the best option to circumvent the baseline issue.

The SB method is simple and intuitively appealing; it has been applied with prior ascertainment of stable endogenous synthesis [3] or without such ascertainment, especially when endogenous levels are small and negligible [4]. A less common alternative consists in allowing the PK model to include a parameter for the constant background, and let the regression algorithm find an estimate for this parameter. In this model with fully adjusted background (FAB), derived PK quantities such as elimination half-life or AUC are obtained via the same standard formula as before, except that the background estimate can be ignored (an example for this is given in the next section). Available software packages commonly address the baseline issue for pharmacodynamic modelling (e.g., to take account for baseline blood pressure), but not for PK modelling [5].

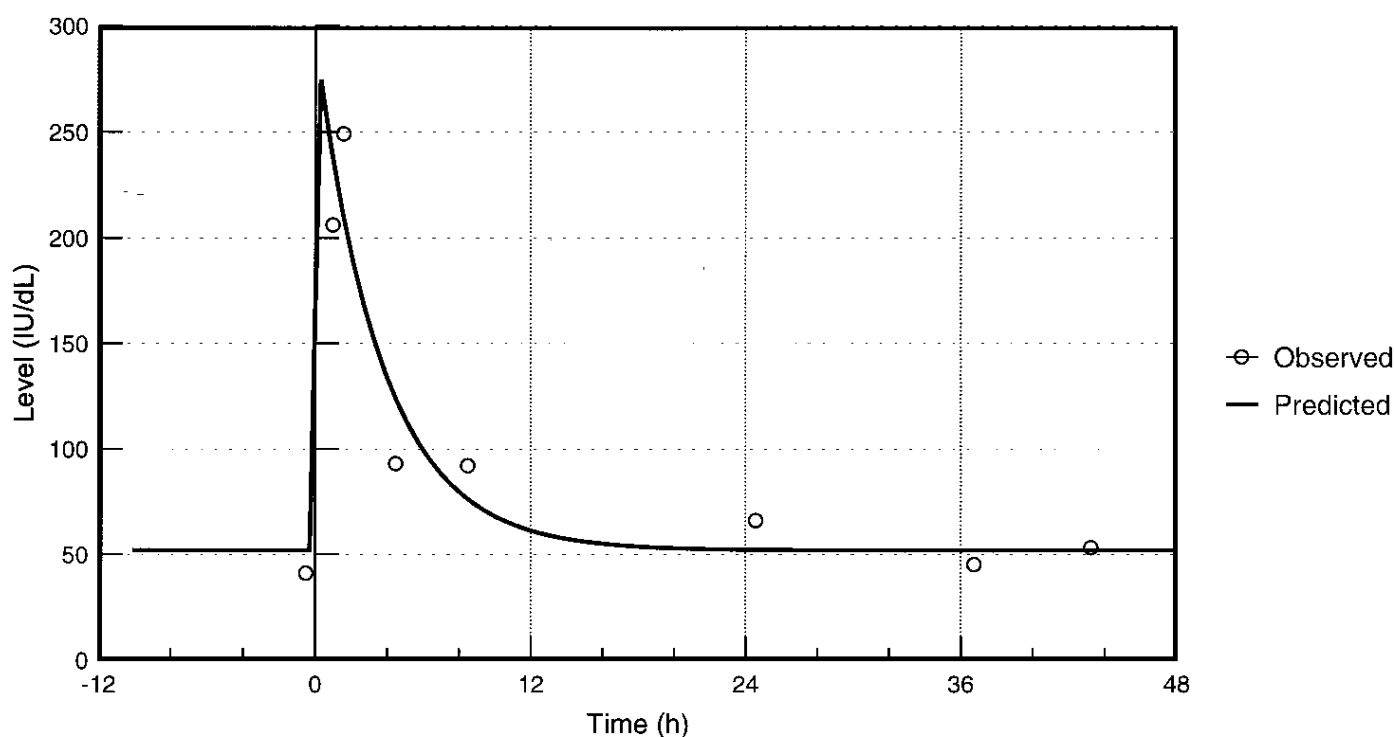


Fig. 1: Observed and predicted levels with adjusted background

The purpose of this paper is to compare the SB and FAB method via simulations under fairly simple circumstances (open one-compartment model). At a first glance, it may appear unclear why the same dataset would lead to different PK results, when analyzed in the one or other way. In fact, the SB method literally takes the baseline value as a known background, whereas the FAB method acknowledges a possible random error of this value and provides a background estimate in the light of all further available data; in this case, the adjusted curve will not necessarily hit the baseline value exactly (see Fig. 1 for an example).

METHODS

Simulations were carried out for an open one-compartment model with constant background; expected concentrations (e.g. in blood, plasma or serum) were assumed to follow the following equation:

$$C(t) = C_0 + C_{\max} \exp(-\lambda t) \quad (1)$$

where $C_0 = 20$ is the endogenous background
 $C_{\max} = 80$ is the dose related peak value
and $\lambda = 0.0693$ is the elimination rate constant.

Two sampling schemes were considered: a rich one, with 10 post dose samples, and a poor one, with only 6 post dose samples (for details see Table 1). A random error was superposed to expected concentrations at these sampling times, corresponding to three different scenarios: normally distributed with constant variance (standard deviation $s = 5$), variance proportional to C ($s = C^{1/2}$), and variance proportional to C^2 ($s = 0.2 C$). The chosen variance relationships were intended to reflect some common types of analytical assay variability. Note that a common variance (with $s = 5$) is reached at a concentration of $C = 25$ for all 3 types.

Table 1: Simulation Characteristics

PK-model	one-compartment with constant background	$C(t) = 20 + 80 \exp(-0.0693 t)$
Sampling scheme	rich (n=11)	- 1 (baseline); 0.25, 0.5, 1, 2, 4, 7, 10, 20, 30, 40
	poor (n=7)	- 1 (baseline); 0.5, 2, 4, 8, 24, 48
Added random error	normal with constant variance	$s = 5$
	variance $\sim C$	$s = C^{1/2}$
	variance $\sim C^2$	$s = 0.2 C$
Number of replicate data sets	5,000 each	(6 scenarios)
Non-linear regression	fully adjusted background (FAB) vs subtracted baseline (SB)	unweighted or iteratively reweighted⁺ least squares
Reported PK parameters⁺⁺	$C_{\max}$, half-life, AUC	

⁺ see text ⁺⁺ expected $C_{\max} = 80$, half-life = 10, AUC = 1,154

For each of the six scenarios (2 sampling schemes times 3 added error types), 5,000 replicated datasets were generated (using PC-SAS® software version 6.12 together with the built-in random generator RANNOR). Non-linear regression analysis (with PC-SAS procedure NLIN) was carried out for each replicate in two different ways:

- (a) SB-method : after subtraction of the baseline value from all subsequent values, possible negative concentrations were set to zero, beforehand. A monoexponential function (equation (1), without constant term C_0) was fitted to the dataset by means of iteratively reweighted least squares. For the constant variance random error type, no weights were applied (unweighted); with error variance proportional to C , weights proportional to the inverse of predicted C were applied ($w \sim 1/C$); and with error variance proportional to C^2 , weights proportional to the inverse of the square of predicted C were applied ($w \sim 1/C^2$).
- (b) FAB-method: the function in equation (1) was fitted directly to the dataset, without prior transformation, by means of iteratively reweighted least squares. Weight schemes remained identical to the SB-method.

The following PK parameters were reported:

- $C_{\max}$, as obtained directly from the regression procedure
- $t_{1/2} = 0.693/\lambda$ (elimination half-life)
- $AUC = C_{\max}/\lambda$ (area under the curve)

For the chosen one-compartment model in equation (1), expected values thus were 80 for $C_{\max}$, 10 for $t_{1/2}$ and 1,154 for AUC.

For each of the six scenarios, the derived PK results were summarized by reporting the mean, coefficient of variation (CV in %) and bias. Bias was calculated as the percent deviation of the mean from the expected value. Datasets where the regression algorithm failed to converge (after 500 iterations) or showed a negative elimination rate were not included, the failure rate was reported nevertheless.

RESULTS

For the rich sampling scheme (Table 2), the FAB method never failed to converge or showed a negative elimination rate, whereas the SB method showed 23 (0.46%) failures with the third weighting scheme ($w \sim 1/C^2$) and none for the unweighted and second scheme ($w \sim 1/C$).

The mean estimates for the background level (with 19.9, 20.3, and 20.6 for the three weighting schemes) found by the FAB method were close to the expected value of 20.

With unweighted regression, FAB and SB method performed equally well. If anything, a tiny bias to the right was observed for the SB-method for half-life (+ 1.3%) and AUC (+ 1.7%), which was less pronounced for the FAB method (+ 0.6% and + 1.2%, respectively). With weighted regression, however, the bias of half-life and AUC became noteworthy for the SB-method: mean elimination half-life was 6.0% too high with the second weighting scheme, and 12.8% with the third. The respective figures for AUC were 5.7% and 14.3%. As for $C_{\max}$, no differences emerged between the FAB- and SB-method. For all three reported PK-parameters, the FAB- and SB-method provided fairly comparable CV-values. However, CV-values were lower for $C_{\max}$ (5% - 12%) than for half-life (15% - 22%) or AUC (13% - 21%).

Table 2: Simulation Results (n = 5,000 each) - rich sampling scheme

Error, weight scheme	Regression method	Cmax = 80			T _{1/2} = 10.0			AUC = 1154		
		Mean	CV %	Bias %	Mean	CV %	Bias %	Mean	CV %	Bias %
SD=5, unweighted	FAB	80.2	5.5	+ 0.3	10.06	16.0	+ 0.6	1168	18.6	+ 1.2
	SB	79.9	6.4	- 0.1	10.13	17.4	+ 1.3	1174	21.2	+ 1.7
SD= C ^{0.5} , w ~1/pred ⁺	FAB	80.4	6.5	+ 0.5	10.04	16.9	+ 0.4	1160	15.7	+ 0.5
	SB	80.1	6.4	+ 0.1	10.60	15.2	+ 6.0	1220	13.0	+ 5.7
SD= 0.2 C, w ~ 1/pred ²	FAB	82.6	11.5	+ 3.2	9.96	22.2	- 0.4	1172	19.0	+ 1.6
	SB*	82.4	12.3	+ 3.0	11.28	20.7	+ 12.8	1319	15.9	+ 14.3

⁺ pred: predicted concentration * algorithm failed in 23 cases

Table 3: Simulation Results (n = 5,000 each) - poor sampling scheme

Error, weight scheme	Regression method	Cmax = 80			T _{1/2} = 10.0			AUC = 1154		
		Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
SD= 5, unweighted	FAB	80.5	6.0	+ 0.6	10.2	20.3	+ 1.6	1182	22.2	+ 2.4
	SB	80.1	6.9	+ 0.1	10.3	21.5	+ 2.9	1193	24.5	+ 3.4
SD= C ^{0.5} , w ~ 1/pred ⁺	FAB	81.1	9.2	+ 1.3	10.1	22.6	+ 0.7	1166	19.9	+ 1.0
	SB*	80.0	9.1	+ 0.1	10.9	21.9	+ 8.7	1241	18.0	+ 7.5
SD= 0.2 C, w ~ 1/pred ²	FAB	83.9	16.0	+ 4.9	9.99	28.8	- 1.1	1176	23.2	+ 1.9
	SB**	83.9	18.0	+ 4.9	11.2	26.9	+ 11.6	1311	20.3	+ 13.6

⁺ pred: predicted concentration * algorithm failed in 1 case ** algorithm failed in 222 cases

For the poor sampling scheme (Table 3), the SB-method failed or showed a negative elimination rate in one ($w \sim 1/C$) and 222 (4.4%) cases ($w \sim 1/C^2$), whereas the FAB-method never failed. The mean estimates for the background level (with 19.8, 20.1, and 20.3 for the three weighting schemes) were close to the expected value of 20. Again, FAB- and SB-method performed comparably well with the unweighted method, with small biases for half-life (SB: 2.9% vs FAB: 1.6%) and AUC (SB: 3.4% vs FAB: 2.4%). With weighted regression, a noteworthy bias again appeared for the SB-method: elimination half-life was 8.7% too high with the second weighting scheme ($w \sim 1/C$) and 11.6% with the third ($w \sim 1/C^2$). The respective figures for AUC were 7.5% and 13.6%. As for $C_{\max}$, no relevant differences emerged between the two methods, but interestingly, both methods provided a biased result (4.9% in each case) with the third weighting scheme ($w \sim 1/C^2$). For all three reported PK parameters, the FAB- and SB-method provided fairly comparable CV-values. Not surprisingly, these were somewhat higher than with the rich sampling scheme. Again, CV-values were lower for $C_{\max}$ (6% - 18%) than for half-life (20% - 29%) or AUC (18% - 24%).

Modifications in program code are fairly simple: Table 4 shows an example of modified code used e.g. in the generation of Figure 1.

Table 4: Modified Winnonlin® program code to incorporate endogenous background parameter

```

model 1
remark    one compartment model - bolus input, first order output
rema    !! constant endogenous background level fitted as asymptote !!
rema
rema      no.      parameter      constant      secondary parm.
rema      ---      -
rema      1        volume        # doses        auc
rema      2        k10           dose 1        k10 half life
rema      3        cback         time, etc     cmax
rema      4                            cl
rema      5                            aumc
rema      6                            mrt
rema      7                            vss
rema*****
rema      I-----I
rema      Bolus    I          I
rema      iv  --> I    Compartment 1    I  --> K10
rema      I          I
rema      I-----I
rema*****
comm
nparm 3
nsec 7
pnames 'VOLUME', 'K10' , 'CBACK'
snames 'AUC', 'K10HL', 'CMAX', 'Cl', 'AUMC', 'MRT', 'VSS'
end

func 1

j = 1
ndose = con(1)

```

```

rema  Count up the number of doses administered up to time x
do i = 1 to ndose
j = j+2
if x <= con(j) then goto red
endif
next

rema  Adjust number of doses if x exactly equals a dosing time
red:
if x = con(j) then ndose = i
  else ndose=i-1
endif

rema  Perform superposition
sum = 0
j = 1

do i = 1 to ndose
j = j + 2
t = x - con(j)
dose = con(j-1)
amt = (dose / volume) * exp(-k10 * t)
sum = sum + amt
next

f=sum + cback
end

seco
dose = con(2)
auc = dose / (volume * k10)
k10hl = -loge(.5) / k10
cmax = dose / volume
cl = dose / auc
aumc = dose / (volume * k10 * k10)
mrt = aumc / auc
vss = cl * mrt
end

eom

```

DISCUSSION AND CONCLUSIONS

In reality, random deviations from the expected concentration profile have more sources than just analytical assay variability, such as biologic fluctuations in a subject (e.g. due to water uptake, circadian influences etc). In practice, however, the choice of a weighting scheme for the regression analysis largely depends on the assay properties, which can be obtained conveniently from a validation experiment prior to the PK study in question. The adequacy of the weighting scheme would only be questioned, if an inspection of the (weighted) residuals reveals some gross imbalances.

Although not given explicitly, the volume of distribution in the chosen model is proportional to the inverse of $C_{\max}$, which was not severely biased by either method. Conversely, systemic clearance, which is inversely proportional to AUC, is subject to bias in the same extent as AUC, except that the bias goes in the other direction (clearance is underestimated).

The bias of the SB-method, especially for the elimination half-life and AUC, might be attributed to the fact that resulting negative concentrations were arbitrarily set to zero. However, when applying an altered SB-method (allowing negative concentrations) to the scenario of a rich sampling scheme and the third weighting scheme ($w \sim 1/C^2$) with otherwise identically replicated data, the observed bias essentially remained: the bias of the mean became + 1.1% for $C_{\max}$, + 22.2% for the elimination half-life and + 20.9% for AUC, while 61 (1.2%) failures occurred.

In conclusion, it appears as if the risk of biased results, which is already inherent to the non-linear regression technique [6], would be enhanced in the presence of inappropriate weighting schemes. In fact, the SB-method, as presented here, leads to inappropriate weights in the regression algorithm, when these are readily transferred from the analytical validation report. In contrast, the FAB-method provides a convenient way to implement the correct weighting scheme. Furthermore, the FAB-method is applicable in situations where a true baseline value does not even exist (an example for this would be a situation, where the first blood sample was erroneously taken a few minutes after start of a longer IV-infusion).

The SB-method would also fail with the unweighted scheme, if by chance the measured baseline value is too low, but final blood samples (taken after 3 - 4 elimination half-lives have passed) reflect a more correct higher value. In this case the SB-method would point to a second, long elimination half-life, whereas the FAB-method would not. With small and negligible backgrounds, the differences of the two methods become irrelevant, e.g. when a sufficiently high dose is studied. However, if a dose linearity study is conducted, background levels may be negligible for the highest, but not for the lowest dose; in this case, an artificial dose dependency might be observed with the SB-method. A similar situation may emerge, when conducting bioequivalence studies with biologic compounds: since AUC values usually are obtained here by curve extrapolation from a terminal portion of data reflecting elimination [7], the true average difference of two formulations may appear as attenuated, when using the SB-method together with a scheme giving more weight to small concentrations.

Finally, the observed distortions of the SB method are likely to become transmitted to population kinetic data analysis, as suggested by the results of the poor sampling scheme. It is therefore advisable to use fully adjusted backgrounds in the data analysis of biologic compounds, regardless of the complexity of the pharmacokinetic model involved [8].

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