J Pharmacokinet Pharmacodyn (2012) 39:453–462
DOI 10.1007/s10928-012-9261-5

ORIGINAL PAPER

Modeling of red blood cell life-spans in hematologically normal populations

Rocío Lledó-García · Robert M. Kalicki · Dominik E. Uehlinger · Mats O. Karlsson

Received: 28 February 2012 / Accepted: 27 June 2012 / Published online: 31 July 2012

Abstract Despite the impact of red blood cell (RBC) Life-spans in some disease areas such as diabetes or anemia of chronic kidney disease, there is no consensus on how to quantitatively best describe the process. Several models have been proposed to explain the elimination process of RBCs: random destruction process, homogeneous life-span model, or a series of 4-transit compartment model. The aim of this work was to explore the different models that have been proposed in literature, and modifications to those. The impact of choosing the right model on future outcomes prediction—in the above mentioned areas- was also investigated. Both data from indirect (clinical data) and direct life-span measurement (biotin-labeled data) methods were analyzed using non-linear mixed effects models. Analysis showed that: (1) predictions from non-steady state data will depend on the RBC model chosen; (2) the transit compartment model, which considers variation in life-span in the RBC population, better describes RBC survival data than the random destruction or homogenous life-span models; and (3) the additional incorporation of random destruction patterns, although improving the description of the RBC survival data, does not appear to provide a marked improvement when describing clinical data.

R. Lledó-García (✉) · R. M. Kalicki · M. O. Karlsson
Pharmacometrics Research Group, Department of Pharmaceutical Biosciences, Uppsala University, Box 591, SE 751 24 Uppsala, Sweden
e-mail: rocio.lledo@gmail.com

Present Address:
R. Lledó-García
UCB Celltech, Department of Modeling and Simulation, GED, 208 Bath Road, Slough SL1 3WE, UK

R. M. Kalicki · D. E. Uehlinger
Department of Nephrology and Hypertension, University of Bern, Inselspital, Bern, Switzerland

Keywords Life-span modeling · Red blood cells · Hba1c · Pharmacometrics · Biotin labeled · Mechanism-based model

Introduction

Erythropoiesis is a complex multistep process encompassing the proliferation and differentiation of hemopoietic stem cells to mature erythrocytes (RBC or red blood cell). A normal homeostasis of the erythropoietic system requires a balance between the rate of red blood cell production and destruction. Red-cell mass is controlled within a narrow range at which rheological properties and delivery of oxygen to tissues are optimal. When red-cell mass decreases, i.e. in bleeding, the kidneys, sensing a decrease in the tissular oxygen content, release erythropoietin (Epo) which increases production and prevents apoptosis of early erythroid precursors [1]. The continuous cell production occurs in bone marrow and is ensured by pluripotent stem cells, which have the unique property of both self-renewal and differentiation through progressive commitment to maturing precursors. During the differentiation process, the cells become progressively and transiently sensitive to Epo due to the appearance of erythropoietin receptors [2, 3].

The typical life-span of a human RBC is reported to be about 120 days, after which the RBC undergoes a process of senescence with morphological changes leading to the removal from circulation through phagocytosis by the macrophages [2, 4]. In addition to senescence, random destruction of red blood cells may also occur, as a result of pathologic factors, such as bleeding, hemolysis or clotting. This process causes the premature disappearance of cells which are not potentially immortal, but have a life-span limited by senescence [5, 6].

Although the mechanisms describing the fate of aged erythrocytes have been of great interest for many years, and a number of candidate pathways have been proposed [7–9], there is still no universally accepted model of red cell senescence and removal from the circulation. Besides physiological considerations and cellular mechanisms involved in this phenomenon, a better understanding of the kinetics describing RBC production, release in the circulation and destruction would be useful in both the clinical and research settings. This is because RBC life-span plays a key role in some pathophysiological models impacting on treatment decisions in individual patients, as well as on analyses and predictions in drug development. The process of haemoglobin glycosylation and the erythropoietic response to rHuEPO, both strongly dependent on the RBC life-span, illustrate the importance of this concept for the management of diabetic and anemic patients.

Several models describing the life-span concept of RBC have been proposed: (1) Uehlinger et al. proposed a homogeneous life-span model in uremic patients, where the life-span concept is considered as a delay time parameter—the life-span of a specific RBC population. This approach thus assumes that each of the patient's erythrocytes has the same life-span [10]. This type of model has also been applied to describe the effect of rHuEPO on reticulocytes and RBC by other authors [11–14]. (2) A second type of model has been proposed, that considers RBC removal from circulation as a random process. This random destruction model was used for example by Petersson et al. [15] and de Winter et al. [16] to describe RBC LS in diabetic patients. In this case, the elimination process follows a first order decay. The life-spans for an individuals' RBC population is considered to follow an exponential distribution. (3) A third type of model, proposed by Hamrén et al. considers a series of 4 transit compartments with transition between compartments and elimination from the last compartment as first order, random, processes. This model, which has life-span distributions which are intermediate to the two preceding types of models, was developed based on HbA1c measurements in diabetic patients [17].

In order to determine the RBC life-span we can either use an indirect approach as in the studies described above, where model based analysis is used to infer this information from e.g.: changes in the glycosylated haemoglobin fraction (HbA1c) or haemoglobin concentration -time profiles under rHuEPO treatment [18]. Alternatively, direct measurements of the life-span, based on different cell labelling methods, allow elimination to be followed for a sample of the RBC population. There are two labelling approaches: cohort labels and population (also called random) labels. The former identifies the RBC released from the bone marrow during a defined time period. At subsequent times all of the labeled RBCs are approximately the same age. In the latter case, labels are placed on RBC of all ages. In humans, these procedures are always done ex vivo, followed by immediate reinfusion of the labeled cells. A recent review on the different techniques can be found in Franco et al. [4].

Cellular biotinylation has been shown to give particularly precise determinations of cell survival for almost the entire RBC life-span, because the number of false positives events in the flow cytometer is extremely low ($\sim 10/10^6$). Compared to the prior gold standard population label technique with ^{51}Cr, cellular biotinylation yields equivalent values for RBC and offers some advantages such as lack of radioactivity and little elution from the cells which makes it more suitable method for entire survival curve analysis [19–24]. Even when some loss of biotin occurs, presumably due to plasma biotinase activity, the cell still falls within the positive analysis region and is therefore counted the same as if there were no loss. The same is true for loss of surface area or hemoglobin as the cells age.

However, there are also some disadvantages compared to ^{51}Cr. The biotin labeling procedure requires more manipulation of the cells, with multiple washes to remove unreacted reagent and reaction by products, which could damage the RBCs. Because the flow cytometric analysis determines labeled cells as a percentage of the total cells, an underlying assumption is that the total number of RBC is at steady-state during the lifespan measurement [4].

A recent study by Cohen et al., based on biotin labeled RBCs in a small number of healthy volunteers and diabetic subjects, showed that the observed variation in RBC survival was large enough to have a significant impact on the interpretation of HbA1c levels [20].

Given the impact of a good description of the RBC life-span in the above mentioned disease areas, and the lack of consensus on the model that best describes the RBC life-span, the aim of this work was to explore the different models that have been proposed in the literature, and to assess modifications to them, by analysing data relevant to the underlying processes involved. The impact that choosing the right model would have on the prediction of future outcomes was also investigated. In order to cover a wider spectrum of cases, both data from indirect and direct life-span measurement methods were used.

Methods

Data analysed

Biotin-labeled data

Survival RBC fraction—time profiles following biotin-labelling for 6 diabetic and 6 non-diabetic participants

reported in Fig. 2 of the publication by Cohen et al. [20] were extracted by digitization (DigitalizeIt software version 1.5). A three replicate digitization was performed and for each time record the average of the replicated digitized values was taken, providing a satisfactory precision (mean SD of 0.4 % units).

rHuEPO-data

Clinical data from 54 hemodialysis patients with end stage renal disease (ESRD) patients, previously reported by Uehlinger et al. [10] was analysed in the present study. The data consisted of haemoglobin concentration–time profiles following initiation of an i.v. rHuEPO substitution in rHuEPO naïve patients receiving long-term high-flux hemodialysis.

HbA1c-data

A dataset of time-courses of fasting plasma glucose (FPG) and HbA1c in 1083 type II diabetic patients [25] was analysed. The data originate from phase II and III studies within the development program of tesaglitazar, an α-γ-PPAR agonist, where both naïve and previously treated patients were followed during wash-out, treatment and post-treatment phase with either placebo or tesaglitazar.

Models assessed

The three datasets presented in the previous paragraph were analysed with a non-linear mixed effect modelling approach using NONMEM VI. The first-order conditional estimation method (FOCE) and subroutine ADVAN6 were used. Inter-individual variability (IIV) in model parameters was assumed to be log-normally distributed. Additive, proportional and additive plus proportional models were assessed as residual error models.

The three previously published models considered are described below.

Homogeneous life-span model

First described by Uehlinger et al. [10], this model treats the RBC ageing and removal as a zero-order process, and assumes that each of the patients' erythrocytes has the same life-span. The survival function is *1* if time is less than the life-span or *0* otherwise. The corresponding differential equation is:

$$\frac{dRBC}{dt} = k_{in}(t) - k_{in}(t - LS) \quad (1)$$

where $k_{in}(t)$ represents the cell production and $k_{in}(t\text{-}LS)$ the cell loss, following a zero-order rate process, which means that the number of dying cells in a fixed time interval (life-span) does not depend on the total number of cells. Each RBC lives for the same period of time *LS* and then disappears as a consequence of senescence. This life-span determines the cell elimination rate, which is the rate of cell production delayed by the time LS.

Random destruction, or turn-over, model

This model treats the RBC removal as a first-order, i.e. random, process. The following equations describe the model:

$$\frac{dRBC}{dt} = k_{in} - k_{out} \cdot RBC \quad (3)$$

$$k_{out} = \frac{1}{LS} \quad (4)$$

where k_{in} is a zero-order RBC production rate constant and k_{out} the first-order rate constant representing the RBC removal process.

Transit compartment model

Hamrén et al. [17] introduced four, in series coupled, transit compartments to describe the RBC ageing, starting with a zero-order release of RBC into circulation (k_{in}). The first-order rate constant (k_{tr}) defines the RBC transition from one age stage to the next until the cell dies, when removed from the last compartment. As in the previous case, the model assumes a distribution of life-span around a mean for a RBC cohort in an individual. The shape of this distribution is given by the number of transit compartments. The equations describing the RBC cohort ageing and removal process for a 4-transit compartment model are as follow. In this case the number of compartment NC = 4.

$$\frac{dRBC_1}{dt} = k_{in} - ktr \cdot RBC_1 \quad (5)$$

$$\frac{dRBC_2}{dt} = ktr \cdot RBC_1 - ktr \cdot RBC_2 \quad (6)$$

$$\frac{dRBC_3}{dt} = ktr \cdot RBC_2 - ktr \cdot RBC_3 \quad (7)$$

$$\frac{dRBC_4}{dt} = ktr \cdot RBC_3 - ktr \cdot RBC_4 \quad (8)$$

$$ktr = \frac{NC}{LS} \quad (9)$$

The random destruction model is equivalent to a transit compartment model with a single transit compartment. The homogenous life-span model corresponds to a transit compartment model with an infinite number of transit compartments. Thus, increasing the number of transit compartments from one and upwards, explores intermediary models between these two extremes. In the software chosen, the

maximum number of compartments possible to implement was 30, 29 and 14 for the biotin-labeled, the rHuEPO- and the HbA1c-data sets, respectively. For the biotin-labeled and rHuEPO-data sets also the exact implementation of the homogenous life-span model was evaluated.

In addition, a random destruction process, in parallel to the transit compartments, and occurring from every compartment was also incorporated into the models and explored for the biotin-labeled and the rHuEPO data sets. Again, the optimal number of transit compartments was assessed. The random destruction process was investigated by considering a linear description of the process with *krd* as a first-order rate constant describing the destruction.

$$\frac{dRBC_1}{dt} = k_{in} - ktr \cdot RBC_1 - krd \cdot RBC_1 \quad (10)$$

$$\frac{dRBC_2}{dt} = ktr \cdot RBC_1 - ktr \cdot RBC_2 - krd \cdot RBC_2 \quad (11)$$

$$\frac{dRBC_3}{dt} = ktr \cdot RBC_2 - ktr \cdot RBC_3 - krd \cdot RBC_3 \quad (12)$$

$$\frac{dRBC_4}{dt} = ktr \cdot RBC_3 - ktr \cdot RBC_4 - krd \cdot RBC_4 \quad (13)$$

(…) successively till 29 compartments.

$$ktr = \frac{NC}{LS} \quad (14)$$

where NC is the number of transit compartments, in this case 29, *krd* the first order constant for random destruction.

Variance of mean residence time

The mean residence time (MRT) represents the average time spent in circulation by the RBCs. The variance in MRT (VMRT) provides a quantitative measure of heterogeneity in life-spans within an individual and the coefficient of variation in MRT (% CVMRT) is a convenient and robust measure [26]. The following equations apply:

$$MRT = \frac{\int_0^\infty t \cdot RBCel \cdot dt}{\int_0^\infty RBCel \cdot dt} \quad (15)$$

$$VMRT = \frac{\int_0^\infty (t - MRT)^2 \cdot RBCel \cdot dt}{\int_0^\infty RBCel \cdot dt} \quad (16)$$

where t is time and *RBCel* the RBCs eliminated at time t, which can be calculated as follows:

$$RBCel = -ktr \cdot RBC_n \quad (17)$$

$$RBCel = -krd \cdot [RBC1 + RBC2 + \cdots + RBC29] - ktr \cdot RBC29 \quad (18)$$

where n in Eq. 17 would correspond to the last compartment, depending on the model and Eq. 18 represents the eliminated RBCs expression in the model considering RBC ageing and random destruction. As we are referring to fractions of RBC eliminated, it can be simplified as follows:

$$MRT = \int_0^\infty t \cdot f \cdot dt \quad (19)$$

$$VMRT = \int_0^\infty (t - MRT)^2 \cdot f \cdot dt \quad (20)$$

$$CVMRT = \frac{\sqrt{VMRT}}{MRT} \cdot 100 \quad (21)$$

where t is time and f the RBCs fraction eliminated at time t.

Model evaluation

OFV mapping

In the models investigated, the OFV was evaluated with varying numbers of transit compartments. The number of estimated parameters remained constant between models during the OFV mapping, so that any improvement was due to the increase or decrease in the number of transit compartments. This was done both with and without the addition of a random destruction component. For two nested models, the difference in OFV is approximately χ^2 distributed with the number of degrees of freedom given by the difference in number of parameters.

Visual predictive checks (VPC)

To evaluate the predictive performance of the model, visual predictive checks were performed using the final model parameter estimates [27]. The visual predictive check illustrates the model's ability to simulate the data that have been used for the model development. Time-courses of RBC count were simulated 1,000 times using the original design of the study in question. Percentiles (5th, 50th and 95th) of the observations were compared to percentiles (including confidence intervals) of the simulated values. The goodness of fit plots and graphical representation of visual predictive performance were performed in Xpose [28].

Impact of model choice on predictive performance

To assess the impact that the choice of a given model would have on future outcomes, the predictive performance applied to two disease areas where RBC life-span has a major impact was studied:

1. Simulations of Hb concentrations versus time were performed in an anemic patient. It was assumed that observations were made at 0 and 30 days after starting treatment with rHuEPO resulting in a constant increase

in RBC mass. Prediction of the Hb concentration beyond 30 days was performed using the different models that had previously been derived using the biotin-labeled data (homogeneous life-span model, random destruction model, 12-transit compartment model and 29-transit compartment model with random destruction).

2. Changes in HbA1c levels were investigated by predicting for a diabetic patient with a FPG level at baseline of 8.2 mmol/L, future HbA1c levels after an assumed immediate 25 % decrease in FPG. The model used for the simulations was based on that published by Hamrén et al. [17] but expanded to 12 transit compartments.

Results

Figure 1 shows the OFV values when analysing the biotin-labeled data with the models described in the "Methods" section. The 12 transit compartments best described the data (OFV = 345.57) while the random destruction model behaved worst (OFV = 579.72), followed by the homogeneous life-span model (OFV = 517.08). Figure 2 shows model predictions for a RBC cohort, the fraction eliminated per day versus time (a) and the fraction remaining to be eliminated (b). This illustrates the high variance on mean residence time (VMRT) for the random destruction model (CV 100 %), followed by the 12-transit compartment model (CV 27 %), whereas in the homogeneous life-span model the CV is 0 %, as all cells will die at the same time. Panel (c) shows the probability that the remaining RBC will be eliminated in the next day, for the different models. For the random destruction model, this probability will be the same every day, whereas for the homogeneous life-span model will be zero, except for the day prior to the life-span where the probability will be of 1. In the transit compartment model, the probability will increase over time.

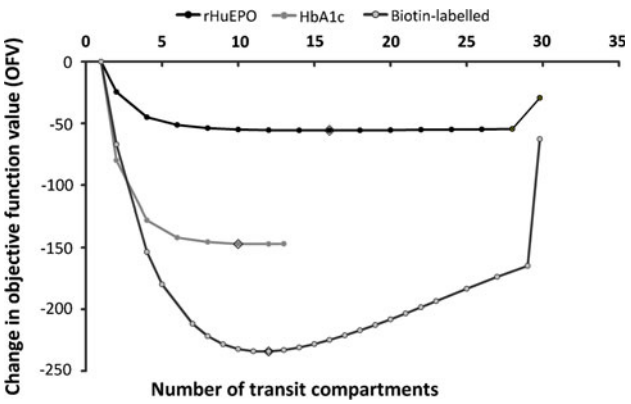

Fig. 1 The OFV mapping for the different data sets, where NC is the number of transit compartment assessed. The result for the homogeneous life-span model is indicated at NC = 30. The diamond shapes high-light the optimal model for each dataset

The visual predictive checks (VPC) showed a better predictive performance of the 12-transit compartment model as compared to the other models assessed (Fig. 3). However, when the transit model with additional random destruction process was assessed, a 29-transit compartment model was found to describe the data better (OFV = 250.7). The improvement was not clearly visible in the VPC as shown in Fig. 3, but may be related to the two more parameters capturing the inter-individual variability better. The estimated life-span was also very similar for the best two models: 92 (12-transit compartment model) and 95 days (29-transit compartment model with random destruction). In addition, calculated CVMRTs were similar in both cases, 27 versus 43 %, respectively. Estimated parameter values for the 4 main models are given in Table 1.

For the two other datasets, the random destruction model again behaved the worst, followed by the homogeneous life-span model. In these cases, the optimal number of transit compartments was 10 for the HbA1c data, and 16 for the rHuEPO data. This is also illustrated in Fig. 1, which illustrates the OFV relative to the random destruction model. The combination of transit compartment model with random destruction process was also investigated for the rHuEPO data. In this case there was no marked improvement when the random destruction process was added as the ΔOFV value of the model with and without random destruction term was −0.243. The estimated rate constant for random destruction was also low, 0.000323/day.

Figure 4 shows the estimated mean life-span for models with different number of transit compartments. For the optimal transit compartment models the mean life-span values were: 91.8 days (12-compartment; biotin-labeled RBC in normal subjects), 75.4 days (16-compartment; Hb data in ESRD), and 87.5 days (10-compartment; HbA1c data in diabetic patients).

The impact of the model choice on individual predictions for long term outcomes was also investigated. In Fig. 5, the homogeneous life-span model would predict the fastest and the random destruction model the slowest improvement in Hb levels for an individual anemic patient based on Hb measurements on days 0 and 30 after start of rHuEPO therapy. In Fig. 6, HbA1c predictions are illustrated for the 12 transit compartment model and the random destruction model. In this case, it is shown how a 25 % decrease of FPG at baseline will translate to different HbA1c levels changes, depending on the life-span model considered.

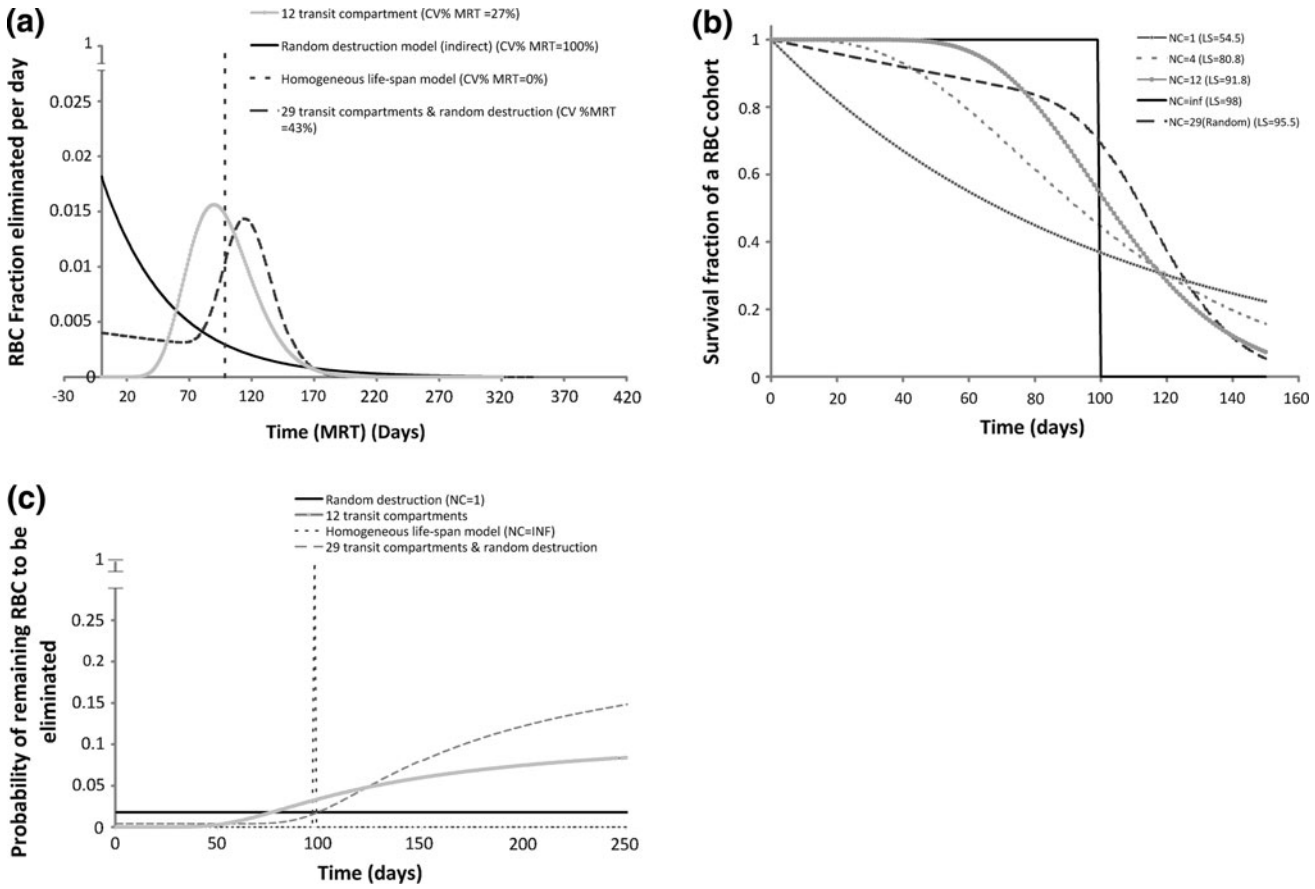

Fig. 2 **a** The daily eliminated RBC fraction from a RBC cohort formed at time zero as predicted by the different models for biotin-labeled data. **b** The survival fraction profiles of a RBC cohort versus time for models for the biotin-labeled data. **c** The probability of a remaining RBC to be eliminated during a day versus time for the different models

Discussion

Three published models for the RBC life-span are: the homogeneous life-span model (Uehlinger et al. [10], Kryzanski et al. [11], Perez-Ruixo et al. [14]), which considers an ageing process where all RBC die at the same time; the random destruction model (e.g. de Winter et al. [16]); and the 4 transit compartment model (Hamrén et al. [17]), which results in a distribution of RBC life-spans intermediate to the previous two models.

After the analysis of the biotin-labeled RBC survival curves from Cohen et al., with the afore mentioned models and some modified ones with varying number of transit compartments, a 12 transit compartment model reached the minimum OFV and showed a better predictive performance (Figs. 1, 3). The trend with regard to the best description of the data was as follows: 12-transit compartment model > 4-transit compartment model > homogeneous life-span model > random destruction model. This pattern was again observed when analysing the other two datasets. However, in these cases, 16 and 10 transit compartment models showed a better description of the rHuEPO and HbA1c data, respectively (Fig. 1).

The LS increase with increasing number of transit compartments is illustrated in Fig. 4. It is also observed that the mean LS obtained with the biotin labeled data and the HbA1c data are similar—91.8 and 87.5 days (with 12 and 10 transit compartments models, respectively)—whereas based on the rHuEPO data in uremic patients this is lower, 75.4 days (with a 10 transit compartment model). It is well known that the RBC LS in uremic patients is decreased, the mechanism for this is unknown but it may be related to the abnormal biochemical environment [29].

Transit compartment models with about 12 compartments or more assumes an increasing probability to be eliminated with increasing age and with an almost zero probability at the youngest ages (Fig. 2). The latter may not be true for patients with a substantial random destruction of RBC caused for instance by bleeding or hemolysis, where the elimination process is insensitive to the cell age. This hypothesis, of a combined ageing process with a random destruction one—occurring at any RBC age—was also

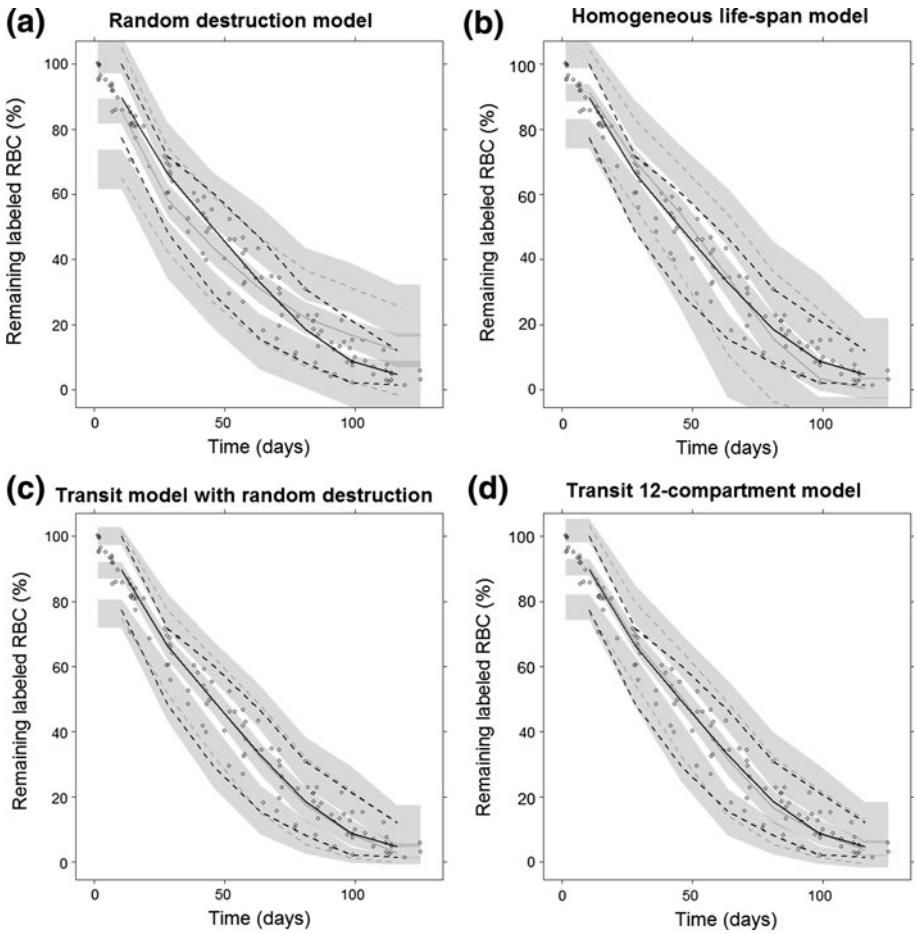

Fig. 3 Visual predictive checks for the most significant investigated models when analysing the biotin-labeled data are shown: **a** Random destruction model (OFV = 579.7); **b** Homogeneous life-span model (OFV = 517.0); **c** Transit compartment with random destruction (OFV = 250.7); **d** Transit 12-compartment model (OFV = 345.6). In each panel: the *dots* represent the observations, the *black line* represents the median of the observations and the *dashed-black lines* the 5th and 95th percentiles of the observed data. Whereas the *grey line* represents the median of the simulated data, the *grey-dashed lines* the 5th and 95th percentiles of simulated data, and the *grey area* the 90 % CI around the simulated median and prediction intervals

Table 1 Parameter values estimated with the life-span models assessed with the biotin-labeled dataset are shown (RSE %)

Parameter		Random destruction model	Homogeneous life-span model	12-Transit compartment model	29-Transit compartment model+ random destruction
OFV	Objective function	579.7	517.1	345.6	250.7
LS (days)	RBC life-span	54.5 (3.7)	98 (3.6)	91.8 (3.6)	95.5 (3.1)
IIV-LS (CV %)	Inter-individual variability on LS	11 ((28)	12.1 % (16)	12.4 (19)	10.7 (21)
krd (1/days)	1st order rate constant of random destruction	–	–	–	0.0053 (15)
IIV-krd (CV %)	Inter-individual variability on krd	–	–	–	47.3 (24)
Additive RE	Residual error (SD)	6.37 (6.7)	4.5 (9)	1.14 (14)	0.51 (18)
Proportional RE	Residual error (CV %)	(*)	(*)	3.96 (12)	2.76 (10)

assessed in the present study for the biotin-labeled and rHuEPO datasets. Transit compartment models with varying number of compartments were evaluated, where both life-span and random destruction parameters were estimated. A 29-transit compartment model with random destruction process best described the biotin-labeled data, as shown in Fig. 3 and Table 1. However, a relatively small difference with the 12-compartment model in terms of visual goodness-of-fit and therapeutically relevant prediction was observed. The average LS estimated with this model (95 days) was very similar to the corresponding one in the 12-transit compartment model without random destruction (91.8 days). While a relative improvement was shown for the biotin-labeled data, no significant improvement by considering the combination of random destruction process with senescense destruction patterns was shown for the rHuEPO data from Uehlinger et al.

Recently Korell et al. have published a survival model that describes the underlying distribution of RBC life-spans [30]. The model uses a human life-span inspired description of the

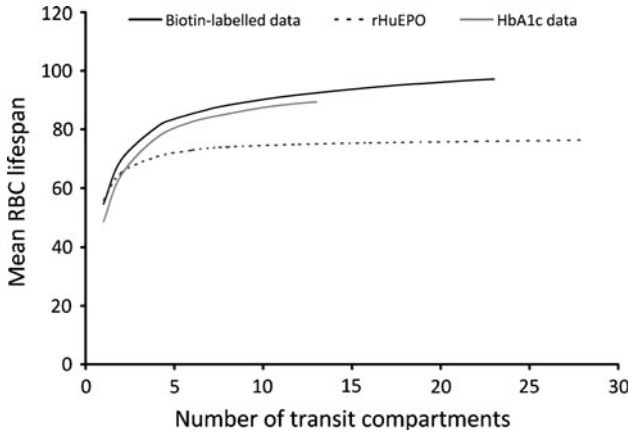

Fig. 4 It is shown how the estimated life-span (LS) increases with the number of transit compartments (NC) for the different models assessed with the different data sets

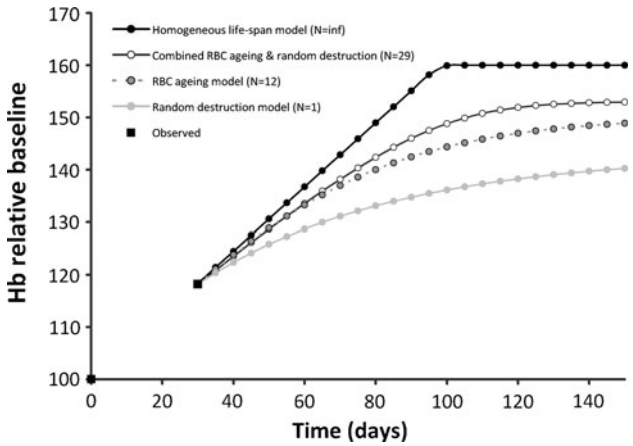

Fig. 5 Predicted Hb time-profiles for an individual patient receiving rHuEPO therapy with LS calculated based on observations at 0 and 30 days after starting treatment with rHuEPO assuming rHuEPO momentarily increases RBC production rate

underlying mechanisms affecting RBC life-span, as it uses a bath tub-shaped hazard curve, and incorporates several mechanisms of destruction of the RBC, including: senescence, random destruction, death due to initial or delayed failures and neocytolisis (early cell mortality). The model, originally derived from a survival analysis in an Indonesian population, was adjusted for the RBC setting and used for simulations of two different scenarios previously published in literature. One of the scenarios Korell et al. reproduce is the model we have derived herein based on the Biotin labeled data, of 29 compartment with random destruction. These authors show to predict a similar typical individual RBC survival profile to the one that our model predicts. However, their model fails to incorporate random effects accounting for inter-individual variability in a population, which is of interest if the model is intended to be used in the clinic for treatment adjustments. Finally, the same authors validate

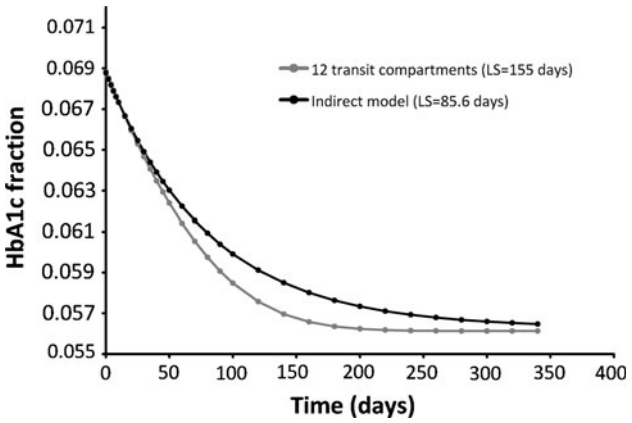

Fig. 6 HbA1c predictions for a hypothetical diabetic patient with an FPG baseline of 8.2 mmol/L are shown when an instantaneous 25 % decrease on FPG occurs at time zero, for the random destruction and the 12 transit compartment models

their model by describing data from a population of 12 patients with chronic kidney disease and 12 healthy subjects, where RBC survival had been measured by ^{51}Cr methodology [31]. Their model is able to describe the data, however not able to estimate all parameters related to destruction processes. When trying to estimate the parameters related to initial or delayed RBC destruction, they found not significant improvement, which yields to the same mechanisms of destruction that have been explored in the present work: senescense and random destruction. In addition, the model presented herein has been validated for three different populations, and describes the mechanisms in a semi-mechanistic manner, where compartments represent age stages of RBCs.

In the present work the RBC distribution was described by life-span models. Krzyzanski [32], has recently pointed out that under some circumstances the models used here and models described by life-span indirect response (LIDR) models are the same. Under some other considerations, results from transit compartment models can be similar. In the present situation, at least the HbA1c model would not have been possible to implement as an LIDR model. In other circumstances the LIDR models may offer advantages as more condensed model description and faster runtimes.

The current study illustrates that different types of data inform differently about the underlying processes involved. Based on the OFV functions values, one can see that the biotin-labeled data is most informative, followed by the rHuEPO data and the least informative HbA1c data. The ΔOFV between the worst and the best model was: 234.15 (biotin-labeled), 55.6 (Hb) and 147.2 (HbA1c). Taking into account the number of individuals in the analysis, the ΔOFV/individual was: 19.5 (biotin-labeled) $>$ 1.03 (rHuEPO) $>$ 0.14 (HbA1c). These values provide a measure of the information in an individual's data with respect to the life-span.

The aim of this study was not only to assess the model that best describes the different types of data, but to evaluate the impact of choosing a given model on predictions in the clinical setting. This is illustrated in Fig. 5 for a typical individual patient following rHuEpo therapy. How would the models predict Hb concentrations, if only observations at baseline and 30 days, after starting the treatment, were available. A considerable variation in predictions is observed, which points out the clinical relevance that a good description of the RBC kinetic mechanisms will have for decision making in regards to treatment adjustments.

Any mathematical model aiming at the prediction of the average HbA1c concentration must be able to account not only for the kinetics of the hemoglobin glycosylation, but also for RBCs formation and elimination processes [33]. It should also take into account the heterogeneity associated with the RBC survival between individuals, which has been shown to be large enough to cause clinically important differences in HbA1c levels for a given mean blood glucose concentration [20]. Again, the better description of these relationships can give answer to questions when changes of state occur, such as how long it will take for a change in HbA1c when initiating treatment. This is illustrated in Fig. 6, which depicts the impact that a 25 % decrease in FPG from baseline (8.2 mmol/L) would have on predicted HbA1c levels of a typical individual patient when implementing different life-span models. This demonstrates again the importance of identifying the appropriate model in order to predict individual changes in HbA1c levels under treatment.

The main conclusions of the present study can be summarised as follows: (1) predictions from non-steady state data will depend on the RBC model chosen. (2) the transit compartment model, which considers variation in life-span in the RBC population (intra-subject or inter-subject) better describes RBC survival data than the random destruction or homogenous life-span models, and (3) the additional incorporation of random destruction patterns although improving the description of the RBC survival data, does not appear to provide a marked improvement when describing clinical data.

Acknowledgments Rocio Lledo-Garcia was supported by a grant from Hoffmann-LaRoche.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Alfrey CP, Rice L, Udden MM, Driscoll TB (1997) Neocytolysis: physiological down-regulator of red-cell mass. Lancet 349(9062): 1389–1390
2. Testa U (2004) Apoptotic mechanisms in the control of erythropoiesis. Leukemia 18(7):1176–1199
3. Fisher JW (2003) Erythropoietin: physiology and pharmacology update. Exp Biol Med (Maywood) 228(1):1–14
4. Franco RS (2009) The measurement and importance of fed cell survival. Am J Hematol 84:109–114
5. Eadie GS, Brown IW Jr (1953) Red blood cell survival studies. Blood 8(12):1110–1136
6. de Lima JJ (1987) Survival equations for red blood cells with statistical distributions in life spans. Eur J Nucl Med 13(2):76–81
7. Kay MM (1984) Localization of senescent cell antigen on band 3. Proc Natl Acad Sci USA 81(18):5753–5757
8. Kay MM (1990) Senescent cell antigen, band 3, and band 3 mutations in cellular aging. Biomed Biochim Acta 49(2–3): S212–S217
9. Rettig MP, Low PS, Gimm JA, Mohandas N, Wang J, Christian JA (1999) Evaluation of biochemical changes during in vivo erythrocyte senescence in the dog. Blood 93(1):376–384
10. Uehlinger DE, Gotch FA, Sheiner LB (1992) A pharmacodynamic model of erythropoietin therapy for uremic anemia. Clin Pharmacol Ther 51(1):76–89
11. Krzyzanski W, Ramakrishnan R, Jusko WJ (1999) Basic pharmacodynamic models for agents that alter production of natural cells. J Pharmacokinet Biopharm 27(5):467–489
12. Krzyzanski W, Jusko WJ, Wacholtz MC, Minton N, Cheung WK (2005) Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after multiple subcutaneous doses in healthy subjects. Eur J Pharm Sci 26(3–4):295–306
13. Ramakrishnan R, Cheung WK, Wacholtz MC, Minton N, Jusko WJ (2004) Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after single and multiple doses in healthy volunteers. J Clin Pharmacol 44(9):991–1002
14. Perez-Ruixo JJ, Kimko HC, Chow AT, Piotrovsky V, Krzyzanski W, Jusko WJ (2005) Population cell life span models for effects of drugs following indirect mechanisms of action. J Pharmacokinet Pharmacodyn 32(5–6):767–793
15. Peterson CM, Jones RL, Koenig RJ, Melvin ET, Lehrman ML (1977) Reversible hematologic sequelae of diabetes mellitus. Ann Intern Med 86(4):425–429
16. de Winter W, DeJongh J, Post T, Ploeger B, Urquhart R, Moules I, Eckland D, Danhof M (2006) A mechanism-based disease progression model for comparison of long-term effects of pioglitazone, metformin and gliclazide on disease processes underlying Type 2 Diabetes Mellitus. J Pharmacokinet Pharmacodyn 33(3):313–343
17. Hamren B, Bjork E, Sunzel M, Karlsson M (2008) Models for plasma glucose, HbA1c, and hemoglobin interrelationships in patients with type 2 diabetes following tesaglitazar treatment. Clin Pharmacol Ther 84(2):228–235
18. Kalicki R, Lledó-García R, Karlsson M (2009) Modeling of red blood cell (RBC) Lifespan (LS) in a Hematologically normal population, in PAGE 2009. 2009: St Petersburg
19. Mock DM, Lankford GL, Widness JA, Burmeister LF, Kahn D, Strauss RG (1999) Measurement of red cell survival using biotin-labeled red cells: validation against 51Cr-labeled red cells. Transfusion 39(2):156–162
20. Cohen RM, Franco RS, Khera PK, Smith EP, Lindsell CJ, Ciraolo PJ, Palascak MB, Joiner CH (2008) Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. Blood 112(10):4284–4291
21. Mock DM, Lankford GL, Widness JA, Burmeister LF, Kahn D, Strauss RG (2004) RBCs labeled at two biotin densities permit simultaneous and repeated measurements of circulating RBC volume. Transfusion 44(3):431–437
22. Franco RS, Lohmann J, Silberstein EB, Mayfield-Pratt G, Palascak M, Nemeth TA, Joiner CH, Weiner M, Rucknagel DL (1998) Time-dependent changes in the density and hemoglobin F

content of biotin-labeled sickle cells. J Clin Invest 101(12):2730–2740
23. Franco RS, Yasin Z, Lohmann JM, Palascak MB, Nemeth TA, Weiner M, Joiner CH, Rucknagel DL (2000) The survival characteristics of dense sickle cells. Blood 96(10):3610–3617
24. Franco RS, Yasin Z, Palascak MB, Ciraolo P, Joiner CH, Rucknagel DL (2006) The effect of fetal hemoglobin on the survival characteristics of sickle cells. Blood 108(3):1073–1076
25. Ribbing J, Hamren B, Svensson MK, Karlsson MO (2010) A model for glucose, insulin and beta-cell dynamics in participants with insulin resistance and patients with type 2 diabetes. J Clin Pharmacol 50(8):861–872
26. Purves RD (1994) Numerical estimation of the noncompartmental pharmacokinetic parameters variance and coefficient of variation of residence times. J Pharm Sci 83(2):202–205
27. Karlsson MO, Holford NH (2008) Tutorial on visual predictive checks. http://www.page-meeting.org/?abstract=1434, in PAGE meeting. 2008: Marseille
28. Jonsson EN, Karlsson MO (1999) Xpose–an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed 58(1):51–64
29. Giovanetti S, Balestri P, Cioni L (1965) Spontaneous in vitro autohemolysis of blood from chronic uremic patients. Clin Sci 29:407–416
30. Korell J, Coulter CV, Duffull SB (2011) A statistical model for red blood cell survival. J Theor Biol 268(1):39–49
31. Korell J, Vos FE, Coulter CV, Schollum JB, Walker RJ, Duffull SB (2011) Modeling red blood cell survival data. J Pharmacokinet Pharmacodyn 38(6):787–801
32. Krzyzanski W (2011) Interpretation of transit compartments pharmacodynamic models as lifespan based indirect response models. J Pharmacokinet Pharmacodyn 38(2):179–204
33. Ladyzynski P, Wojcicki JM, Bak M, Sabalinska S, Kawiak J, Foltynski P, Krzymien J, Karnafel W (2008) Validation of hemoglobin glycation models using glycemia monitoring in vivo and culturing of erythrocytes in vitro. Ann Biomed Eng 36(7):1188–1202