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Can we predict chemo-induced hematotoxicity in elderly patients treated with pegylated liposomal doxorubicin? Results of a population-based model derived from the DOGMES phase II trial of the GINECO

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ARTICLE INFO

Article history:
Received 26 December 2011
Received in revised form 31 May 2012
Accepted 27 June 2012
Available online 3 August 2012

Keywords:
Modeling
Pharmacodynamics
Age-related toxicity

ABSTRACT

Introduction: Use of anthracyclines is often limited in older patients due to cardiac and hematologic toxicities. Thanks to its reduced toxicity profile, Pegylated Liposomal Doxorubicin (PLD) allows an extended use of doxorubicin to this population. We aimed at modeling PLD-induced hematotoxicity in patients with metastatic breast cancer ≥ 70 years old and at finding predictive factors of neutrophil nadir value.

Methods: Sixty patients, enrolled in the DOGMES prospective multicentric phase II trial, were treated with PLD at 40 mg/m^2 every 28 days during six cycles. Trial design included geriatric covariates assessment at inclusion and monitoring of cells count every week for three cycles. A population model was developed to describe hematopoiesis and hematopoietic reserve in these patients. The effect of co-administered G-CSF (granulocyte colony-stimulating factor) was also examined.

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1879-4068/$ – see front matter
doi:10.1016/j.jgo.2012.06.004

Frailty
Hematotoxicity
PLD

Results: A pharmacodynamic model was built using data from 53 patients not receiving G-CSF. This model assumed an instantaneous effect of PLD on the system. Based on this model, exact neutrophil nadir value was computed and ranged between 0.069 K/mm^3 and 2.63 K/mm^3 confirming the weak hematotoxicity of PLD. The same model was then applied to the 7 patients receiving G-CSF and showed that basal neutrophil count was higher for these patients. No other difference was found between both cohorts. Among the covariates collected, three were predictive of neutrophil nadir value: diabetes, frailty syndrome and assistance at home.

Conclusion: This developed model allowed the identification of predictive factors of nadir ANC and the identification of patients that are more likely to develop hematotoxicity that should be monitored with attention.

1. Introduction

Breast cancer is the first cause of cancer mortality in women worldwide. Nowadays, more than 50% of breast cancers are diagnosed in women age 65 or older and cancer-related mortality rate is threefold higher in this population. Due to the increase in life expectancy, a rise in cancer incidence and mortality is expected in the next decades.[1,2] Despite an improvement in breast cancer care and overall survival, these improvements did not benefit patients over 65 because of a trend towards under-treatment in the elderly and the lack of specific recommendations.[3] It is thus necessary to characterize the age specificity of breast cancer and understand the risks and benefits of treatment in older adults.[4,5]

Anthracyclines play an important role in the management of breast cancer. Due to their cardiac toxicity, they are usually administered with caution to elderly patients and often at adapted dose. A cumulated doxorubicin dose greater than 400 mg/m^2 significantly increases the risk of congestive heart failure for patients older than 65.[6]

Toxicity remains a dose-limiting parameter in clinical trials evaluating anthracyclines in geriatric populations. As compared to the CMF (cyclophosphamide, methotrexate, fluorouracil) protocol, adjuvant anthracyclines induced more grade 3 or 4 toxic events, hospitalizations due to febrile neutropenia according to the Memorial Sloan–Kettering records.[7] Nevertheless, no official recommendation exists to adapt doxorubicin dose with age even if doses lower than 50 mg/m^2 are often preferred in elderly patients.[8] Development of liposomal formulation associated with lower risk of cardiotoxicity allows extending the use of doxorubicin to the geriatric population.

Doxil® is a pegylated liposomal formulation of doxorubicin (PLD): the active substance is encapsulated in liposomal vesicles, which preferentially penetrates tumor tissues, thus reducing plasma concentrations of doxorubicin and its active metabolite, doxorubicinol. Comparisons between standard doxorubicin and PLD have shown that PLD was better tolerated with an equivalent efficacy even though hand-and-foot syndrome was more frequent with PLD.[9,10]

Nowadays the adaptation of medical and surgical care in the elderly is often a matter of debate. Among the leading questions is the better assessment of their individual vulnerability and toxicity risk prediction.[11]

Physiological modifications and comorbidities have a non-negligible influence on drug pharmacokinetics (PK) and pharmacodynamics (PD), translated into an increased inter-individual variability within the geriatric population. Moreover previous studies have shown a progressive decrease of neutrophil nadir upon time in patients over 65 treated with 4 cycles of doxorubicin–cyclophosphamide, whereas it is maintained over time in a younger population.[12] This phenomenon is hypothesized to illustrate the exhaustion of hematopoietic reserve in the elderly.[12]

Hematotoxicity is often dose-limiting[13] and international guidelines consider age as a vulnerability criterion to be taken into account for hematopoietic growth factor use without any insight into hematopoiesis dynamics.[14–16]

The prospective DOGMES phase II study aimed at evaluating response and tolerance of PLD in patients with metastatic breast cancer aged over 70. The impact of age, comorbidities and geriatric covariates on PLD hematotoxicity was also explored.

In this paper, we propose a population pharmacodynamic modeling approach to describe the hematopoietic reserve, hematopoiesis and neutropenic effects of PLD in elderly patients. This model has been used to correlate the risk of neutropenia to patients' characteristics.

2. Patients and Methods

2.1. *Patients*

Sixty patients with metastatic breast cancer over 70 years old were enrolled in the "Doxorubicine liposomale pégylée en Oncologie Gériatrique — Metastases du cancer du Sein" (DOGMES) prospective multicentric phase II study. The main objective of the DOGMES study was to evaluate, in an elderly population, PLD efficacy in terms of objective response.[17] The main eligibility criteria were histologically proven HER2/neu negative invasive breast adenocarcinoma (ductal or lobular) and cytologically or histologically proven first line hormone-resistant metastatic disease at the time of study entry. Details and clinical results of the DOGMES study are reported elsewhere.[18]

Patients received PLD at 40 mg/m^2 every 28 days for at least 6 cycles. Absolute neutrophil count (ANC) was monitored at inclusion and every week for three cycles. In total sixty-six covariates (demographic, biologic, oncologic and geriatric) were assessed at inclusion. Geriatric assessment domains included comorbidities, nutritional assessment, functional assessment, cognitive status, psychological states and autonomy.

2.2. Data Analysis

2.2.1. Model Development

Semi-mechanistic myelosuppression models have been proposed previously. The one we based our analysis on consists of a proliferation compartment representing stem cells and proliferative precursor cells in the bone marrow, proliferating at the rate constant k_{prol} (Fig. 1).[19] Cells transit then to the blood circulation through three transit compartments mimicking the maturation process in the bone marrow in a mean maturation time (MMT). Transfer rate (K_{tr}) is constant along the transit chain and is defined as $K_{tr} = (n+1)/MMT$, where n is the number of transit compartments. K_{circ} represents the rate of neutrophil entry into the tissue. A feedback mechanism describes the regulation of the hematological system by endogenous growth factors (i.e. G-CSF) and is modeled as the ratio of ANC in the blood compartment at baseline and at time t, powered to a feedback factor Υ. Production of cells in the proliferation compartment is increased when cell count is below baseline level, mimicking the rebound effect, and is decreased when above baseline level.

This model includes five system-related parameters describing both bone marrow compartment and peripheral blood compartment independent of the cytotoxic drug effect: k_{prol}; MMT, mean maturation time of non-proliferative cell in bone marrow; Υ, feedback on the proliferation rate from the circulating cell count; and K_{circ}. Cytotoxic drugs predominantly affect mitotic cells. Drug effect was implemented as decreasing the proliferation rate (k_{prol}). Resulting k_{prol} could thus be negative, corresponding to a net elimination of cells from the proliferation compartment.

Three different drug effect models were tested:

- "Constant drug effect" that does not integrate drug concentration kinetics but a constant drug effect.
- "K-PD linear drug effect model" that does integrate the drug effect kinetics[20] and describes the relationship between the drug concentrations and effect with a linear model.
- "K-PD E_{max} drug effect model" that does integrate the drug effect kinetics[20] and describes the relationship between the drug concentrations and effect with an E_{max} model.

Model building and evaluation are presented in Appendix 1.

Inter-individual variability (IIV) and Inter-occasion variability (IOV) were explored on all parameters and were described by log-normal distributions. The residual variability was described with an additive error model (constant variance).

2.3. Prediction of ANC Nadir Values

Final model structure and empirical Bayes estimates (EBE, i.e. individual parameter values) were used to simulate the ANC profile for each individual. The nadir value was estimated directly from the ANC profile. Sixty-six covariates were tested for a potential relationship with nadir. Relationships between categorical covariates and nadir values were assessed using the non-parametric Mann–Whitney test. Continuous covariate relationships were tested using a linear regression. Covariate effect was judged as significant if associated to a p-value below 0.05.

2.4. Effect of Recombinant G-CSF Co-Administration

Patients receiving recombinant G-CSF were analyzed separately, using the same model structure. To evaluate the effect of recombinant G-CSF, system-related parameter estimates from both cohorts were compared using z-tests. Parameter estimates were considered as different if the associated p-value was lower than 0.05.

3. Results

3.1. Patients

Sixty patients were enrolled in the DOGMES study. Fifty-three patients did not receive recombinant G-CSF and were considered for model development. Seven patients received recombinant G-CSF for several cycles during the study. These patients were analyzed separately and compared to the former subgroup. Patients' baseline characteristics are presented in Table 1. Twelve ANC observed values per patient were available.

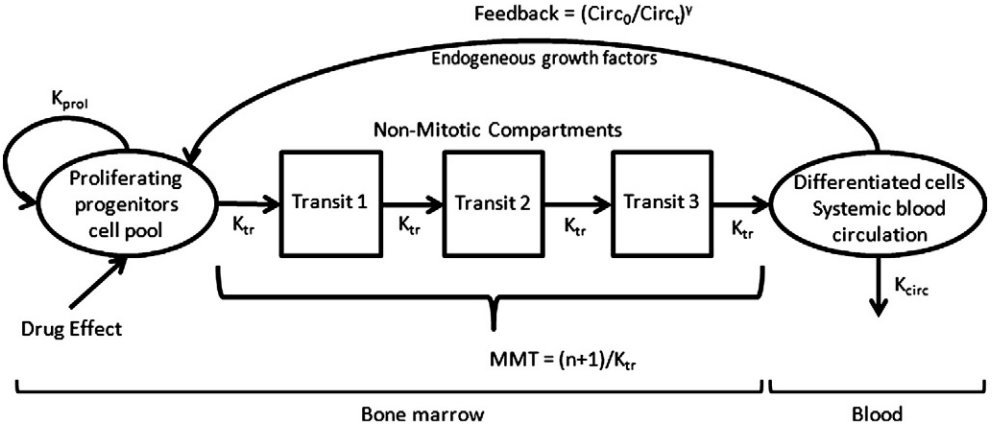

Fig. 1 – Scheme of the PLD induced myelosuppression model. Schematic representation of the myelosuppression model by Friberg et al.[19] Model parameters are: baseline neutrophil count (ANC_0), mean maturation time ($MMT = (n+1)/K_{tr}$), feedback parameter on neutrophils proliferation (γ), rate of neutrophils entry into tissues (K_{circ}) and drug related parameters (Drug Effect).

Table 1 – Patients' baseline characteristics.

Number of patients	Patients not receiving recombinant G-CSF		Patients receiving recombinant G-CSF	
	53		7	
	Median	Range	Median	Range
Age (years)	77	70–88	80	72–85
Weight (kg)	64.5	43–100	63	47–75
Height (cm)	160	143–170	157	154–165
BSA (m^2)	1.70	1.31–2.04	1.65	1.43–1.82
ADL	6	2.5–6	6	5–6
IADL	24	13–27	25	11–27
	No. of patients	Relative frequency (%)	No. of patients	Relative frequency (%)
Performance status [a]				
0	9	16.9%	2	28.6%
1	37	69.9%	3	42.8%
2	6	11.4%	2	28.6%
3	1	1.8%	–	–
Number of concomitant treatments				
0–3	18	33.9%	2	28.6%
4–6	17	32.2%	3	42.8%
>6	18	33.9%	2	28.6%
Frailty syndrome				
No syndrome	42	79.2%	1	14.3%
Pre-syndrome+syndrome	11	20.8%	6	85.7%
Diabetes [b]				
0	47	88.6%	6	85.7%
1	6	11.4%	1	14.3%
Normal ECG	36	67.9%	6	85.7%
Hormone status at initial diagnosis				
HR+ (ER+ PR+)	34	64.2%	5	71.4%
ER– PR+ or ER+ PR–	12	22.6%	1	14.3%
HR–	5	9.4%	1	14.3%
Unknown	2	3.8%	–	–
HER2 status at study entry				
Positive	–		–	
Negative	39	73.6%	7	100%
Unknown	14	26.4%	–	–
Prior treatment				
Yes	51	96.2%	6	85.7%
No	2	3.8%	1	14.3%
Time to metastatic disease				
<5 years	28	52.8%	0	0%
>5 years	25	47.2%	7	100%
Metastatic site involvement				
Breast	3	5.6%	1	14.3%
Bone	37	69.8%	4	57.1%
Lymph nodes	20	37.7%	3	42.9%
Visceral	45	84.9%	5	71.4%
Other	18	34%	2	28.6%

HR: hormone receptor, ER: estrogen receptor, PR: progesterone receptor.
Other: presternal, retro-ocular, sub-cutaneous, pre-pectoral, mediastinum, kidney, subcoronary, pelvis, pericardium.
[a] Performance status according to the Eastern Cooperative Oncology Group.
[b] Type I and type II diabetes, 0: presence, 1: absence.

3.2. *Myelosuppression Model for ANC*

From the three drug effect models described in the Patients and Methods section, the "constant drug effect" model has been chosen on the basis of objective function value (OFV), Akaike criterion and goodness-of-fit (GOF) plots (detailed in Appendix 1). The two other K-PD models did not show any improvement in terms of OFV nor GOF. According to the parsimony principle, the simpler model was chosen. Moreover, bootstrap analysis for both K-PD models showed that drug-related parameters (i.e. E_{max}, EC_{50} or slope) were associated to infinite confidence intervals and were thus not estimable.

Since only one dose level was tested in this population and no ANC profile was reported while off treatment, the proliferation constant and the drug effect were not distinguishable. A common parameter k_{pool} was thus estimated, representing the progenitor proliferation rate in cell pool resulting from hematopoiesis and PLD action.

Inter-individual variability (IIV) was added on four parameters: $Circ_0$, MMT, k_{pool} and Υ and Inter-Occasion Variability (IOV) on MMT.

Parameter estimates for patients not receiving G-CSF of the myelosuppression model are presented in Table 2. GOF plots are shown in Fig. 2a.

K_{circ} was estimated at 0.39 day^{-1} corresponding to a half-life of 1.78 days. Mean maturation time (MMT) was estimated at 9.17 days with an IIV estimated at 5.7% and an IOV at 6.3%. Resulting proliferation constant k_{pool} was estimated at 0.34 day^{-1} corresponding to a proliferation half-life of 2 days with an IIV of 6.1%.

All typical parameters were estimated with a good precision (relative standard error (RSE) < 10%). RSE was larger for variability parameters.

3.3. *Comparison with Patients Receiving Recombinant G-CSF*

The myelosuppression model presented above was re-estimated for the seven patients receiving recombinant G-CSF. Parameter estimates in this subgroup are presented in Table 3.

Table 2 – Parameter estimates (patients not receiving G-CSF).

Parameters	Typical mean value IIV (%CV), IOV (%CV)	% Relative standard error
$Circ_0$ (10^9/L)	4.7	4.2%
	28.8%, –	19.6%, –
MMT (d)	9.2	2.6%
	5.7%, 6.3%	72%, 48%
k_{pool} (day^{-1})	0.34	8.9%
	6.1%, –	80.6%, –
K_{circ} (day^{-1})	0.39	2.8%
	–, –	–, –
Υ	0.70	8.1%
	11.1%, –	56%, –

IIV: inter-individual variability.
IOV: inter-occasion variability.
%CV: coefficient of variation (%).
"–": no variability.

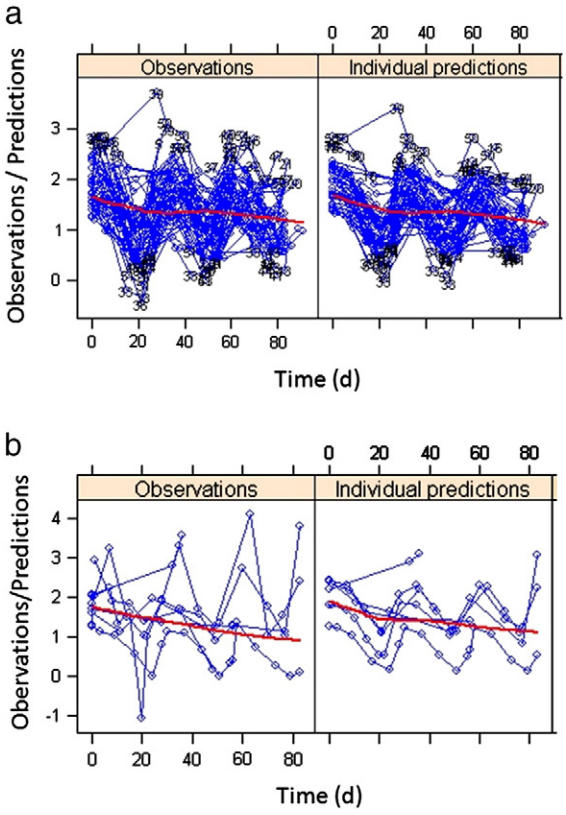

Fig. 2 – Basic goodness of fit plots. Basic goodness of fit plots in a) patients not receiving G-CSF (N = 53) and b) patients receiving G-CSF (N = 7) compare the observed (left) to predicted (center, right) absolute neutrophil counts (10^9/L) on the Box-Cox scale. Absolute neutrophil counts (ANC) are shown at each time point (points connected with lines) for the population prediction (right), the individual prediction (center) and the observations (left). The median ANC over time is shown in each panel (heavy red line).

Due to the low number of patients, IIV was only estimable on two parameters: $Circ_0$ and k_{pool}. GOF plots are shown in Fig. 2b: the model is able to well describe the data observed in this subgroup (cf. Model Evaluation section). In order to characterize the action of recombinant G-CSF, system-related parameters were compared. Baseline ANC ($Circ_0$) was greater (5.9 versus 4.74, p-value = 0.004) in patients receiving G-CSF.

3.4. *Model Evaluation*

Basic diagnostic plots helped evaluate the quality of the model: the model could adequately describe the data. The goodness-of-fit plots showed that the model predictions at the individual level were able to mimic the observed profiles over time for both cohorts (Fig. 2a and b). As expected the normalized prediction distribution errors (NPDEs) were normally distributed, centered around 0 with a variance of 1, without trend with time (not shown).

Table 3 – Parameter estimates (patients receiving G-CSF).

Parameters	Typical mean value IIV (%CV), IOV (%CV)	% Relative standard error
$Circ_0$ (10^9/L)	5.6 35.9%, –	13.9% 53.7%, –
MMT (d)	9.1 –, –	13.7% –, –
k_{pool} (day^{-1})	0.30 4.4%, –	21.2% 76.2%, –
K_{circ} (day^{-1})	0.35 –, –	28.1% –, –
Υ	0.85 –, –	24.2% –, –

IIV: inter-individual variability.
IOV: inter-occasion variability.
%CV: coefficient of variation (%).
"–": no variability.

The visual predictive check (VPC) showed a good predictive performance of the model in both cohorts (Fig. 3a and b). The 90% non-parametric confidence intervals of the 2.5th, 50th and 97.5th simulated percentiles over time were plotted together with the corresponding observed ones. The observed percentiles were enclosed within the 90% CI as required for model validation.

Thus the model was able to describe ANC profiles adequately in both cohorts.

3.5. *Predictive Factors for ANC Nadir Values*

Few severe neutropenic events were observed in the DOGMES patients, treated with PLD: observed nadir values (at observation time, i.e. once a week) ranged between 0.6 and 7.2 K/mm^3, corresponding to 26% grade I, 25% grade II, 6% grade III, 0% grade IV. No significant relationship was found between covariates and observed nadir values. Based on final model structure and individual parameter values, model-based ANC nadir values for each cycle and patient were calculated. ANC nadir values ranged between 0.096 K/mm^3 and 2.63 K/mm^3, corresponding to 32% grade I, 40% grade II, 13% grade III and 9% grade IV. The median time spent in grade 2 neutropenia was estimated at 4 days [1.1–4.8].

Among the 66 tested covariates for potential relationship with ANC nadir, 3 were significantly correlated with the individual

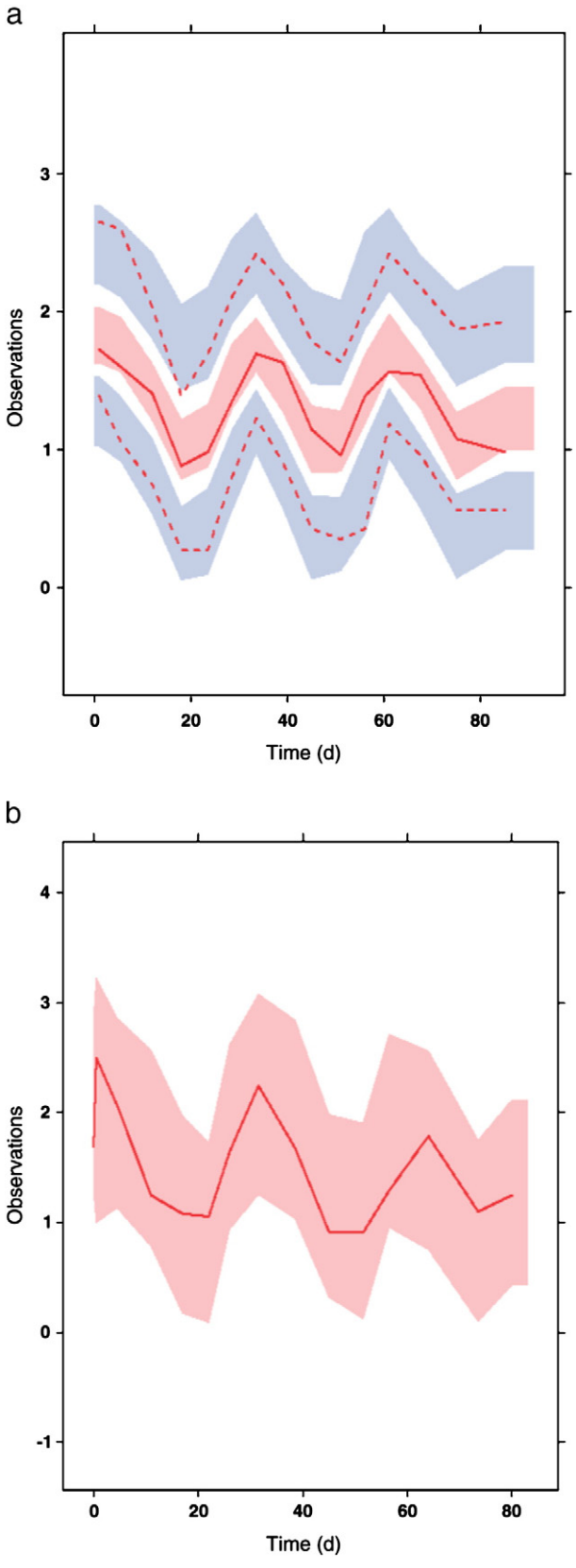

Fig. 3 – a) Visual predictive check for patients not receiving G-CSF. Visual predictive check (VPC) plots for patients not receiving G-CSF comparing the 90% non-parametric confidence interval of median (pink area), 2.5th (bottom blue area) and 97.5th (top blue area) percentiles of absolute neutrophil count on the Box-Cox scale obtained after simulations to the observed ones (median: heavy red line, 2.5th percentile: bottom dashed red line, 97.5th percentile: top dashed red line). b) Visual predictive check for patients receiving G-CSF. Visual predictive check (VPC) plot for patients receiving G-CSF comparing the 90% non-parametric confidence interval of median (pink area) of absolute neutrophil count on the Box-Cox scale obtained after simulations to the observed one (heavy red line).

ANC nadir values: diabetes, assistance at home and frailty syndrome. To identify patients with frailty syndrome, 5 items were analyzed based on the criteria on the Women's Health and Aging study 1 (WHAS-1).[3]

Diabetic patients (type I/II) were found to have higher ANC nadir values (p-value<0.0001, Fig. 4). Pre-frail and frail patients were found to have lower ANC nadir values compared to non-frail patients (p-value=0.065, Fig. 4). Despite a p-value at the significance limit, frailty was included due to its clinical relevance. Finally, patients needing assistance at home were found to have higher nadir values (p-value=0.037, Fig. 4).

A linear regression was performed to compute the percentage of variability explained by these predictive factors. Taken together, 18.5% of the variability in ANC nadir values can be explained by diabetes, frailty syndrome and assistance at home.

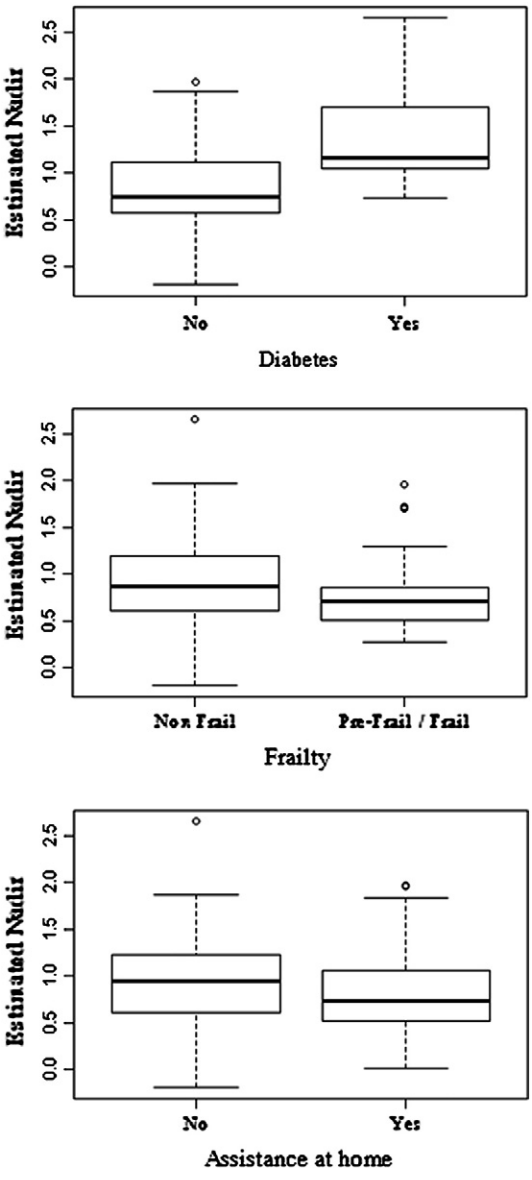

Fig. 4 – Relationship between neutrophil nadir value and covariates. Effect of covariates on estimated neutrophil nadir value is presented. Each boxplot shows the range, the 5th, 95th and the median (heavy horizontal line) of nadir value in each group for each covariate.

4. Discussion

Neutropenia remains one of the major – and often dose-limiting – toxicities with anticancer chemotherapies. It is even more critical to control the evolution of ANC in elderly patients with cancer who are generally frailer and subject to comorbidities.

We propose a dynamic model for the neutrophil count evolution in patients over 70, treated with pegylated liposomal doxorubicin (PLD). Data were issued from the DOGMES clinical study which aimed at evaluating response and tolerance to this treatment in patients with metastatic breast cancer over 70.

The model we propose takes into account the physiological processes of neutrophil maturation in the bone marrow, the circulation to blood, the differentiation system- and drug-related parameters. Similar models for ANC were already proposed with various treatments: Kloft et al. reviewed the different kinds of models proposed in the literature for ANC kinetics[21]: empirical models,[22,23] semi-mechanistic models,[24–26] and physiology-based platform models consistent across drugs.[19] To our knowledge, this is the first ANC model built in a geriatric population.

All the parameters were estimated in our analysis, including system-related parameters. In a previously reported model in adults,[27] neutrophil half-life in blood was fixed at 7 days, whereas it was estimated to be 1.78 days in our geriatric population. This unexpected difference could be explained by immune-senescence (i.e. coexistence of inflammation and immunodeficiency) and chronic inflammation (inflamm-aging) which are associated with an increased level of pro-inflammatory cytokines leading to a decline of the immunological function in geriatric patients.[28] Meanwhile mean maturation time of neutrophils was estimated to be 9.17 days in elderly patients, and reported to be 6.75 days by Quartino et al. Those parameters are in agreement with the longer recuperation period and lower ANC levels, expected in geriatric populations.

Given the data and the study design, the proliferation constant and the drug effect could not be distinguished. To be able to separate both components, ANC profiles should have been observed off treatment and under a wide range of PLD dose levels. The model in its current form cannot be used directly to extrapolate ANC profiles to different drug regimens. Nevertheless, using the model, sixty-six covariates were tested for potential relationships with the neutrophil nadir values.

Diabetic patients were found to have higher nadir values. With lipoprotein levels being affected by diabetes, the disposition of liposomes may be modified as shown earlier for liposomal amphotericin B.[29] Our results are compatible with PLD entering preferably into tissues leading to lower levels of circulating drugs and lower exposure in the bone marrow.

The frailty syndrome was found to induce lower nadir values. Pre-frail and frail patients are thus more sensitive to the hematotoxic action of the drug in accordance with the

definition of frailty as a loss of functional reserve. Frailty was evaluated on the basis of the criteria of the Woman's Health and Aging Study 1.[3] Age has also been tested for potential predictive value, but did not reveal any significant relationship. This tends to confirm that the chronological age is not adapted to geriatric patients whereas other indicators, such as the Comprehensive Geriatric Assessment[30,31] or the frailty syndrome[32] correlate better with the real physiological/biological status.

Assistance at home is thought to be confounded with the others factors and no clear clinical explanation could be found for this factor.

Interestingly, no significant relationship was found between nadir value and albumin level, renal function or hepatocyte function. These three factors were usually used to drive the dose adaptation. Our analysis reveals that geriatric covariates and comorbidities were more relevant for PLD dose adaptation in geriatric patients. This is in line with pharmacokinetic PLD analysis from Bononi et al. where geriatric covariates were significantly associated with PLD disposition.[33]

In the DOGMES study, 7 patients received recombinant G-CSF. Model parameters were separately estimated in this cohort, and were compared to the former. Significant difference has been found on baseline ANC parameter and not on the other system related parameters. Recombinant G-CSF is expected to accelerate the neutrophil maturation process, thus increasing the number of circulating neutrophil and shortening the recuperation period. Nevertheless, due to the unbalanced number of individuals in each subgroup (53/7) and the very few patients receiving G-CSF, the parameter comparison should be considered with caution. In order to evaluate the effect of G-CSF on system-related parameters, data from a controlled randomized study where PLD is administered alone or in combination with G-CSF in elderly patients should be modeled. Quartino et al.[34] modeled simultaneously ANC and endogenous/recombinant G-CSF. In this model, the feedback function on ANC proliferation was dependent on the G-CSF levels (instead of ANC levels) and the mean maturation time was shortened with higher G-CSF concentrations. We could not apply a similar approach to the DOGMES data because of the few numbers of patients receiving G-CSF and the absence of G-CSF concentration measurements.

Observed nadir values could not be successfully correlated to any covariates. Our modeling approach lets us predict the "true" nadir value, which is correlated to frailty syndrome, diabetes and assistance at home. In clinical practice, it is not possible to obtain this value since an infinite number of samples per patients would be necessary. In silico approach allows one to virtually increase the number of samples per patients to obtain this value.

No study aimed to compare conventional doxorubicin in a geriatric population versus a non-geriatric population. Despite the lack of direct comparison of doxorubicin and PLD in the elderly, PLD appears to have manageable hematotoxicity in the elderly at the adapted dose of 40 mg/m^2.

Using a population modeling approach, we were able to describe ANC dynamics in geriatric breast cancer treated with PLD and to find clinical relevant predictive factors of the ANC nadir. Population approach allows extending recruitment of geriatric patients in clinical trials since sparse study design requiring less patients and less samples per patients is possible. Nevertheless, to take maximum advantages of this approach and in the context of a study based only on the geriatric population, it is necessary to include heterogeneous patients and patients treated with different doses to increase predictive properties of the model.

Our model-based analysis allowed the identification of predictive factors of the nadir ANC value: frail and diabetic patients are more at risk of severe neutropenia, and should be monitored with attention.

Disclosures and Conflict of Interest Statements

None of the authors have any financial conflict of interest to declare with respect to this manuscript.

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Appendix 1

1.1. *Population Modeling*

Data were analyzed using non-linear mixed effects modeling, so called "population" approach.[35] The term "mixed effects" refers to "fixed" and "random" effects that are simultaneously estimated in the model. Fixed effects correspond to typical values (mean) of each parameter P within a population. Random effects are estimated on three different levels: between subjects (inter-individual variability, IIV), between occasions defined as treatment cycles within a subject (inter-occasion variability, IOV), and within a subject (residual variability, RV).

Each observation for an individual can be expressed as follows:

$$y_{ikj} = f(X_{ikj}, P_{ik}) + \varepsilon_{ikj} \quad (1)$$

where y_{ikj} is the jth observation on kth occasion for the ith individual, f(…) is the individual prediction described by the variables X_{ikj} and parameters of individual i at occasion k, P_{ik}; and ε_{ikj} is the residual error defined as the difference between the individual prediction and the corresponding observed value. Residual errors are expected to be normally distributed with a 0 mean and a σ^2 variance.

Individual parameters on each occasion P_{ik}, are expressed as a function of the typical value for the population (θ) and the deviation from this typical value for a given individual at a given occasion. A log-normal distribution of individual parameters is commonly used:

$$P_{ik} = \theta \times e^{\eta_i} \times e^{\kappa_{ik}} \quad (2)$$

where θ is the typical value of parameter P, η_i is the random parameter that quantifies the difference between the population and the ith individual; κ_{ik} is the random parameter that describes the variability at occasion k within individual i. The η_i and κ_{ik} values are expected to be normally distributed with a 0 mean and ω^2 and μ^2 variance respectively.

1.2. *Model building and evaluation*

Absolute Neutrophil Count (ANC) data were Box-Cox transformed as follows:

$$ANC_{BOX-COX} = \frac{ANC^0 \cdot 2 - 1}{0} \cdot 2 \quad (3)$$

The value of the power factor (0.2) has been established previously and allows the obtainment of centered and normally distributed Box-Cox transformed weighted residuals [(22)].

Model parameters were estimated using the First Order Conditional Estimates method with Interaction (FOCEI) [(36)] implemented in NONMEM 7 software [(37)].

Model development was guided by the Objective Function Value (OFV), the criterion to be minimized by NONMEM; precision of parameter estimates; and goodness of fit (GOF) plots. OFV was used to differentiate between two nested models using a log-likelihood ratio test. Precision of parameter estimates was computed from the NONMEM covariance step and was expressed in terms of percentage of Relative Standard Error (%RSE). The XPOSE4 program was used for graphical evaluation of the model fit [(38)].

Simulation-based diagnostics were performed to check model predictive performance. Two diagnostic tools were used: Normalized Predictive Distribution Errors (NPDE) and Visual Predictive Checks (VPC). NPDEs should be following a centered and reduced normal distribution. The model was considered as invalid when NPDEs were not normally distributed with a 0 mean and variance of 1 [(39)]. VPC consists of simulating ANC profiles in virtual patients, given model structure and parameter estimates. We simulated 1000 replications of the original data set; the median and the 95% prediction interval (PI), for each time bin, were calculated for each replicate. The 90% confidence interval (CI) of simulation medians, 2.5th and 97.5th percentiles were computed and compared to the original data set [(40)]. The model was considered as invalid when the 90% non-parametric confidence interval does not include the observed quantiles. PsN toolkit was used for the execution of simulations and VPC calculation [(41)].

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