Thesis

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Title: Imatinib pharmacokinetic/pharmacodynamic analysis in COVID-19 patients

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Doel

In de gerandomiseerde, dubbelblinde en placebogecontroleerde (CounterCOVID) studie bleek imatinib een reductie in de mortaliteit en behandelduur van mechanische ventilatie bij patiënten met ernstige COVID-19 infectie te geven. (1) Het doel van deze huidige studie was om de farmacokinetiek (PK) en farmacodynamiek (PD) bij de COVID-19 patiënten, die met imatinib behandeld zijn in de CounterCOVID studie, te bestuderen.

Methode

COVID-19 patiënten van de CounterCOVID studie met minstens één PK-monster waren in deze huidige studie geïncludeerd. Deze patiënten hadden een oplaaddosis van 800 milligram (mg) orale imatinib gekregen met vervolgens gedurende negen dagen eenmaal daags 400 mg orale imatinib. Met behulp van nonlinear mixed-effects modelling en een voorheen gepubliceerd semi-mechanistisch eiwitbinding PK-model werd de grootte van blootstelling aan imatinib geschat. (2) De individuele PK-profielen zijn beschreven in totale en ongebonden oppervlak onder de plasmaconcentratie-tijdcurve (AUC), de dalspiegels (C_{trough}) gedurende steady state (C_{ss}) en de ongebonden maximum concentratie (C_{max}).
De bestudeerde PD-uitkomsten zijn de verandering in de P/F-ratio, het primair eindpunt van de CounterCOVID studie (1), tijd tot bevrijding van zuurstofsuppletie en beademing langer dan 48 uur en levend, en mortaliteit (binnen 90 dagen). Verder is bestudeerd of de parameters leeftijd, geslacht, alfa-1-zuur glycoproteïne (AAG), roken, comorbiditeiten, behandeling met dexamethason en ernst van de ziekte confounders of effect modifiers zijn. De continue data is met behulp van lineaire regressie geanalyseerd en ANOVA testen zijn gebruikt voor de categorische data. Verder zijn tijd-tot event analyses door middel van cox regressie en Kaplan-Meier, correlatie- en distributiegrafieken en univariabele- en multivariabele regressie toegepast voor het analyseren van de data.

Resultaten

Van de 197 patiënten, die in de CounterCOVID studie behandeld waren, hadden 168 patiënten minstens één PK-monster. Behandelduur was 1-10 dagen, met mediaan van 9 dagen, met 33% van de patienten < 5 behandeldagen. De totale en ongebonden concentraties vertoonden een hoge correlatie (correlatie: 0.880, R^2=0.813, p-waarde<0.0001 bij C_{ss} op dag 3). Vanwege deze hoge correlatie was het niet mogelijk om de individuele effecten van deze concentraties te bestuderen. Leeftijd en AAG bleken effect modifiers, hiervoor zijn in de totale concentratie-respons analyses de uitkomsten gecorrigeerd. Bij de P/F-ratio en het primair eindpunt van de CounterCOVID was een omgekeerd verband tussen hogere totale C_{ss} (blootstelling aan imatinib) en relatief lagere verandering in de P/F-ratio gevonden. Verder was er geen significant verband tussen de mate van blootstelling aan imatinib en de mortaliteit (binnen 90 dagen) gevonden.

Conclusie: CounterCOVID PKPD data analyses bleken met de huidige limited PK-imatinib sampling en response metingen bleek onvoldoende sensitief om een optimale correlatie tussen expositie en COVID-19 uitkomst te bepalen. Confounding factors waren verhoogde eiwitbinding, lage metabole snelheid leeftijdsafhankelijke PK en niet continu toepassen van de behandeling. Verfijning van het PK-model, verdere analyses van de ongebonden monsters en analyses met betrekking tot het metabolisme zijn nodig om de PK-PD relatie onafhankelijk en nauwkeurig te bestuderen.

Abstract

Purpose:
In the recent randomized double-blind placebo-controlled clinical trial (CounterCOVID) imatinib showed a reduction in mortality and duration of mechanical ventilation in patients with severe COVID-19. (1) The aim of this current study was to evaluate the pharmacokinetics (PK) and pharmacodynamics (PD) relationship in the COVID-19 patients treated with imatinib in the CounterCOVID trial.

Methods:
COVID-19 patients from the CounterCOVID trial with at least one imatinib PK sample were included in this study. These patients were treated with a loading dose of 800 milligrams (mg) oral imatinib followed by 400 mg once daily imatinib on nine subsequent days. The observed treatment period ranged between 1 and 10 days, with a median of 9 day, but 33% had less than 5 days of treatment. The magnitude of imatinib exposure was estimated using nonlinear mixed-effects modelling with a previously published semi mechanistic protein binding PK-model. (2) Individual PK profiles were expressed as total and unbound plasma imatinib area under the concentration-time curve (AUC), trough concentration (C_{trough}) at steady state (C_{ss}) and unbound maximum concentration (C_{max}). The pharmacodynamics outcomes of interest were change in P/F ratio, the primary endpoint of the CounterCOVID trial (1) - time to liberation from oxygen supplementation and ventilation free for more than 48 hours and alive – amount of intubation days and mortality (within 90 days). The possibility of the parameters age, gender, alpha-1-acid glycoprotein (AAG), smoking, comorbidities, dexamethasone treatment and disease severity being confounders or effect modifiers was evaluated. Linear regression for continuous data, ANOVA tests for categorical data, time-to-event analysis using cox regression and Kaplan-Meier, correlation and distribution plots, univariation regression analysis and multivariate analysis were used.

Results:
168 Patients out of 197 patients treated with imatinib had at least one imatinib PK-sample. Total concentrations and unbound concentrations highly correlated (correlation: 0.880, R^2=0.813, p-value<0.0001 C_{ss} at day 3). Due to this high correlation it was not possible to independently evaluate the unbound and total exposure - effect assocations. Age and AAG were effect modifiers, therefore the total concentrations –response analyses were adjusted for age and AAG. An association between higher total C_{ss} (higher exposure) and relatively lower change in P/F ratio was observed. This inverse correlation was also observed for the CounterCOVID primary endpoint. The magnitude of imatinib exposure did not show a significant effect on the mortality (within 90 days).

Conclusion:

CounterCOVID PKPD data analyses using limited PK-imatinib exposure sampling and response measurements was insufficient for optimal correlation with response COVID-19, due to confounding factors, such as increased protein binding, low metabolic rate total exposure of this highly protein bound drug and non-continuous drug use.

Introduction

Coronavirus disease 2019 (COVID-19) is caused by infection with the SARS-CoV-2 virus. Patients with severe COVID-19 infections may develop acute respiratory distress syndrome (ARDS). In patients with ARDS inflammation in the lungs leads to increased permeability of the capillaries in the alveoli, resulting in alveolar damage, endothelial injury and accumulation of fluids in the lungs. (3-7) Anti-inflammatory drugs (such as dexamethasone and tocilizumab) and antiviral drugs (such as molnupiravir) have been shown to be effective for some patients. (6-13)

Imatinib is a tyrosine kinase inhibitor which may exert its biological effect in COVID-19 by inhibiting Abl-related gene (Arg) and therefore inhibiting the cytosolic tyrosine-protein kinase ABL2. Inhibition of ABL2 in ARDS may decrease vascular permeability and increase the amount of antioxidant enzymes. (1, 2, 5, 14-16) The use of oral imatinib as a treatment has been studied in the multicenter, randomized placebo-controlled CounterCOVID clinical trial. (1) Imatinib did not show a significant effect on the primary endpoint: time to liberation from oxygen supplementation and ventilation free for more than 48 hours and alive, but patients treated with imatinib (N=197) had a significantly lower mortality and shorter duration of invasive mechanical ventilation when compared to patients receiving placebo (N=188).(1)

Imatinib has a half-life of 18 hours and a 98% bioavailability. Imatinib has a high protein binding (95%), where it is predominantly bound to albumin and alpha-1-acid glycoprotein (AAG). Imatinib is metabolised by cytochrome P450 (CYP) enzymes, more specifically the CYP3A4, CYP3A5 and CYP2C8 and has an active metabolite, which has a half-life of 40 hours. (1, 5, 13, 17) The previous PK research by Bartelink, *et al*. showed that total imatinib area under the concentration-time curve (AUC), maximum concentration (C_{max}) and trough concentration (C_{trough}) were 2.32- (CI95% 1.34-3.29), 2.31- (1.33-3.29) and 2.32-fold (1.11-3.53) higher in COVID-19 compared to CML/GIST patients, while unbound concentrations were comparable. (2) This effect was attributed to increased binding to alpha-1-acid glycoprotein (AAG) during COVID-19 infection. Furthermore, large interpatient variability in total and unbound PK was observed. Besides AAG; CRP, albumin, bodyweight, age and ICU admission showed to be predictive of total imatinib oral clearance. (2)

The aim of the current study was to evaluate the correlation between the total and unbound exposure to imatinib and the pharmacodynamics (PD) response in all imatinib-treated patients of the CounterCOVID trial. Based on the findings of the CounterCOVID trial, the hypothesis was that higher unbound concentrations of imatinib would lead to improved PD outcomes. (1) To test this hypothesis a semi-mechanistic AAG binding model was applied to predict the total and unbound concentration-time profiles in all CounterCOVID patients. The individual PK parameters were calculated by numerical integration using nonlinear mixed-effects modeling and associated with multiple primary and secondary outcomes, such as mortality and early biomarkers of response fraction of inspired oxygen (FiO2) and the arterial pO2 divided by FiO2 (P/F ratio). (18, 19)

Methods

In this study all the COVID-19 patients from the imatinib randomized double-blind placebo-controlled clinical trial - the CounterCOVID - with at least one imatinib PK sample were included. These COVID-19 patients had received 800 mg imatinib orally as a loading dose, followed by 400 mg once daily on

nine subsequent days. Further description of the PK sample collection and PK analysis can be found in the previous publication by Bartelink, *et al*. (2).

Exposure PK parameters

A previously published semi-mechanistic protein binding AAG-PK model was used to estimate the magnitude of imatinib exposure. (2) The AAG binding model and all parameters were re-estimated to optimally assess the total and unbound concentration-time profiles as described in the Supplement. Solely the unbound fraction can be distributed from systemic circulation to tissues, therefore the efficacy of imatinib may depend on the imatinib unbound fraction. Thus independent estimates of the unbound concentration-time profiles were included besides the total imatinib plasma levels. (2)

For the individual PK profile the following PK parameters were used as a measure for exposure:

- AUC total on day 1 ($AUCt_{day1}$) represents the cumulative AUC of the total concentrations for day 1.
- AUC unbound on day 1 ($AUCu_{day1}$) represents the cumulative AUC of the unbound concentrations for day 1
- Day 10 cumulative AUC total ($AUCt_{cum}$) represents the cumulative AUC of the total concentrations during the 10 days
- Day 10 cumulative AUC unbound ($AUCu_{cum}$) represents the cumulative AUC of the unbound concentrations during the 10 days
- Unbound maximum concentration on day 1 ($Cmaxu_{day1}$)
- Total trough concentrations (C_{trough}) on day 3 represent the total concentrations at steady state (C_{ss}total)
- Unbound trough concentrations (C_{trough}) on day 3 represent the unbound concentrations at steady state (C_{ss}unbound)

The exposure data was divided in low, medium and high exposure to visually evaluate the correlation between the exposure and categorical responses.

Outcomes

The outcomes of interest were change in P/F ratio, the primary endpoint of the CounterCOVID trial (1) - time to liberation from oxygen supplementation and ventilation free for more than 48 hours and alive – amount of intubation days and mortality (within 90 days).

Potential confounders/effect modifiers

Based on biological plausibility, correlation and distribution plots, the parameters age, gender, AAG (for total concentrations only), smoking, comorbidities (hypertension and diabetes), use of dexamethasone and disease severity (WHO clinical score) were explored as potential confounders and/or effect modifiers. Highly correlated parameters (Pearson's correlation above 0.7) were excluded in further analysis. (20)

Analysis

The exposure, response biomarkers and demographic data were selected based on correlation and distribution plots. Linear regression was performed for the analysis of the continuous data and ANOVA tests for categorical data for the outcome P/F ratio and FiO2. For the outcomes mortality and time to liberation from oxygen supplementation and ventilation free for more than 48 hours time-to-event analyses were performed using cox regression and the Kaplan-Meier method. For the selection

of confounders and/or effect modifiers stepwise univariation regression analysis was performed. The parameters which showed a significant effect ($p<0.05$) were further used in the multivariate analyses. When data was missing the median value was used.

Software

The data were stored in Castor EDC https://data.castoredc.com. Rstudio (version.4.0.3; R Foundation for Statistical Computing, Vienna, Austria) was used to perform analyses and to produce graphs. Nonlinear mixed-effects modelling software NONMEM (version 7.3, Globomaxx LLC, Hanover, MD, USA) with Piraña Software (version 3.0, Certara) and Perl-speaks-NONMEM (PsN) were used for modeling. Visual predictive check graphs (VPCs) were created using the VPC package (version 1.0.1; R).

Results

This study includes in total 168 patients out of the 197 patients from the CounterCOVID trial, with an addition of 34 patients compared to the previously published imatinib PK study by Bartelink, *et al* (1, 2). In total 648 total samples and 46 unbound samples were included. The observed treatment period ranged between 1 and 10 days, with a median of 9 day, but 33% had less than 5 days of treatment. The median age of the patients was 65 years and the median bodyweight was 84 kg. With 76.8% the majority of the patients were male. The most common comorbidities were hypertension (32.1%) and diabetes (22.0%). Out of the 168 patients 43 patients (25.6%) had a BMI above 30. Other patient characteristics are shown in ***Table 1*****.**

The final covariate AAG-PK model was used to predict the individual PK-parameters in these 168 imatinib treated patients in the CounterCOVID study as described in detail in the supplement (***Appendix 1: final PK model***). The correlation plots of individual PK-parameter estimates (***Appendix figure 7***) showed a high correlation between most parameters***.*** The total and unbound concentrations on day 3 at C_{ss} (correlated: 0.880, R^2=0.813, p-value<0.0001), AUC on day 1 (R=0.759) and cum AUC at day 10 (R=0.884), ***Appendix figure 7***. Due to this high correlation, it was not possible to independently evaluate the effect of the total and unbound concentrations at the same timepoint. Furthermore, goodness of fit, VPC and Bland Altman plots showed that the individual estimates, were similarly predictive at C_{max} and C_{trough}. In further analyses total concentrations were used only and C_{max} estimates were excluded.

The results for the outcomes change in P/F ratio, mortality, and time to liberation from oxygen supplementation and ventilation for more than 48 hours and alive are shown in ***Table 2***. At baseline, in all 168 patients, P/F ratio were performed, in contrast to 139 at day 3 and only 50 on study day 9. Therefore the results of the outcomes change in P/F ratio are presented at day 3.

(WHO clinical score, amount of intubation days and comorbidities are not included in this table, since these analyses are ongoing).

The parameter age showed to have a significant effect on the outcomes change in P/F ratio (p=0.0004), mortality (p=0.00002) and time to liberation from oxygen supplementation and ventilation for more than 48 hours and alive (p=0.00174) in univariate analyses and is an effect modifier. Therefore the multivariate analyses were adjusted for age. The p-values of the parameter AAG were not below 0.05 in the univariate analysis, but AAG was used in multivariate analysis and all

total concentrations where adjusted for AAG as the previous publication by Bartelink, *et al*. showed AAG to be highly correlated with the total concentrations.

The parameter total C_{ss} (p=0.0187) had a significant effect on the change in P/F ratio at day 3 in the univariate analysis. Lower total C_{ss} were correlated with higher change in P/F ratio (***Table 2***, ***Figure 1***). Furthermore, the cumulative total AUC (p=0.012) also had an inverse significant effect on the change in P/F ratio on day 3 (***Table 2***, ***Figure 1***). In the multivariate analyses besides age (Beta: -1.72, p=0.006) the parameter of interest total C_{trough} on day 3 (total C_{ss}) (Beta: -0.02, p=0.040) showed to have an inverse significant effect on the change in P/F ratio on day 3 (***Table 2***, ***Figure 1***). This inverse effect, where higher exposure is correlated with lower change in P/F ratio has also been observed in the plots of change in P/F ratio on day 1 (***Appendix figure 9***).

The parameters total cumulative AUC (p=0.0179), total C_{trough} on day 3 (total C_{ss}) (p= 0.0114), the use of dexamethasone (p=0.0317) and gender (p=0.0278) showed to have a significant effect on the outcome time to liberation from oxygen supplementation and ventilation for more than 48 hours and alive in the univariate analysis (***Table 2***). In the multivariate analysis besides age (HR: 0.98, CI95%: 0.9662-0.9931, p=0.0033), AAG (HR: 0.65, CI95%: 0.4401-0.9475, p=0.0253), gender (HR: 0.58, CI95%: 0.3979-0.8568, p=0.00597) and dexamethasone use (HR: 0.48, CI95%: 0.2326-0.9940, p=0.04814) also showed to have a significant effect. However the parameter of interest, total C_{trough} on day 3 (total C_{ss}), did not have a significant effect in the multivariate analysis on the CounterCOVID primary outcome (p=0.07151).
The Kaplan-Meier plots of cumulative total AUC and total C_{trough} on day 3 (total C_{ss}) illustrate that the group with high exposure has a lower cumulative event of time to liberation from oxygen supplementation and ventilation for more than 48 hours and alive (***Figure 2***). The Kaplan-Meier plots of the parameters that did not show a significant effect on the outcome time to liberation from oxygen supplementation and ventilation for more than 48 hours and alive are shown in ***Appendix figure 10***. The dexamethasone Kaplan-Meier plot shows that the patients treated with dexamethasone had a lower cumulative event of time to liberation from oxygen supplementation and ventilation for more than 48 hours and alive (***Appendix Figure 11***). The female patients had a higher cumulative event for the outcome time to liberation from oxygen supplementation and ventilation for more than 48 hours and alive (***Appendix Figure 12***).

As shown in ***Table 2,*** age was the only parameter that had a significant effect on the outcome mortality in the univariate analysis. Older age also showed to have a significant effect on the mortality in the multivariate analysis (HR: 1.13, CI95%: 1.07-1.19, p<0.00001). For some parameters the group with high exposure initially showed a lower cumulative mortality, however after 90 days the group with high exposure had a cumulative mortality equal to or higher than the low and medium exposure groups (***Appendix figure 13***).

Discussion

Total C_{ss} had a significant effect on the change in P/F ratio (in univariate and multivariate). However, the effect was for both small. Total cumulative AUC and total C_{ss} had a significant effect on the CounterCOVID primary endpoint, time to liberation from oxygen supplementation and ventilation for more than 48 hours and alive, in univariate analysis. AAG has shown to affect PK. AAG values at

baseline showed to have a significant effect on the CounterCOVID primary endpoint. Older age was an effect modifier in all PD outcomes. Dexamethasone and gender had a significant effect on the CounterCOVID primary endpoint (in univariate and multivariate analysis). In multivariate analysis in addition.

The results of the change in P/F ratio analysis and plots and Kaplan-Meier plots (of cumulative total AUC and total C_{trough} on day 3 showed) showed, contradictory to the hypothesis, a small but inverse correlation where lower exposure was correlated with more favorable outcomes. As described above and in the Supplement (***Appendix 1: final PK model***) the PK parameters are highly correlated (***Appendix figure 7***). Because of a high correlation between the total concentrations and unbound concentrations (R^2=0.813, p-value<0.0001) the effect of the total concentration and unbound concentrations could not be independently assessed. The limited number of unbound samples are an important limitation of this study. Currently only 46 unbound samples of were included. As discussed in ***Appendix 1: final PK model,*** further PK analysis with more unbound samples and PK model refinement are needed. Because of these limitations further analysis on the unbound concentrations have not yet been performed. The third limitation of this study is the decrease in available PK samples over time, this made it statistically not possible to continuously evaluate the change in P/F ratio over the course of the treatment days. (2)

The significant effect of cumulative total AUC on the change in P/F ratio and CounterCOVID primary endpoint and the significant effect of cumulative unbound AUC on the change in P/F ratio in the univariate analysis are sensitive to bias. Early discontinuation of treatment in some patients and mortality in some other patients adds bias to the parameter cumulative AUC (total + unbound). After the treatment is discontinued or the patient has passed away, the AUC does not increase over time, resulting in a lower cumulative AUC than if the treatment had been continued.

The group treated with dexamethasone showed a lower cumulative event of the outcome time to liberation from oxygen supplementation and ventilation for more than 48 hours and alive, which means that the group without dexamethasone treatment had a more favorable outcome. Since not all patients were treated with dexamethasone, it is possible that patients with more severe COVID-19 infection received dexamethasone treatment, which could explain this lower cumulative event in the treated group. Out of the 168 patients 119 (70.8%) had received dexamethasone, which means that the group sizes are unequal and this could lead to a less accurate comparison. The Kaplan-Meier curve showed that female patients had a higher cumulative event of time to liberation from oxygen supplementation and ventilation for more than 48 hours and alive. This is in agreement with the expectation and observation where male patients show worse prognosis. (21, 22) In this study 129 out of 168 patients (76.8%) were male, which also lead to unequal group sizes.

The metabolism of imatinib might be affected in COVID-19 patients. Increase in cytokine levels, which occurs during a COVID-19 infection, could inhibit the activity of CYP enzymes. Inhibition of the imatinib metabolism could result in lower imatinib clearance, but also lower levels of the active metabolite. Interleukin-6 (IL-6) is elevated during COVID-19 infection and is known to inhibit CYP-enzymes, including CYP3A4, during inflammation. (7, 13, 23, 24) In an *in vitro* study more than 40% decrease in CYP-enzymes expression was observed. (23) The amount of CYP3A4 inhibition, mediated by IL-6, has been observed to vary between patients. Other in COVID-19 elevated cytokines, such as interferon gamma (IFNγ), tumor necrosis factor alfa (TNFα), transforming growth factor (TGF) and interleukin-1 (IL-1) significantly inhibit CYP3A4 expression. IL-2 and IL-10 are increased during COVID-

19 infection and could possibly also inhibit CYP3A4. (7, 13, 23, 24) Decreased clearance would result in higher total imatinib concentrations, however the exact magnitude of total imatinib concentrations increase by reduction of metabolism and the effect on the unbound imatinib concentrations is currently unknown. (13, 23) A small prospective observational study showed in 43% of the COVID-19 patients change in the CYP3A phenotype from normal metabolizer to poor metabolizer or from ultra-rapid metabolizer to normal metabolizer. This study also showed a significantly (22.8%) decrease in CYP3A metabolism. (24) Further research to evaluate the change in metabolism in COVID-19 patients, the effect of this on imatinib and active metabolite levels and the pharmacodynamics effects of the active metabolite are needed.

Further sensitivity analyses to support the current unexpected finding of the reversed exposure P/F response relationship are suggested: Oxygen supplementation before and after intubation is performed through different methods. The amount of FiO2 differ for different oxygen supplementation methods. (18) To improve the comparison, the different supplementation methods can be taken into account by dividing the patients into intubated and non-intubated subsets for a more accurate evaluation of the change in P/F ratio. Furthermore, the correlation may be driven by only a limited number of extreme cases, suggesting that non-parametric tests, data transformation or other assessment of the effect of potential outliers is needed. The analysis using the WHO clinical score is ongoing and these results could also give more insight into the effect of imatinib on the change in disease severity. Furthermore, instead of only using the variables with a p-value<0.05 in multivariate analysis and adjusting for the variables who showed a significant effect in further analysis all baseline characteristics could be evaluated and the most important confounders (such as age and sex) will be adjusted for. Lastly, including the results of the outcomes change in FiO2, WHO clinical score and amount of intubation days would show a more complete exposure-response correlation.

In conclusion, CounterCovid PKPD data analyses using limited PK-imatinib exposure sampling and response measurements was insufficient for optimal correlation with response COVID-19, due to confounding factors, such as increased protein binding, low metabolic rate total exposure of this highly protein bound drug and non-continuous drug use. PK model refinement, further analyses of unbound samples and metabolism are needed to adequately assess the independent effect of unbound imatinib levels, exposure-response relationship and the clinical effects. Age was an effect modifier on the change in P/F ratio, CounterCOVID primary endpoint and mortality.

Acknowledgments

I am grateful to dr. Imke Bartelink and the other researchers for allowing me to participate in this study. I would like to thank dr. Imke Bartelink for her guidance, feedback and the valuable knowledge about PKPD, modeling, coding and programming she has taught me. I would also like to thank Nadia Baalbaki for the PD analysis and her feedback. In addition, I would like to thank Amanda Evelo and Ivo Tielbeek for their help with coding. I am thankful to everyone mentioned and also Anke Leeuwerik and Zobeir Amiri for the feedback and contribution during discussions regarding the progress of the study. I would like to thank Richard Honeywell as well for the guidance during the tasks related to the laboratory.

Table 1 *patient characteristics and biomarker values at baseline*

	COVID-19 patients (N=168)
	Median (IQR)
Age (years)	65 (57-73)
Male (N; %)	129 (76.8)
Bodyweight (kg)	84.0 (75.0-95.3)
Height (cm)	175 (169-180)
BMI (kg/m2)	27.19 (24.78-30.47)
Smoke (no, yes, former) (N; %)	109, 7, 52 (64.9, 4.2, 31)
ICU admission (N; %)	33 (19.6)
Nasogastric tube (N; %)	17 (10.1)
eGFR (ml/min/1.73 m^2)	87 (72-90)
Albumin (g/L)	34.0 (30.0-38.0)
AAG (g/L)	1.88 (1.62-2.18)
CRP (g/L)	0.095 (0.033-0.153)
ALAT (U/L)	36.0 (26.0-55.0)
ASAT (U/L)	46.0 (35.0-61.8)
Bilirubin (mg/dL)	8.0 (6.0-10.0)
GGT (U/L)	60.5 (37.8-104.3)
Hb (mmol/L)	8.4 (7.8-9.1)
Leukocyte (*10^{-9}/L)	7.65 (5.58-10.50)
Dexamethasone (N; %)	119 (70.8)
LDH (U/L)	348.0 (274.0-441.3)
NLR	6.44 (3.62-9.82)
PF0	320.7 (267.9-373.1)
BMI > 30 (N; %)	43 (25.6)
Comorbidity COPD/asthma (N; %)	28 (16.7)
Comorbidity diabetes (N; %)	37 (22.0)
Comorbidity renal failure (N; %)	6 (3.6)
Comorbidity cardiovascular diseases (N; %)	32 (19.0)
Comorbidity heart failure (N; %)	8 (4.8)
Comorbidity hypertension (N; %)	54 (32.1)
Comorbidity rheumatic disease (N; %)	8 (4.8)
Comorbidity hepatic disease (N; %)	1 (0.60)
Comorbidity pulmonary embolism (N; %)	2 (1.2)
Comorbidity neurological (N; %)	14 (8.3)
Comorbidity coronary artery disease (N; %)	17 (10.1)
Comorbidity atrial fibrillation (N; %)	10 (6.0)
No comorbidities (N; %)	42 (25.0)

AGE = age, BMI = body mass index, AAG = alpha-1-acid glycoprotein, CRP = C-reactive protein, ALAT = alanine amino transaminase, ASAT = aspartate aminotransferase, GGT = gamma glutamyl transferase, Hb = hemoglobin, LDH = lactate dehydrogenase, NLR = neutrophil to lymphocyte ratio, PF0 = fraction of inspired oxygen (P/F ratio) at baseline. The eGFR values were calculated with the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI). The IQR are the interquartile range 0.25-0.75 values.

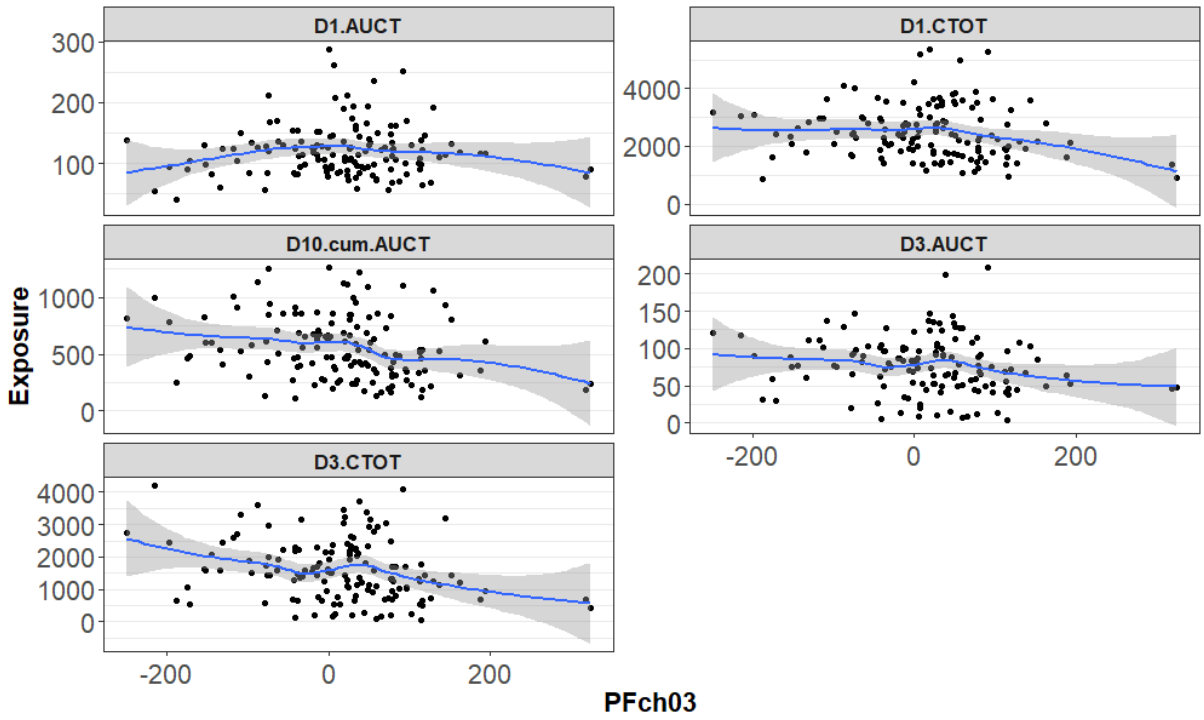
Table 2 *The results of the statistical analysis for the P/F ratio, mortality, and time till more than 48 hours liberation from ventilation and oxygen supplementation and alive (CounterCOVID primary outcome) of the parameters and multivariate analyses adjusted for AAG and age.*

		P/F ratio*			Mortality N=15			48h liberation from ventilation & oxygen supplementation N=168		
Parameter		**Beta**	**R^2**	**p-value**	**HR**	**CI95%**	**p-value**	**HR**	**CI95%**	**p-value**
$Cmaxu_{day1}$ (µg/L)**	linear	0.162	-0.003	0.482	0.9996	0.98 - 1.015	0.958	1.001	0.9965 - 1.006	0.592
$AUCt_{day1}$(mg*h/l)	linear	0.02	-0.007	0.901	1.001	0.991 - 1.012	0.79	0.9985	0.9948 - 1.002	0.427
$AUCt_{cum}$(mg*h/l)	linear	-0.07	0.037	0.012	1.00	0.99 - 1.001	0.619	0.9992	0.9986 - 0.9999	0.0179
C_{ss}total (µg/L)	linear	-0.02	0.033	0.0187	1.72	0.99-1.00	0.403	0.9997	0.9996 - 0.9999	0.0114
Dexamethasone	yes	8.81	-0.005	0.603	1.11	0.35 - 3.49	0.857	0.6741	0.4703 - 0.9661	0.0317
Smoking	current	-13.44	-0.0001	0.323	1.84	0.84 - 3.978	0.122	0.7674	0.569 - 1.035	0.0827
Age (years)	linear	-2.14	0.079	0.0004	1.12	1.06 - 1.174	0.00002	0.9799	0.9676 - 0.9925	0.00174
Gender	female	-15.51	-0.0025	0.418	2.05	0.46 - 9.11	0.343	0.653	0.4468 - 0.9545	0.0278
AAG (mg/L)	log	14.91	-0.003	0.307	1.05	0.2893 - 3.814	0.94	0.6913	0.467 - 1.023	0.065
CRP	log	36.38	0.0004	0.704	-	-	-	-	-	-
Multivariate analyses (adjusted for AAG and age)										
$Ctrought_{day3}$ (µg/L)	linear	-0.02	0.096	0.040	1.00	0.999 - 1.00	0.42	1.00	0.9996 - 1.0000	0.07151
Age (years)	linear	-1.72	0.096	0.006	1.13	1.07 - 1.19	0.000003	0.98	0.9662 - 0.9931	0.0033
AAG (mg/L)	Log	45.1	0.096	0.223	4.90	0.41 - 59.17	0.211	0.65	0.4401 - 0.9475	0.0253
Gender	female	-	-	-	-	-	-	0.58	0.3979 - 0.8568	0.00597
Dexamethasone	Yes	-	-	-	-	-	-	0.48	0.2326 - 0.9940	0.04814

P/F ratio = change in fraction of inspired oxygen between baseline and day 3, HR = hazard ratio, $Cmaxu_{day1}$ = unbound maximum concentration on day 1 , AUCt = total area under the concentration-time curve (AUC), , cum = cumulative , Css = steady state concentration, which is the trough concentration on day 3, AAG = alpha-1-acid glycoprotein, CRP = C-reactive protein.

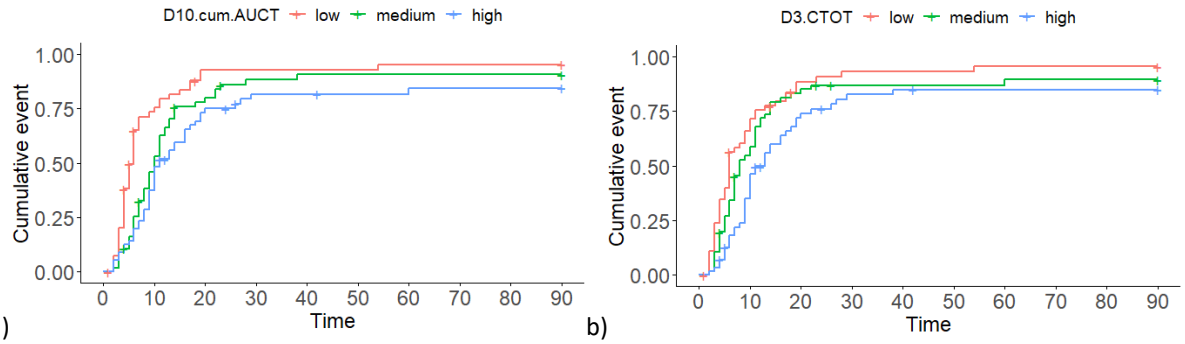
**linear regression for continuous (normally distributed) data and logistic ANOVA for categorical data vs change from baseline at day 3.*
***Unbound C_{max} to be replaced by total C_{max}*

Figure 1 *Exposure plots for the change in P/F ratio on day 3*



CMAXU = unbound maximum concentration on day 1, D = day, CTOT = total trough concentration (C_{trough}), CFREE = unbound C_{trough}, AUCT= total area under the concentration-time curve (AUC), AUCU= unbound AUC, cum. AUC= cumulative AUC over the full 10 day treatment period.

Figure 2 Kaplan-Meier plots for the outcome time till more than 48 hours liberation from ventilation and oxygen supplementation and alive split in low, medium and high exposure
a) Cumulative total AUC on day 10. b) Total trough concentrations (C_{trough}) on day 3



Supplementary material

Appendix 1: final PK model

Introduction

In the published study by Bartelink, *et al*. (2) the accuracy of predictions for COVID-19 patients using the previously published CML/GIST pharmacokinetics (PK) models were evaluated. Here, the final covariate relationships in the AAG-PK model was used for evaluation of PK in all imatinib treated patients in the CounterCOVID study.

Methods

'For the current PKPD study, the CounterCOVID PK-data of 168 patients with > 1 PK sample were combined with total and unbound imatinib concentration-time profiles from rich sampled data from two previously published studies in 15 CML and 83 GIST patients (53 males and 45 females). The dose at steady state ranged between 100 and 800mg in CML/GIST, and plasma samples were collected at steady state.' (2) The full dataset was used to re-estimate the parameters of the AAG-PK model and estimate the effect of final covariate model presented in CPT-CTS on CL_U/F (unbound clearance), Vd_U/F (unbound volume of distribution) and K_A (absorption rate). Natural log transformation was applied to the covariates before including these into the final model and for the population parameters an exponential scale was used. 'The applied AAG-PK-Model is a first order absorption, one-compartment model in which imatinib binds nonlinearly to AAG and forms a complex, with an *in vitro/in vivo* estimated dissociation constant K_D and linear elimination of the unbound fraction only, as shown in ***Appendix figure 1***.' (2) Additionally, effects of the use of a nasogastric tube on the PK were assessed. Correlation plots, forest plots, visual predictive checks (VPC), Bland Altman plot and goodness-of-fit plots and boxplots were used to evaluate the results.

Results and discussion

The re-estimation of the PK parameters, performed using the combined (CML/GIST and COVID) datasets using the AAG-PK model, is shown in ***Appendix table 1***. The covariates ICU admission, age, bodyweight, CRP and albumin showed a significant effect, similar to the prior PK-analyses results ***(Appendix figure 2***), whereas AAG predicted most of the variability in PK (***Appendix figure 3A)***. In total 33 patients out of 168 patients (19.6%) were admitted to the ICU and 17 of these patients (10.1%) had used a nasogastric tube. The use of a nasogastric tube had a significant effect on the bioavailability (***Appendix figure 2***). The bioavailability in patients with a nasogastric tube was 66% (CV 9%), (ΔOFV = -10 (P=0.0016) compared to the model with ICU on clearance), affecting the total and unbound exposure. ***Appendix figure 3B*** shows a lower median area under the concentration-time curve (AUC) for the patients with a nasogastric tube. In addition, decrease in variability with the use of a nasogastric tube can be observed. (2) Possible causes of this difference in bioavailability and variability should be assessed in future studies.

The forest plot demonstrates that covariates bodyweight, albumin and age had a statistical, but not a clinically significant effect on unbound clearance (***Appendix figure 2***). The underlying mechanism of the effect albumin on unbound clearance are a topic of further study: Either low albumin may decrease protein binding in the interaction with AAG-imatinib complex or low albumin may reflect

disease severity, which in turn may affect the metabolic rate of imatinib (***Appendix figure* 2**). (2, 25, 26)

The goodness-of-fit plots showed a better fit of the model for the total concentrations compared to the unbound concentrations, with some overprediction of the population at high AAG values, but adequate individual predictions (***Appendix figure 4***). The Bland Altman plot showed that overall the model showed adequate predictions (mean prediction error of +13.4% (± SD 78.9%) for total and 15.2% (± SD 74.0%) for unbound. Although at the early timepoints (0-4h post dose) C_{total} concentrations in COVID-19 was adequately predicted, with a mean prediction error of 1.1% (± SD 64.3%) with underprediction at $C_{unbound}$ of -21% (± SD 35.5%) (***Appendix figure 5***) the lack of early PK-sampling prevented C_{max} exploration in the PKPD analyses. Also, C_{trough} total in COVID-19 was adequately predicted, with a mean prediction error of 15.1% (± SD 67.4%) with underprediction at C_{max} unbound of -17.3% (± SD 31.8%), suggesting that further improvement of the bound/bound concentration time profile is possible.

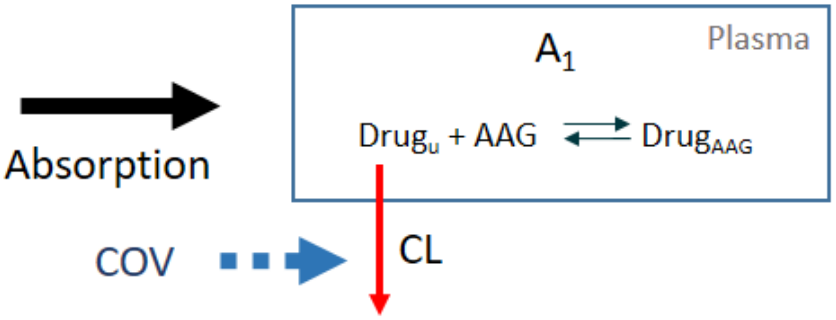
The prediction-corrected VPC plot of the combined dataset-final model shows a consistent distribution of observed and predicted total imatinib concentrations between cancer and COVID-19 patients (***Appendix figure 6***). The VPC stratified for AAG show that at high AAG values, the observed C_{max} of the total and unbound concentrations are relatively high compared to the predicted concentrations. Overall the VPCs for ICU patients with and without a nasogastric tube show accurate predictions with a slight overprediction in the unbound concentrations for patients without a nasogastric tube.

The correlation plots of the PK parameters (***Appendix figure 7***) showed a high correlation between most parameters**.** The total concentrations on day 3 and unbound concentrations on day 3 are also highly correlated (Correlation: 0.880, R^2=0.813, p-value<0.0001). Due to this high correlation it was not possible to independently evaluate the effect of the total and unbound concentrations.

Conclusion & future directions

The AAG-PK model showed accurate individual predictions for imatinib total concentration time profiles in all 168 CounterCOVID patients. Future study PK-sample analyses of unbound concentrations and imatinib metabolites using oral and intravenous imatinib data may help to further characterize absorption, metabolism, and clearance of the unbound and bound fraction in COVID-19. These data will further improve the accuracy of the independent bound and unbound concentration time profile predictions.

Appendix figure 1 *The AAG-PK model which shows the imatinib binding to AAG and clearance of the unbound fraction*
$Drug_u$= unbound drug, AAG = alpha-1-acid glycoprotein, $Drug_{AAG}$ = drug bound to AAG, CL = clearance, COV = covariates

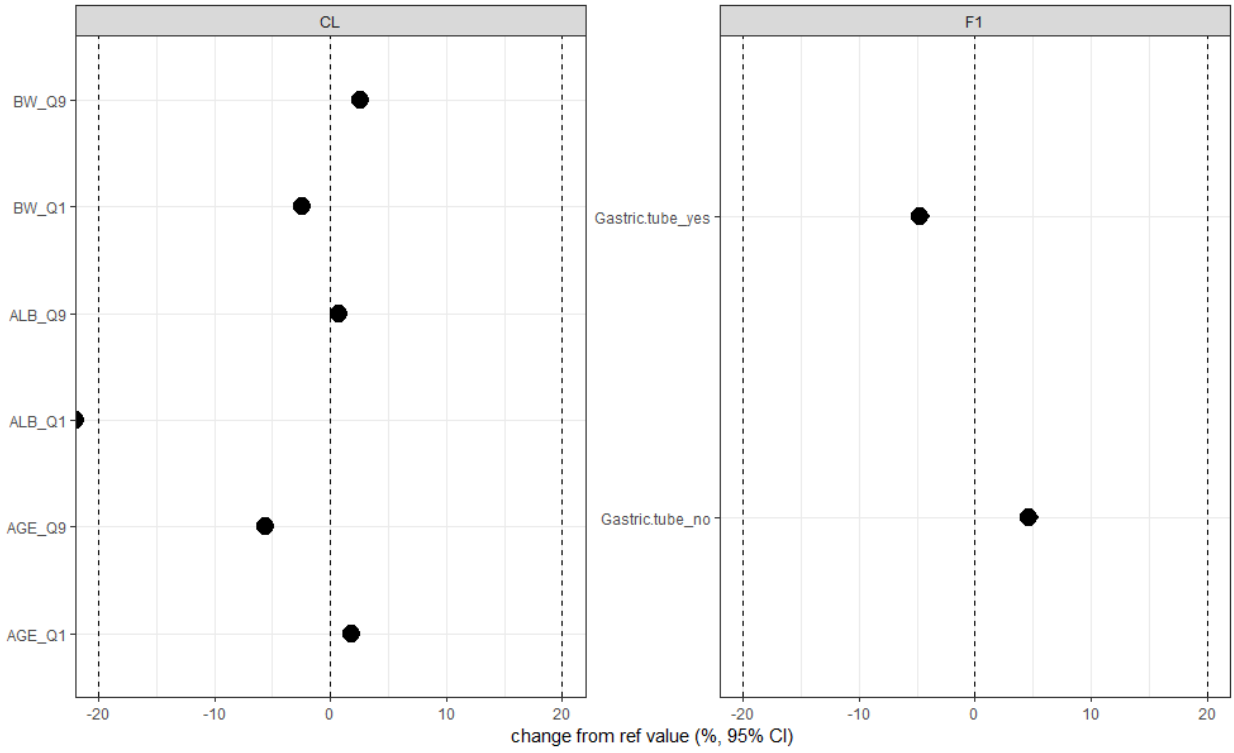


Appendix Table 1 The PK parameter estimates using the final PK model

Fixed effect parameters*	**Estimate (CV%, % shrinkage)**
K_A (L/h)	0.682 (1.7%)
CL_u (L/h)	307.4 (0.3%)
Bodyweight (kg)	1.38 (3.7%)
Age (years)	0.594 (1.9%)
Albumin	1.174 (3.2%)
Vd/F_u (L)	7785.3 (0.3%)
$F_{nasogastric\ tube}$	0.656 (8.7%)
IIV components	
ω2- CL	29.5% (5.6%, 28%)
ω2- V	35.5% (6.9%, 38%)
corr. IIV ClxV	0.549 (15.9%)
ω2- KA	129.5% (5.6%, 58%)
Box dis	0.1755 (56.8%)
Residual var	
$Prop\ error_{total}$	16.2% (4.1%, 7%)
$Prop\ error_{unbound}$	14.4 (1.8%, 15%)
Corr. Error	0.0218 (0%)

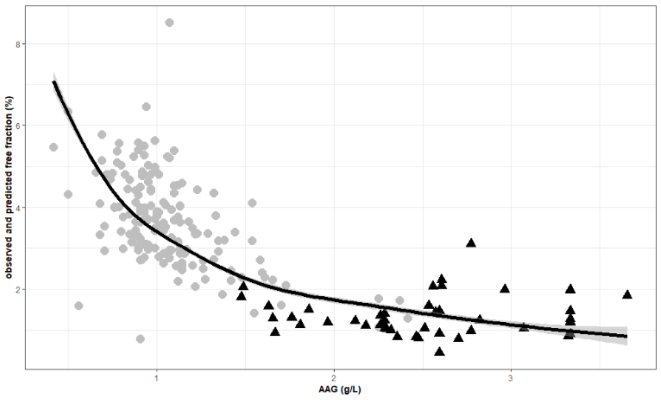
Footnote appendix table 1: K_A = absorption rate, CL_u = clearance unbound fraction, Vd/F_u = unbound volume of distribution, $F_{naso\ gastric\ tube}$ = bioavailability with the use of a nasogastric tube, IIV = inter-individual variability.

Appendix figure *2* *Forest plots with covariates in AAG-PK model for CL and F1*

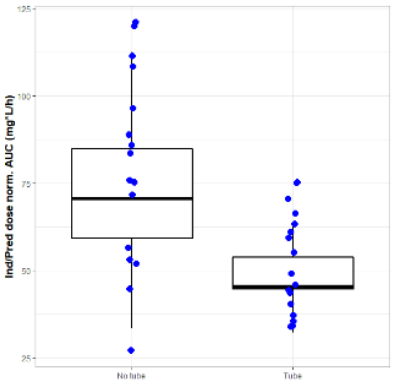


BW: bodyweight, ALB: albumin, AGE: age, gastric.tube: use of nasogastric tube, CL: clearance, F1: apparent bioavailability

Appendix figure 3: *Clinically significant covariate-PK relationships in the final model: A) AAG-free fraction predictions, B) Exposure in ICU patients with / without nasogastric tube*



A)

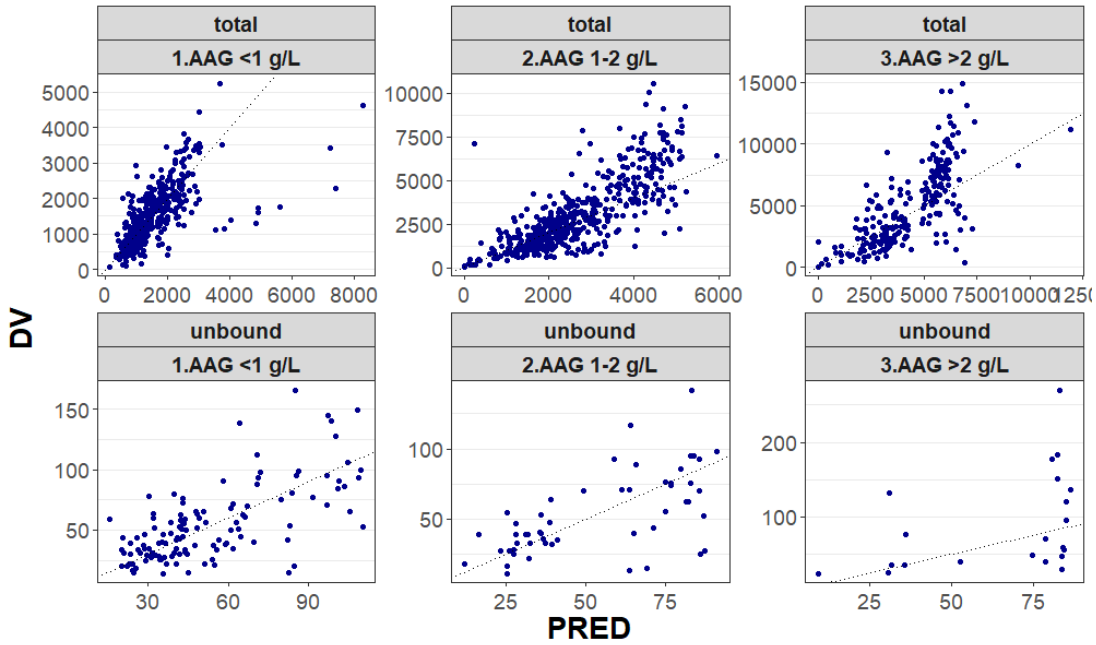


B)

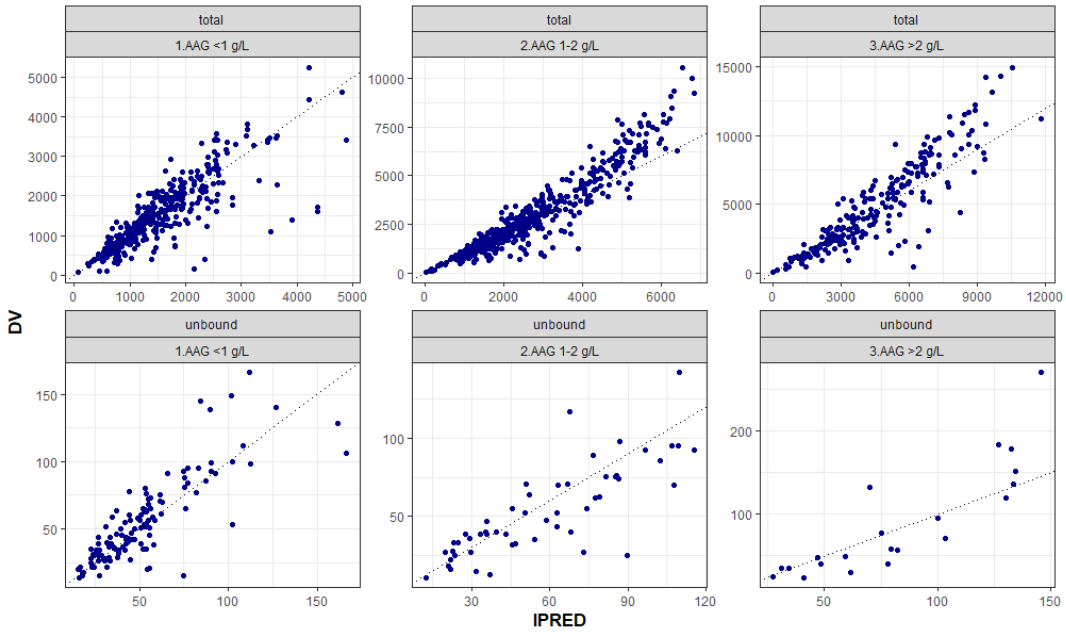
AUC = area under the concentration-time curve

Footnote Appendix figure 3: Greys dots = predicted values using for CML/GIST, black triangles = predicted values for COVID-19, black line = mean values.

Appendix figure 4 *Goodness of fit plots of the observed (total + unbound) predicted data (A) and vs individual predicted data (B), divided by the alpha-1-acid glycoprotein (AAG) levels <1 g/L, between 1-2 g/L and >2 g/L*

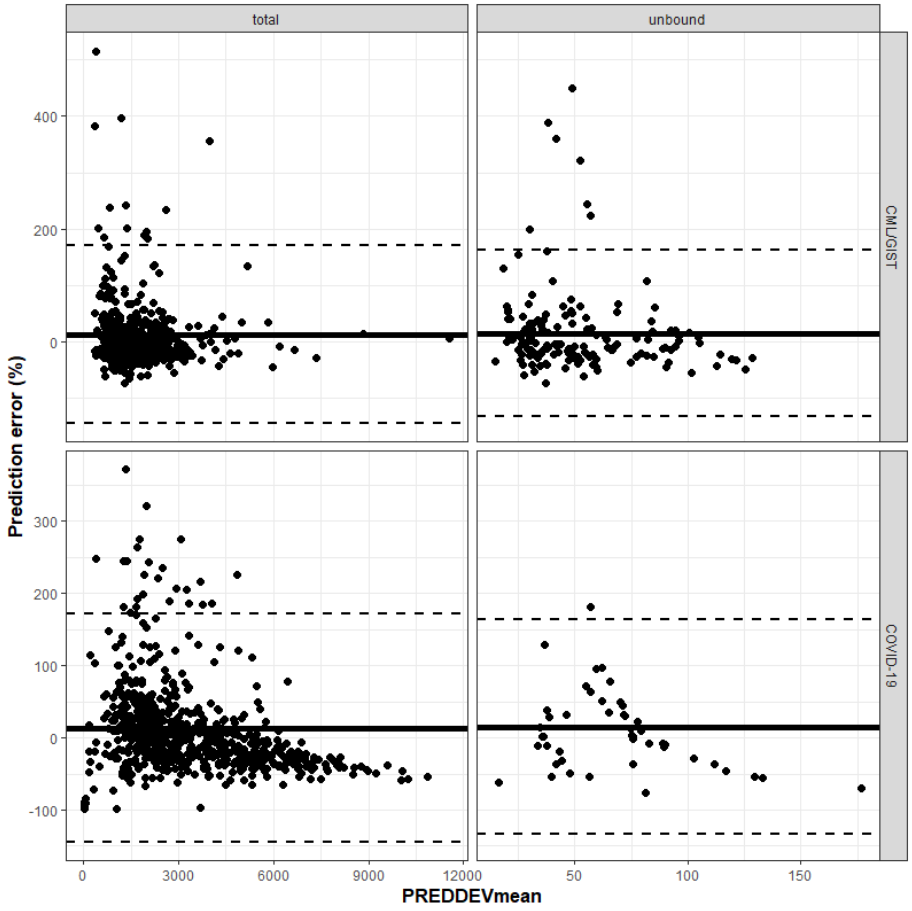


A)



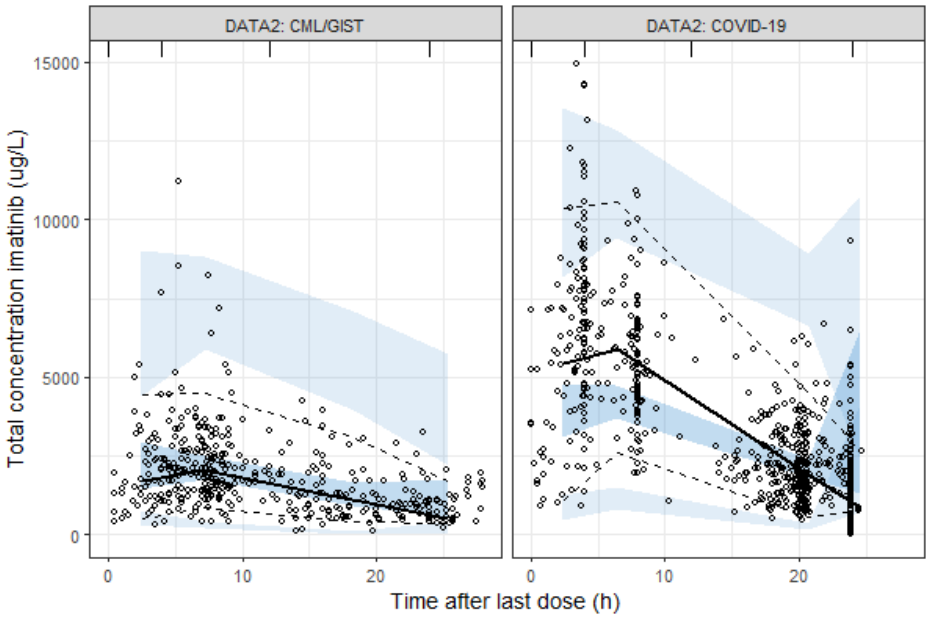
B)

Appendix figure 5 *Bland Altman plot for the CML/GIST and COVID-19 subsets for the total and unbound concentrations*

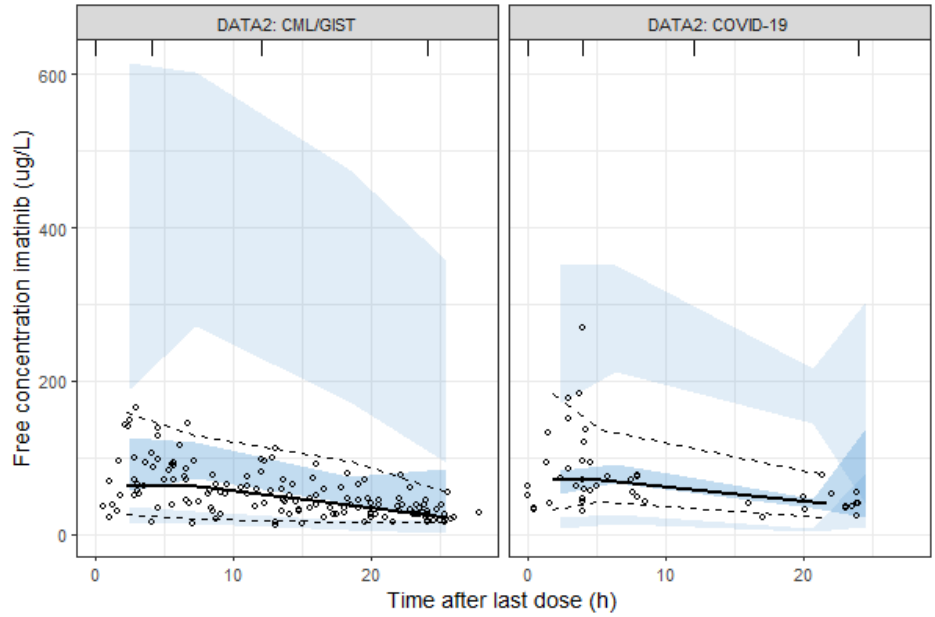


The straight line is the mean value and the dashed lines are the mean + 1.96 standard deviation of the prediction error.

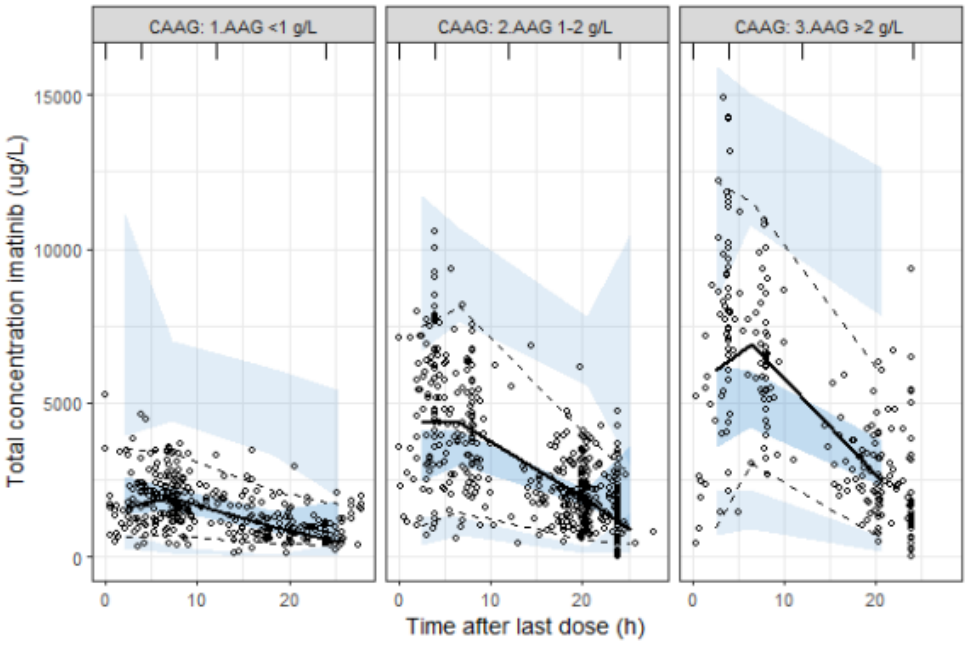
Appendix figure 6 *VPC of the total and unbound concentrations stratified for the CML/GIST and COVID-19 dataset (A, B), per AAG levels <1 g/L, between 1-2 g/L and >2 g/L (C,D) and VPC of the total (E) and unbound concentrations (F) using the final PK model, stratified for nasogastric tube.*



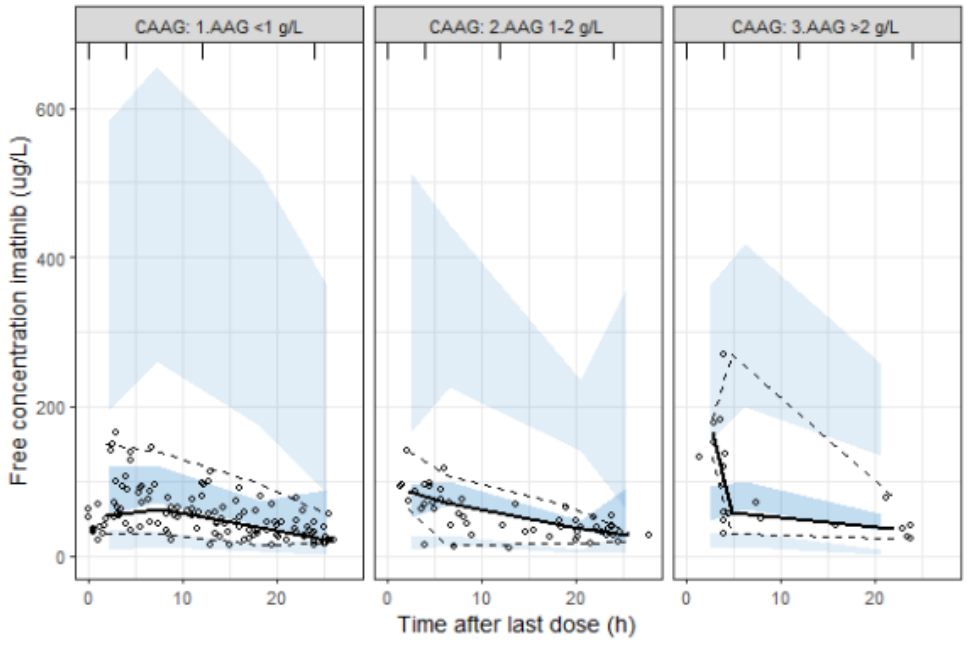
A)



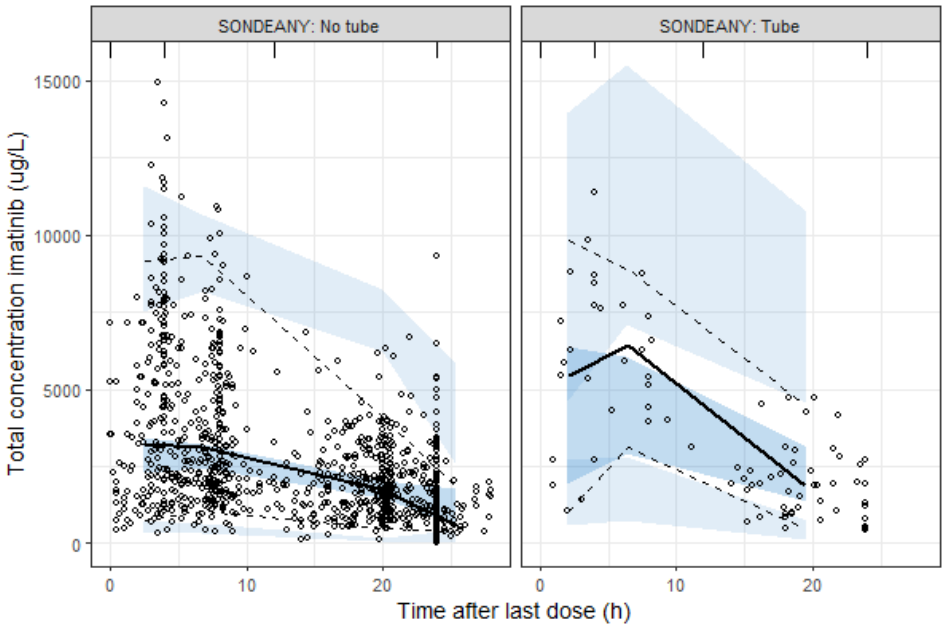
B)


CAAG: 1.AAG <1 g/L
CAAG: 2.AAG 1-2 g/L
CAAG: 3.AAG >2 g/L
Total concentration Imatinib (ug/L)
15000
10000
5000
0
0
10
20
Time after last dose (h)

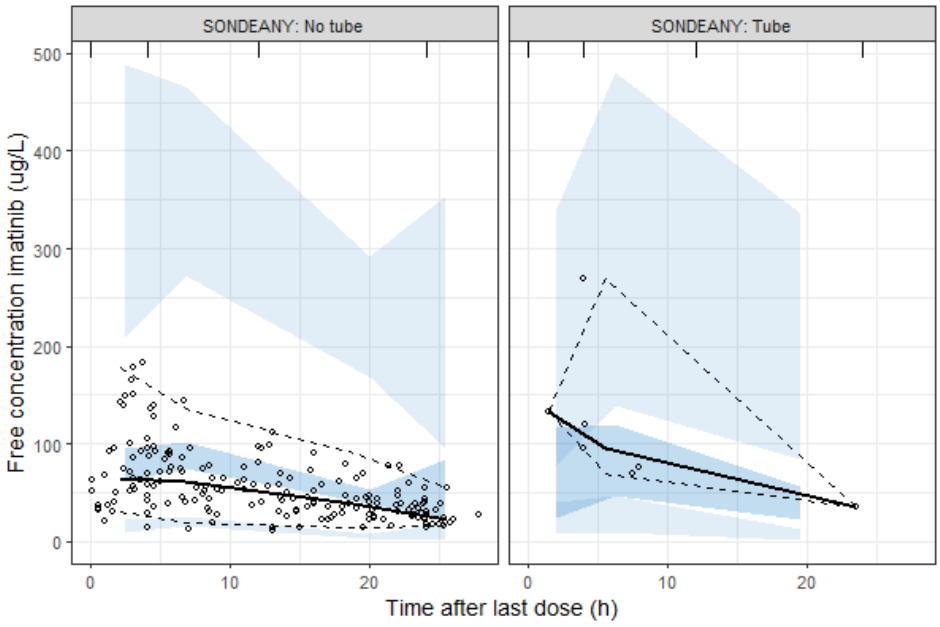
C)


CAAG: 1.AAG <1 g/L
CAAG: 2.AAG 1-2 g/L
CAAG: 3.AAG >2 g/L
Free concentration Imatinib (ug/L)
600
400
200
0
0
10
20
Time after last dose (h)

D)



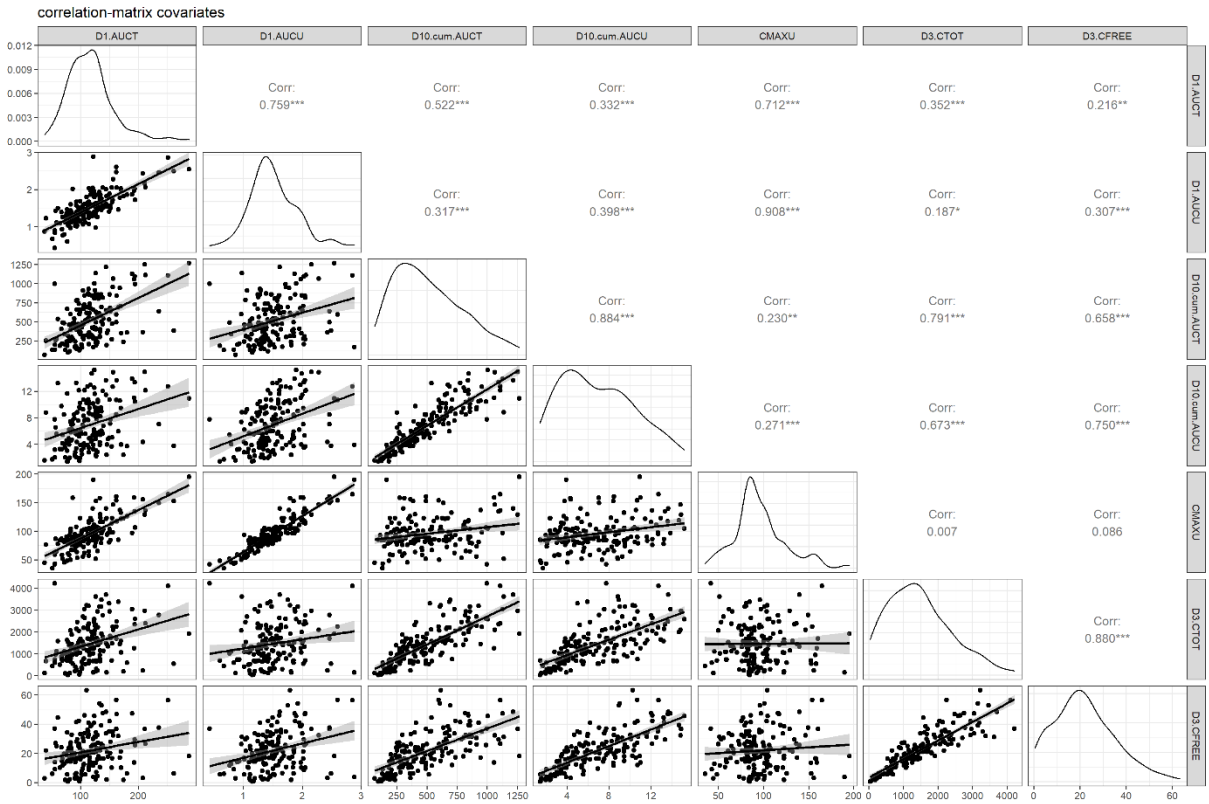
E)



F)

The dots = observed data, dashed *black lines* = 5th percentile and 95th percentile of observed concentrations, solid black line = median *of observed* concentrations, semitransparent dark blue field= *simulation-based 95% confidence interval*, AAG = alpha-1-acid glycoprotein, CAAG = AAG concentration, SONDEANY = use of nasogastric tube.

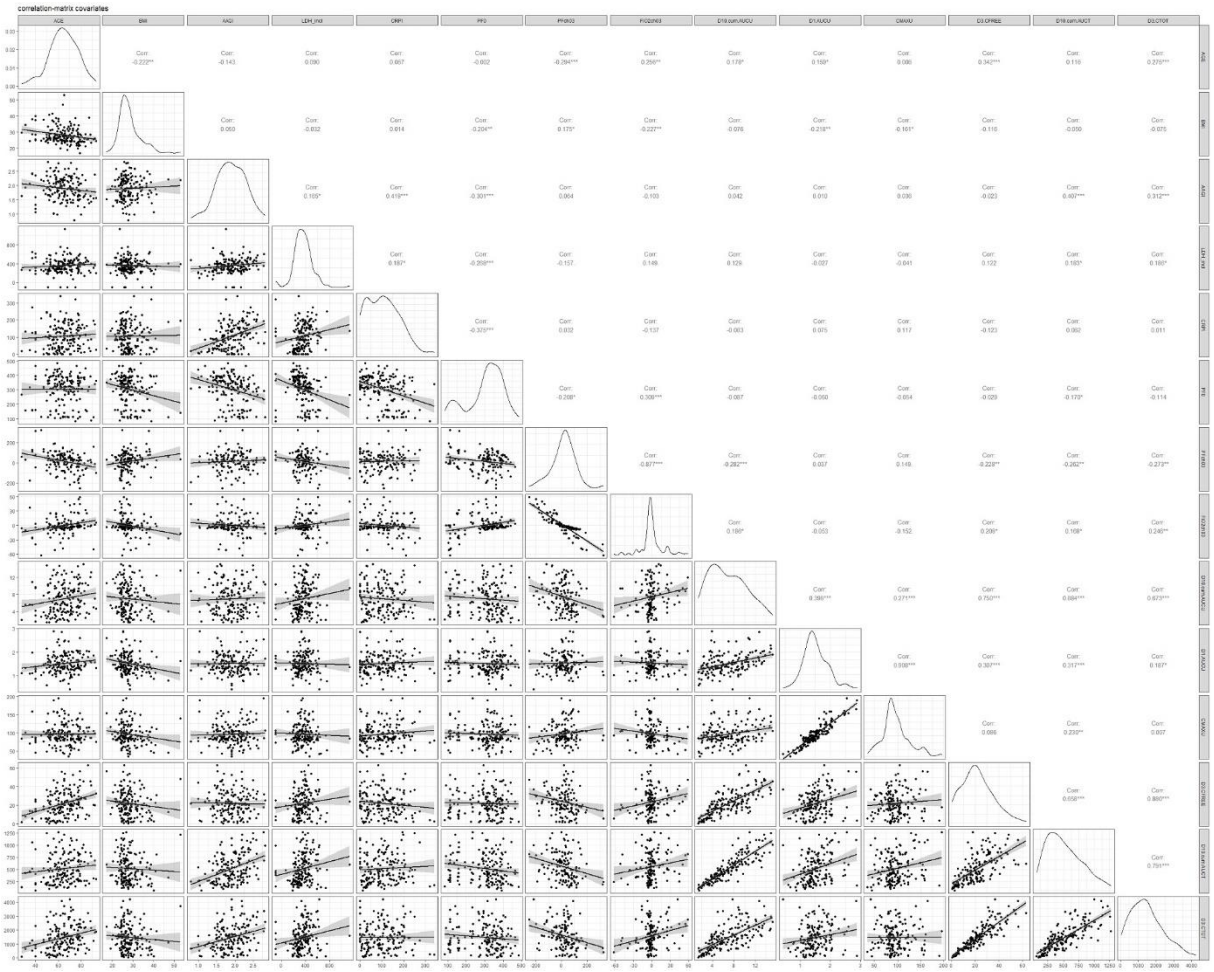
Appendix figure *7:* correlation plots of the PK exposure parameters



D= day, CTOT = total trough concentration (C_{trough}), CFREE = unbound C_{trough}, AUCT= total area under the concentration-time curve (AUC), AUCU= unbound AUC, CMAXU= unbound maximum concentration (C_{max}) on day 1, cum. AUC= cumulative AUC over the full 10 day treatment period.

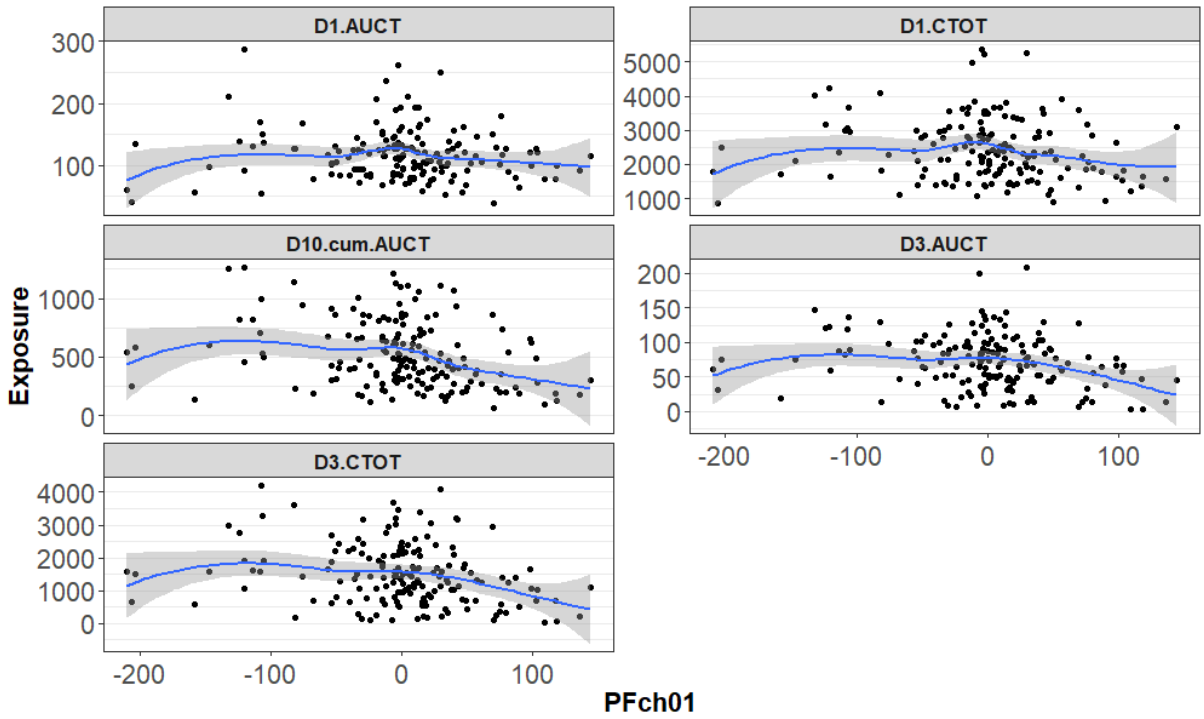
Appendix 2: PD analysis

Appendix figure 8 *correlation plots of baseline biomarkers and PD parameters*



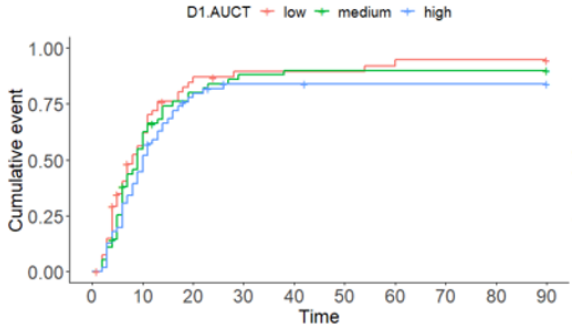
AGE = age, BMI = body mass index, AAGI = imputed alpha-1-acid glycoprotein, ALBI = imputed albumin, NLR = neutrophil to lymphocyte ratio, LDH_incl = lactate dehydrogenase at inclusion, CRPI = imputed C-reactive protein, PF0 = fraction of inspired oxygen (P/F ratio) at baseline, PFch03 = change in P/F ratio on day 3, FiO2ch03 = change in FiO2 on day 3, CMAXU = unbound maximum concentration on day 1, D = day, CTOT = total trough concentration (C_{trough}), CFREE = unbound C_{trough}, AUCT= total area under the concentration-time curve (AUC), AUCU= unbound AUC, cum. AUC= cumulative AUC over the full 10 day treatment period

Appendix figure 9 *Exposure plots for the change in P/F ratio on day 1*

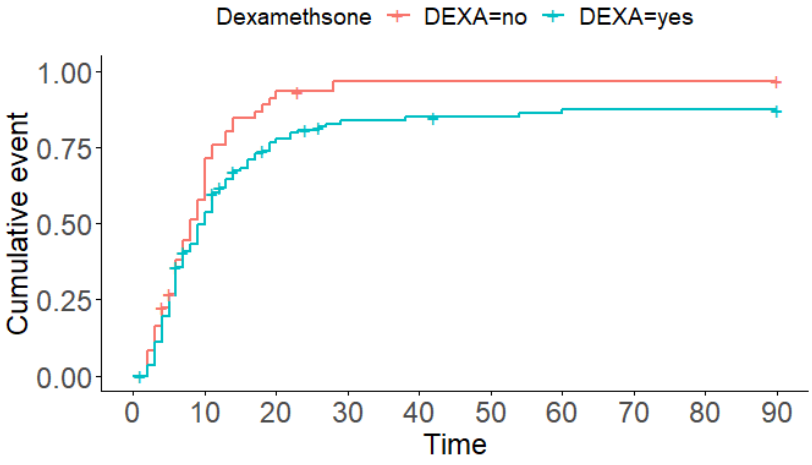


CMAXU = unbound maximum concentration on day 1, D = day, CTOT = total trough concentration (C_{trough}), CFREE = unbound C_{trough}, AUCT= total area under the concentration-time curve (AUC), AUCU= unbound AUC, cum. AUC= cumulative AUC over the full 10 day treatment period.

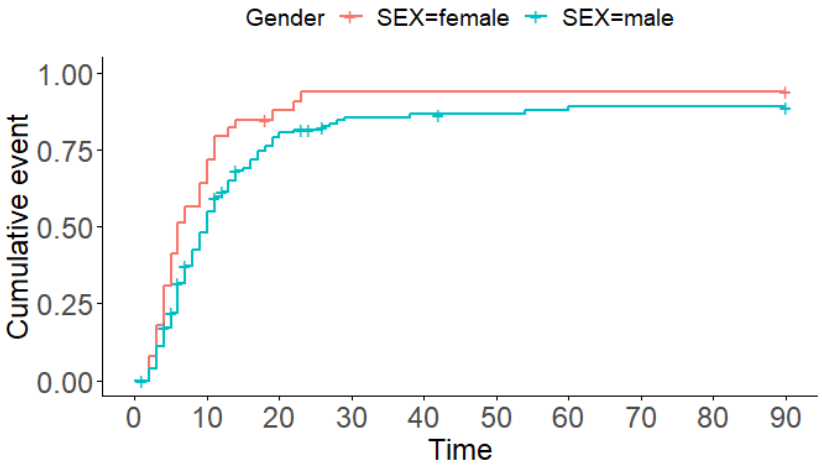
Appendix figure 10 *Kaplan Meier plots for the outcome time till more than 48 hours liberation from ventilation and oxygen supplementation and alive split in low, medium and high exposure Total Area under the concentration time curve (AUC) on day 1.*



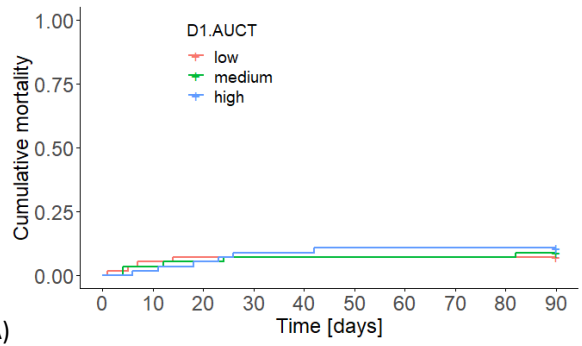
Appendix figure 11 Kaplan Meier plot for the outcome time to liberation from ventilation and oxygen supplementation for more than 48 hours and alive split in treatment or no treatment with dexamethasone



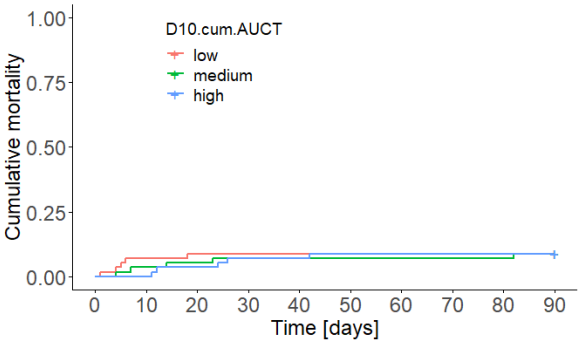
Appendix figure 12 Kaplan Meier plot for the parameter gender and outcome time to liberation from ventilation and oxygen supplementation for more than 48 hours and alive split in female and male



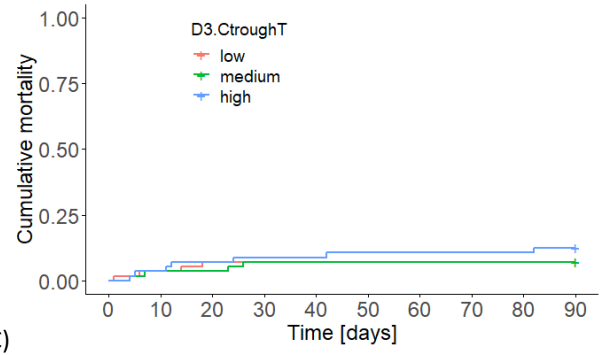
Appendix figure 13 *Kaplan Meier plots of the outcome mortality split in low, medium and high exposure*
A) Total Area under the concentration time curve (AUC) on day 1. B) Cumulative total AUC on day 10. C) Total trough concentrations (C_{trough}) on day 3.



A)



B)



C)

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